9-30-2020

The *Matsucoccus* Cockerell, 1909 of Florida (Hemiptera: Coccomorpha: Matsucoccidae): Potential pests of Florida pines

Muhammad Z. Ahmed

Charles H. Ray

Matthew R. Moore

Douglass R. Miller

A journal of world insect systematics

INSECTA MUNDI

0810

The *Matsucoccus* Cockerell, 1909 of Florida (Hemiptera: Coccomorpha: Matsucoccidae): Potential pests of Florida pines

Muhammad Z. Ahmed

Florida State Collection of Arthropods
Division of Plant Industry, Florida Department of Agriculture and Consumer Services
1911 SW 34th Street
Gainesville, FL 32608, USA
zaheerento@gmail.com

Charles H. Ray

Department of Entomology and Plant Pathology
Auburn University Museum of Natural History
Room 301, Funchess Hall
Auburn University, AL 36849, USA

Matthew R. Moore

Molecular Diagnostics Laboratory
Division of Plant Industry, Florida Department of Agriculture and Consumer Services
1911 SW 34th Street
Gainesville, FL 32608, USA

Douglass R. Miller

Florida State Collection of Arthropods
Division of Plant Industry, Florida Department of Agriculture and Consumer Services
1911 SW 34th Street
Gainesville, FL 32608, USA

Date of issue: October 30, 2020

Center for Systematic Entomology, Inc., Gainesville, FL

Ahmed MZ, Ray CH, Moore MR, Miller DR. 2020. The *Matsucoccus* Cockerell, 1909 of Florida (Hemiptera: Coccomorpha: Matsucoccidae): Potential pests of Florida pines. Insecta Mundi 0810: 1–31.

Published on October 30, 2020 by
Center for Systematic Entomology, Inc.
P.O. Box 141874
Gainesville, FL 32614-1874 USA
http://centerforsystematicentomology.org/

Insecta Mundi is a journal primarily devoted to insect systematics, but articles can be published on any non-marine arthropod. Topics considered for publication include systematics, taxonomy, nomenclature, checklists, faunal works, and natural history. Insecta Mundi will not consider works in the applied sciences (i.e. medical entomology, pest control research, etc.), and no longer publishes book reviews or editorials. Insecta Mundi publishes original research or discoveries in an inexpensive and timely manner, distributing them free via open access on the internet on the date of publication.

Insecta Mundi is referenced or abstracted by several sources, including the Zoological Record and CAB Abstracts. Insecta Mundi is published irregularly throughout the year, with completed manuscripts assigned an individual number. Manuscripts must be peer reviewed prior to submission, after which they are reviewed by the editorial board to ensure quality. One author of each submitted manuscript must be a current member of the Center for Systematic Entomology.

Guidelines and requirements for the preparation of manuscripts are available on the Insecta Mundi website at http://centerforsystematicentomology.org/insectamundi/

Printed copies (ISSN 0749-6737) annually deposited in libraries:

CSIRO, Canberra, ACT, Australia
Museu de Zoologia, São Paulo, Brazil
Agriculture and Agrifood Canada, Ottawa, ON, Canada
The Natural History Museum, London, UK
Muzeum i Instytut Zoologii PAN, Warsaw, Poland
National Taiwan University, Taipei, Taiwan
California Academy of Sciences, San Francisco, CA, USA
Florida Department of Agriculture and Consumer Services, Gainesville, FL, USA
Field Museum of Natural History, Chicago, IL, USA
National Museum of Natural History, Smithsonian Institution, Washington, DC, USA
Zoological Institute of Russian Academy of Sciences, Saint-Petersburg, Russia

Electronic copies (online ISSN 1942-1354, CDROM ISSN 1942-1362) in PDF format.

Printed CD or DVD mailed to all members at end of year. Archived digitally by Portico.
Florida Virtual Campus: http://purl.fcla.edu/fcla/insectamundi
University of Nebraska-Lincoln, Digital Commons: http://digitalcommons.unl.edu/insectamundi/
Goethe-Universität, Frankfurt am Main: http://nbn-resolving.de/urn/resolver.pl?urn:nbn:de:hebis:30:3-135240

The *Matsucoccus* Cockerell, 1909 of Florida (Hemiptera: Coccomorpha: Matsucoccidae): Potential pests of Florida pines

Muhammad Z. Ahmed

Florida State Collection of Arthropods
Division of Plant Industry, Florida Department of Agriculture and Consumer Services
1911 SW 34th Street
Gainesville, FL 32608, USA
zaheerento@gmail.com

Charles H. Ray

Department of Entomology and Plant Pathology
Auburn University Museum of Natural History
Room 301, Funchess Hall
Auburn University, AL 36849, USA

Matthew R. Moore

Molecular Diagnostics Laboratory
Division of Plant Industry, Florida Department of Agriculture and Consumer Services
1911 SW 34th Street
Gainesville, FL 32608, USA

Douglass R. Miller

Florida State Collection of Arthropods
Division of Plant Industry, Florida Department of Agriculture and Consumer Services
1911 SW 34th Street
Gainesville, FL 32608, USA

Abstract. *Matsucoccus krystalae* Ahmed and Miller, **new species**, (Hemiptera: Coccomorpha: Matsucoccidae) is described based on morphological characters of adult females and third-instar males. We **designate** the lectotype of *Matsucoccus alabamae* Morrison. *Matsucoccus alabamae* Morrison and *M. gallicolus* Morrison are redescribed, also based on adult females and third-instar males. Detailed illustrations and descriptions are presented for each species and an identification key for the five species occurring in the eastern U.S. is provided. Analysis of 18S, 28S D2/D3, and 28S D10 loci were performed to support morphological determination. Barcodes using 5′ COI of *M. alabamae* and *M. krystalae* were generated, the first such data from authoritatively identified *Matsucoccus* species. Of particular interest is that most of the specimens in the study were taken in Lindgren funnel traps.

Key words. Distribution, southern US, taxonomy, Lindgren funnel trap, scale insects.

ZooBank registration. urn:lsid:zoobank.org:pub:803B5050-F60D-4E96-8199-7E100258A4C4

Introduction

We became interested in the *Matsucoccus* Cockerell, 1909 of Florida when Krystal Ashman (Florida State Collection of Arthropods, Division of Plant Industry, Florida Department of Agriculture and Consumer Services) noticed third-instar males of an unknown species in Lindgren funnel traps set out as part of a Cooperative Agricultural Pest Survey (CAPS) survey in 2017. At that time, the only published record of *Matsucoccus* in Florida was a species identified as *M. gallicolus* Morrison based on a single collection. We have since determined that these specimens were a new species, not *M. gallicolus*, and are here identified as *M. krystalae*, **new species**. Recently Ahmed and Miller (2020) reported *M. alabamae* from Florida that was collected in Lindgren funnel

traps. Because specimens of both species are often found in Lindgren funnel traps (Ahmed and Miller 2020) and because some species of the genus can cause significant damage to their pine hosts globally, we decided that it would be useful to study the species that occur in Florida.

The genus *Matsucoccus*, commonly called pine scales, pine bast scales or matsucoccids, includes a group of scale insects that occur in North America, East Asia and Europe. There are 32 extant and six extinct *Matsucoccus* species, 17 (16 native and 1 invasive) are reported from the U.S. and five occur in the eastern U.S., all feed on trees in the Pinaceae. Herbert (1919) described *Matsucoccus fasciculensis* from California which was the first report of a *Matsucoccus* species in the United States. *Matsucoccus matusmurae* (Kuwana), a Japanese species, was later reported to occur in the eastern U. S. (Herbert 1921; Morrison 1928). However, it was eventually determined that the eastern U. S. specimens were two different species, *M. alabamae* Morrison and *M. gallicolus* Morrison (Morrison 1939).

The generalized life history of *Matsucoccus* species is as follows (Fig. 1). Eggs are laid in an ovisac that is attached to the body of the adult female and to the pine host. The eggs hatch and first-instar nymphs seek out

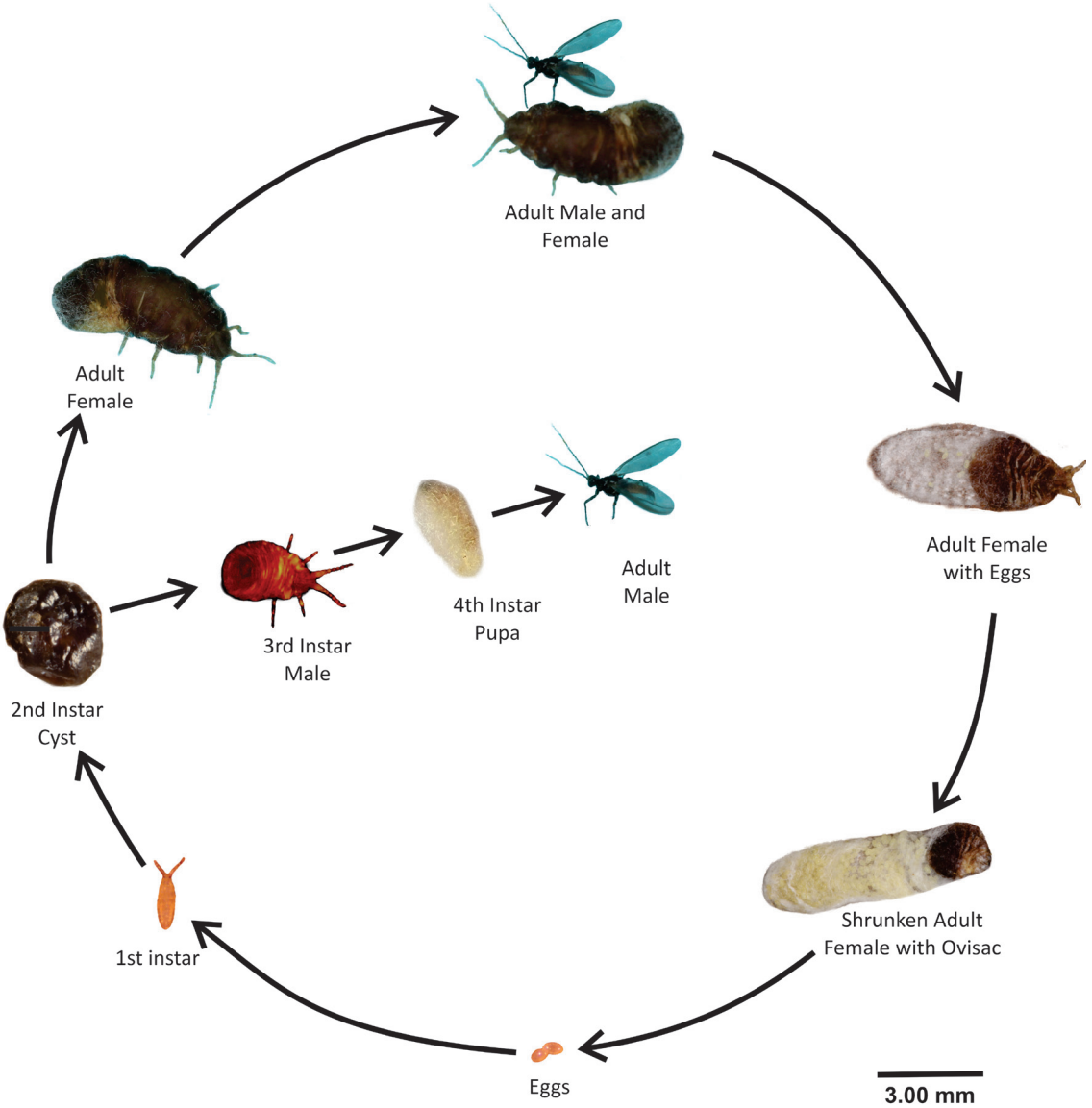

Figure 1. Life cycle of *Matsucoccus alabamae*. With two exceptions, cyst is of *Matsucoccus gallicolus* and 4th instar pupa is of *Matsucoccus josephi*.

a feeding site either under bark, on new growth, or in pine-needle fascicles. Crawlers enlarge to the extent that the larger specimens have erroneously been labeled as second-instar nymphs (Herbert 1919; McKenzie 1943), but they achieve their large body size without molting (Boratynski 1952). When they molt, they transform into a second-instar cyst, which is legless and nearly devoid of useful characters other than large mouthparts and conspicuous spiracles. *Matsucoccus acalyptus* Herbert is an exception, having a second instar between the crawler and the cyst (Unruh and Luck 1987). Second-instar female cysts molt to adult females which have legs and wax pores, but lack mouthparts or mouthparts are rudimentary. Adult females move to exposed parts of the host, bend the abdomen up, and produce a pheromone (Young et al. 1980; Dunkelblum et al. 1993) that attracts the adult males and, in some cases, natural enemies (Mendel et al. 2004).

Males undergo a more complicated life cycle. After molting to the third-instar nymph they develop into a legged instar similar in appearance to the adult female (McKenzie 1942). This instar has been called the prepupa (Foldi 2005). They produce a waxy sac that encloses the body and molt to the fourth-instar pupa. Unlike most male scale insects, some *Matsucoccus* species have only one pupal instar, while others have a short lived prepupa with small wing buds and a pupa with larger wing buds (McCambridge and Pierce 1964). This is the case for *M. josephi* Bodenheimer and Harpaz (Mendel et al. 1997). Adult males emerge from the pupal sac and seek out newly emerged adult females. In at least one case, it was suggested that mating must occur for the female to produce eggs (Ray 1982). Some populations of *M. pini* (Green) are reported to be parthenogenetic (Boratynski 1952), but others have functional adult males (Rieux 1976). *Matsucoccus macrocicatrices* Richards is parthenogenetic (Mech et al. 2013). Most *Matsucoccus* species have a single generation each year, while *M. macrocicatrices* may take two years to complete a generation in some populations, *M. pini* has one or two generations each year (Boratynski 1952), and *M. josephi* may have five or six generations each year (Mendel et al. 1997).

Several species of *Matsucoccus* have been implicated in causing damage, even serious damage, to pines in many parts of the world. They include: *M. acalyptus* (McCambridge and Pierce 1964); *M. bisetosus* Morrison (McKenzie 1942); *M. feytaudi* Ducasse (Riom and Fabre 1979); *M. gallicolus* (Parr 1939; Aughanbaugh 1949); *M. josephi* (Mendel et al. 1997); *M. massonianae* Young and Hu (Young et al. 1976); *M. macrocicatrices* (Mech et al. 2013); *M. matsumurae* (Kuwana) (McClure 1981; Shin et al. 2003); *M. monophyllae* McKenzie (McKenzie 1941); *M. vexillorum* Morrison (McKenzie et al. 1948); *M. yunnanensis* Ferris (Qi and Wang 1981). In light of significant economic damage caused by several species of *Matsucoccus*, it is important to document as much information as possible about the species that occur in Florida.

The purpose of this paper is to summarize the available data on the two species of *Matsucoccus* that presently occur in Florida, including line drawings of slide-mounted third-instar males and adult females; descriptions of third-instar males and adult females; 18S, 28S, and COI sequence data for each species; life history data; economic importance; and distribution information.

Materials and Methods

Illustrations were made using a Leica DMRB compound microscope and a camera lucida. Terminology follows that of Morrison (1939). Numerical values were taken from a minimum of five specimens from as many localities as possible and include the observed range followed by the values given in Ray (1982) in parentheses. Roman numerals were used for abdominal segments. All specimens are deposited in the Florida State Collection of Arthropods, Gainesville (FSCA) unless otherwise indicated. Other depositories include: AUMNH (Auburn University Museum of Natural History, Auburn University, Alabama); UCDC (Bohart Museum, University of California, Davis); NHMUK (The Natural History Museum, London); CSCA (California Department of Food and Agriculture, Sacramento); USNM (United States National Museum of Natural History, scale insect collection, Beltsville, Maryland).

The Lindgren funnel trap was developed by Lindgren (1983). It is composed of a cover at the top of the trap, a series of vertically stacked funnels, and a bottom cup that contains propylene glycol. The traps also have lures that are attached to one or more of the funnels. They are held in plastic packets and are attached near the top of the trap, usually on funnels two through four. If there is more than one packet, they may be attached to the same funnel or a different one, but if on the same funnel they are placed on different sides. Lures used in traps that

captured *Matsucoccus* species were ethanol, Alpha-Pinene, and Monochamol. Traps are usually suspended from a tree branch, so the bottom cup is not reachable when standing on the ground (Fig. 2). This high placement is so that casual observers cannot easily disturb a trap.

DNA extraction, PCR, and sequencing. Four *M. krystalae* (FDACS 2019-3259 and FDACS 2020-1594) and four *M. alabamae* (FDACS 2019-1868 and FDACS 2019-1869) specimens were used for DNA extraction with a Qiagen Blood and Tissue Kit per the manufacturer recommendations. DNA extracts were quantified on a Nanodrop 2000 spectrophotometer and subsequent PCRs utilized an input of at least 20 ng of genomic DNA extract. Previous studies (e.g., Booth and Gullan 2006) have used slowly evolving ribosomal genes for molecular study of *Matsucoccus* species and populations. We targeted these same ribosomal gene regions for sequencing, while also targeting the standard 5′-COI barcode fragment. PCRs were set up as 25 µL reactions using Kapa HiFi HotStart ReadyMix kits. PCR primers and basic thermocycle conditions are listed in Table 1. All PCRs used 35 melting, annealing, and extension thermocycles.

Positive PCRs were purified with Qiagen QIAquick PCR Purification Kits. Purified PCR products were then prepared for sequencing with an Applied Biosystems BigDye Terminator v.3.1 Cycle Sequencing Kit. Bidirectional sequencing was performed on the Applied Biosystems SeqStudio platform at the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida. Sequence traces were trimmed and assembled into sequence contigs in Sequencher 5.4.6 (Gene Codes Corporation). All newly generated sequence data from this study were deposited in GenBank (Table 2).

Distance and phylogenetic analyses. GenBank was datamined for 18S, 28S D2/D3 expansion region, and 28S D10 expansion region sequences (Table 2). Sequences from Booth and Gullan (2006) for *M. gallicolus* were provided to us by the authors (pers. comm. with Janie Booth, Lyn Cook, and Penny Gullan in September 2019). Based on the results of Vea and Grimaldi (2016), we selected the margarodid *Eumargarodes laingi* Jakubski as

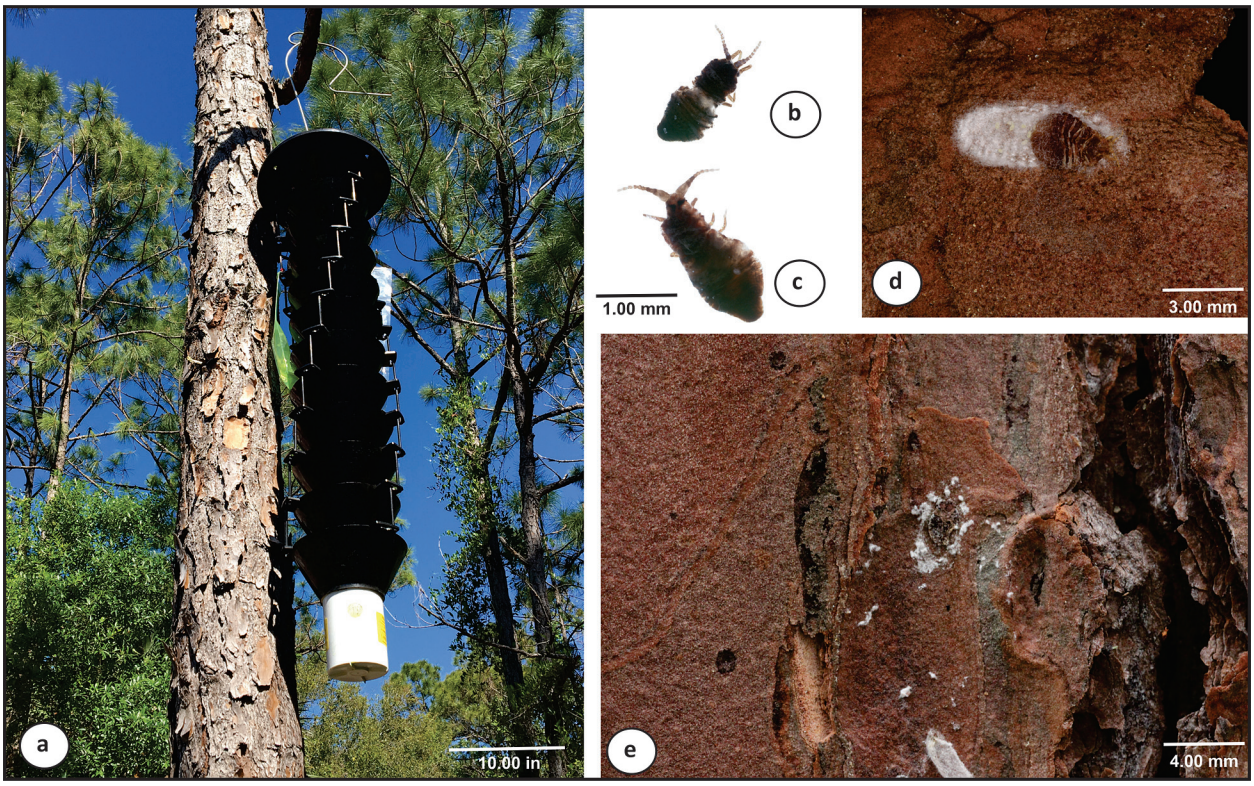

Figure 2. Field appearance of *Matsucoccus krystalae* and *M. alabamae*. **A)** Lindgren funnel trap. **B)** *M. krystalae* third-instar male from Lindgren funnel trap. **C)** *M. krystalae* adult female from Lindgren funnel trap. **D–E)** Old shriveled female mummies of *M. alabamae* with egg sacs on pine trees.

Table 1. PCR primers and thermocycling conditions.

Gene region	Primer name and sequence	Primer citation	Annealing temp. (°C)	Extension time (s)
18S 24-585	18S-2880: CTGGTTGATCCTGCCAGTAG	Tautz et al. (1988)	59	45
	18S-B: CCGCGGCTGCTGGCACCAGA	von Dohlen and Moran (1995)		
28S D2/D3 expansion region	S3660: GAGAGTTMAASAGTACGTGAAAC	Dowton and Austin (1998)	58	45
	A335: TCGGARGGAACCAGTCTAACTA	Whiting et al. (1997)		
28S D10 expansion region	None: GAATGGATTAACGAGATTCTCAA	Booth and Gullan (2006)	53	60
	None: CACAATGATAGGAAGAGCC	Dietrich et al. (2001)		
COI-5′	PcoF1: CCTTCAACTAATCATAAAAATATYAG	Park et al. (2010)	50	60
	LEP-R1: TAAACTTCTGGATGTCCAAAAA	Hebert et al. (2004)		
	LCO1490: GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)	50	60
	HCO2198: TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)		

Table 2. GenBank accession numbers for sequences used in this study.

Species	Voucher/isolate	18S	28S D2/D3	28S D10	COI
Eumargarodes laingi	AMCC: 200602	KT199035	KT199058	KT199078	—
Matsucoccus alabamae	1868-1	MT621659	MT622178	MT622189	MT621224
Matsucoccus alabamae	1868-2	MT621660	MT622179	MT622190	MT621225
Matsucoccus alabamae	1869-1	MT621661	MT622180	MT622191	MT621226
Matsucoccus alabamae	1869-2	MT621662	MT622181	MT622192	MT621227
Matsucoccus krystalae	3259-1	MT621663	MT622182	MT622193	MT621228
Matsucoccus krystalae	3259-2	MT621664	MT622183	MT622194	MT621229
Matsucoccus krystalae	1594-2	MT621665	MT622184	MT622195	—
Matsucoccus krystalae	1594-5	—	MT622185	MT622196	—
Matsucoccus gallicolus	JMB023	MT621666	MT622186	—	—
Matsucoccus gallicolus	JMB024	MT621667	MT622187	—	—
Matsucoccus gallicolus	JMB036	MT621668	MT622188	—	—
Matsucoccus macrocicatrices	LGC02231	KF053072	KF040554	KF040584	—
Matsucoccus macrocicatrices	LGC02229	KF053074	KF040556	KF040583	—
Matsucoccus macrocicatrices	LGC02226	KF053077	KF040559	KF040582	—
Matsucoccus macrocicatrices	LGC02225	KF053078	KF040560	KF040581	—
Matsucoccus macrocicatrices	LGC02224	KF053079	KF040561	KF040580	—
Matsucoccus macrocicatrices	LGC02221	KF053082	KF040563	KF040579	—
Matsucoccus macrocicatrices	LGC02220	KF053083	KF040564	KF040578	—
Matsucoccus macrocicatrices	LGC02219	KF053084	KF040565	KF040577	—
Matsucoccus macrocicatrices	LGC02218	KF053085	KF040566	KF040576	—
Matsucoccus macrocicatrices	LGC02217	KF053086	KF040567	KF040575	—
Matsucoccus macrocicatrices	LGC02216	KF053087	KF040568	KF040574	—
Matsucoccus macrocicatrices	LGC02214	KF053089	KF040570	KF040573	—
Matsucoccus matsumurae	GH	MH574839	—	MH574783	—

Table 2. Continued.

Species	Voucher/isolate	18S	28S D2/D3	28S D10	COI
Matsucoccus matsumurae	SC	MH574840	—	MH574784	—
Matsucoccus matsumurae	BA	MH574841	—	MH574785	—
Matsucoccus matsumurae	TA	MH574842	—	MH574786	—
Matsucoccus matsumurae	PH	MH574843	—	MH574787	—
Matsucoccus matsumurae	SU	MH574844	—	MH574788	—
Matsucoccus matsumurae	JJ	MH574845	—	MH574789	—
Matsucoccus matsumurae	IMA	MH574846	—	MH574790	—
Matsucoccus sp.	IMV-2016	KT199029	KT199053	KT199072	—

an outgroup with appropriate data coverage for three genes (18S, 28S D2/D3, 28S D10). Sequences were aligned using MUSCLE (Edgar 2004), with default settings, as implemented in MEGA X (Kumar et al. 2018). The resulting alignments were maintained as standalone alignments for distance analyses and concatenated for phylogenetic analyses (553 bp 18S; 722 bp 28S D2/D3; 702 bp 28S D10; 594 bp COI). Sequences were also assembled and examined using SeqMan Pro ver. 7.1.0 (DNASTAR star, Inc., USA). Alignment of DNA sequences was also conducted online using MAFFT ver. 7 package (Katoh et al. 2019) on the server (http://mafft.cbrc.jp/alignment/software/).

Gene sequence translation was verified in MEGA X (Kumar et al. 2018) for the presence of inframe stop codons and indels, which can indicate nuclear mitochondrial pseudogenes (NUMTs), generally known to be impediments to DNA barcoding (Song et al. 2008; Leite 2012). For the aligned dataset, phylogenetic trees were constructed using the neighbor joining (NJ) method algorithm with support analysis (1,000 replicates) included in MEGA X (Kumar et al. 2018) based on the Kimura- 2-Parameter (K2P) model. This has been the most widely used method for DNA barcoding analyses (e.g., Yeh et al. 1997; Shin et al. 2013; Gwiazdowski et al. 2015; Wu et al. 2016; Amouroux et al. 2017; Kanturski et al. 2018; Song et al. 2018), including previous works on other hemipterans (Lee et al. 2008; Kang et al. 2010; Katoh et al. 2013, 2014; Chen et al. 2018).To compare our results with those papers, we elected to use the same methodology despite some potential limitations, such as a poor fit of the K2P model at the species level (Srivathsan and Meier 2011; Collins et al. 2012). Pairwise distances were also computed using MEGA X (Kumar et al. 2018). Distance analyses were conducted to assess degree of intra- and interspecific divergences in 18S, 28S D2/D3, 28S D10, and COI in *Matsucoccus* species. *Matsucoccus* sequences were aligned without the outgroup *E. laingi* with the default setting of MUSCLE (Edgar 2004). K2P (Kimura 1980) distances were then calculated for each gene region using partial deletion of missing data with a site coverage cutoff of 95% in MEGA X (Kumar et al. 2018).

Maximum-likelihood analyses of the concatenated matrix were conducted in W-IQ-TREE (Trifinopoulos et al. 2016). The matrix was partitioned by gene (18S, 28S D2/D3, and 28S D10) and codon position (COI). The best fit model of sequence evolution for each partition (K2P+G4 for 18S; TN+F+G4 for 28S D2/D3; TN+F+I for 28S D10; HKY+F for COI first position; F81+F for COI second position; HKY+F for CO1 third position) was selected by ModelFinder (Kalyaanamoorthy et al. 2017) using Bayesian information criteria. Bootstrap support values were calculated using 100 standard ML bootstrap replicates, 10,000 ultrafast ML bootstrap replicates (Hoang et al. 2017), and 10,000 SH-aLRT replicates (Guindon et al. 2010). Parsimony tree searches were performed in MPBoot (Hoang et al. 2018). Heuristic searches were conducted using default parsimony ratchet search options. Bootstrap analyses used the same default parsimony ratchet search options and included 10,000 bootstrap replicates.

Results

Lindgren Funnel Traps

Lindgren funnel traps collect mobile adult females and third-instar males, but we have never recovered other mobile forms such as first-instar nymphs or adult males. Several observations are of interest when examining

these data: 1) both *M. alabamae* and *M. krystalae* have been collected in the traps, 2) only adult females of *M. alabamae* have been recovered from the traps, 3) third-instar males of *M. alabamae* have never been taken in the traps even though third-instar males of *M. krystalae* are the most commonly collected specimens, 4) *Matsucoccus krystalae* specimens were common in numerous traps, and 5) because there is a top on the Lindgren funnel traps, trapped specimens must have actively crawled into the funnels. It is unlikely that they would be windblown since only some instars were collected in the traps even though others are in the vicinity.

A possible explanation for the differences in species counts in traps might be that the life history of *M. alabamae* takes place principally on the lower tree trunk, whereas that of *M. krystalae* apparently occurs on small branches and twigs higher in the tree. Lindgren funnel traps usually are attached to smaller branches of trees (the habitat of *M. krystalae*) and are not as accessible to activity of *M. alabamae*. Another possible explanation might be differential lure attractiveness between the two species. Traps captured both species across Florida.

We noticed the posterior end of the abdomen in many adult female and third-instar male *M. krystalae* were bent under the body resulting in the abdominal apex lying beneath abdominal segments IV, V, or VI. Our hypothesis is that the propylene glycol in Lindgren collection cups may cause this distortion, but we have not observed similar distortion in *M. alabamae*.

Distance Analyses

Intraspecific divergences were low for the analyzed genes (Table 3), ranging from 0% to 1.5%. Interspecific divergences ranged from 1.3% (28S D10) to 14.6% (28S D2/D3).

The sequences of *M. krystalae* were strongly divergent for the comparable 18S and 28S D2/D3 data (Table 3). The genetic distance of *M. krystalae* was 0.4–1.4% from *M. gallicolus* based on 18S and 28S D2/D3. COI barcodes have generally not been used for molecular identification of scale insects due to PCR difficulties (see Park et al. 2010). We report the first COI barcodes from authoritatively identified *Matsucoccus* species, *M. krystalae* and *M. alabamae*. These barcodes have a 4.3% K2P distance from each other. GenBank Blastn searches and BOLD identification searches yielded only one significant match (89% and 85%) to an unidentified Hemiptera specimen

Table 3. Intraspecific and interspecific K2P distances of tested species based on following genes 18S rDNA /28S D2/D3 rDNA /28S D10 rDNA/COI.

Species	Mean (%)	Min (%)	Max (%)
Intraspecific K2P distances			
M. krystalae	0.0/0.04/0.0/0.0	0.0/0.0/0.0/0.0	0.0/0.15/0.0/0.0
M. gallicolus	0.0/0.04/NA/NA	0/0/0.0/NA/NA	0/0.15/NA/NA
M. alabamae	0.04/0.22/0.0/0.0	0/0.15/0.0/0.0	0.19/0.29/0.0/0.0
M. marcocicatrices	0.0/0.0/0.03/NA	0.0/0.0/0.0/NA	0.0/0.0/0.34/NA
M. matsumurae	0.0/NA/0.0/NA	0.0/NA/0.0/NA	0.0/NA/0.0/NA
Interspecific K2P distances			
M. krystalae–M. gallicolus	0.4/1.4/NA/NA	0.4/1.3/NA/NA	0.4/1.5/NA/NA
M. krystalae–M. alabamae	2.4/8.8/1.3/4.4	2.2/8.0/1.2/4.3	2.4/8.8/1.4/4.5
M. krystalae–M. marcocicatrices	3.7/13.0/5.0/NA	3.6/12.8/5.0/NA	3.7/13.2/5.1/NA
M. krystalae–M. matsumurae	6.5/NA/8.1/NA	6.4/NA/8.0/NA	6.6/NA/8.1/NA
M. gallicolus–M. alabamae	2.2/9.0/1.3/NA	2.1/8.8/NA/NA	2.3/9.2/NA/NA
M. gallicolus–M. marcocicatrices	3.7/13.0/NA/NA	3.6/12.8/NA/NA	3.8/13.3/NA/NA
M. gallicolus–M. matsumurae	6.9/NA/NA/NA	6.6/NA/NA/NA	6.9/NA/NA/NA
M. alabamae–M. marcocicatrices	3.2/11.5/5.5/NA	3.2/11.4/5.4/NA	3.2/11.6/5.6/NA
M. alabamae–M. matsumurae	5.3/NA/7.6/NA	5.2/NA/7.5/NA	5.4/NA/7.7/NA
M. marcocicatrices–M. matsumurae	1.5/NA/1.6/NA	1.4/NA/1.4/NA	1.6/NA/1.6/NA

(BIOUG11208-B11; BIN BOLD:ACM6572) from Nova Scotia, Canada. Based on the images of the male specimen, we identified it as a *Matsucoccus* species, most likely *M. macrocicatrices* (pers. comm. with Jayme Sones, June 2020).

Phylogenetic Analyses

A total of 22 aligned sequences for 18S and 28S D2/D3 rDNA (Fig. 3) and 570 bp and 725 bp sequence fragments respectively with outgroups were analyzed (Table 2). All four *Matsucoccus* species are characterized by a distinctive set of 18S and 28S D2/D3 rDNA sequences that form well-supported clusters in the NJ-trees (bootstrap values of 96–100%; Fig. 3–4). Three populations of northern *M. gallicolus* show no significant divergence from each other based on both 18S and 28S D2/D3 rDNA (Fig. 3–4) and clustered in a separate well-supported subclade sister to *M. krystalae*.

MPBoot heuristic tree searches recovered most parsimonious trees of score 657 steps. Analyses of the concatenated matrix recovered two nodes that where strongly supported by all resampling tests (>80 standard ML bootstrap; >95 UF ML bootstrap; >95 UF MP bootstrap; >80 SH-aLRT) (Fig. 5). An additional five nodes were strongly supported by some, but not all resampling tests (Fig. 5). One of these moderately supported nodes forms a clade of *M. krystalae* + *M. gallicolus*. No other sister relationships between *Matsucoccus* species were strongly supported in these analyses.

Taxonomy

Key to *Matsucoccus* species from the eastern U.S. based on adult females

1. Venter with two sizes of setae; setae anterior of hind coxae and in medial areas of abdomen noticeably longer than other ventral setae **2**
— Venter with one size of ventral setae; setae anterior of hind coxae approximately same length as other ventral setae . . . . . ***M. alabamae* Morrison (Fig. 6)**

2(1). Cicatrices present on metathorax or abdominal segment I **4**
— Cicatrices absent from metathorax and abdominal segment I **3**

3(2). Cicatrices present in four rows on abdominal segments III–VI; cicatrices greater than 15 μm in diameter; with less than 100 cicatrices . . . . . ***M. macrocicatrices* Richards**
— Cicatrices present in five rows on abdominal segments II–VI; cicatrices less than 15 μm in diameter; with more than 100 cicatrices . . . . . ***M. matsumurae* (Kuwana)**

4(2). With 20–32 multilocular pores; with 170–378 cicatrices; cicatrices in medial and mediolateral areas, but not in lateral areas . . . . . ***M. gallicolus* Morrison (Fig. 10)**
— With 36–72 multilocular pores; with 437–900 cicatrices; cicatrices in medial, mediolateral, and lateral areas on some segments . . . . . ***M. krystalae* Ahmed and Miller, new species (Fig. 12)**

Matsucoccus alabamae Morrison, 1939

(Fig. 6–7)

Matsucoccus matsumurae (Kuwana); Herbert 1921: 15; Morrison 1928: 3. misidentification (Morrison 1939: 13). (in part; also see *M. gallicolus*).

Matsucoccus alabamae Morrison 1939: 2. Accepted valid name.

Adult Female

(Fig. 6)

Diagnosis. Cicatrices usually in four rows, anterior row narrower than remaining rows; body setae all about same size; vulva present near posterior end of abdomen; fleshy setae present on distal four antennal segments.

Description. Body elongate, parallel sided, 4.0–6.9 (4.4–6.7) mm long, 1.8–2.2 (1.8–2.2) mm wide. Dorsum: Setae uncommon, inconspicuous, arranged in rows, of one size, 8–10 (7–11) μm long. Bilocular tubular ducts scattered over surface, uncommon on head and within rows of cicatrices; tubes sometimes slightly divergent. Cicatrices in four rows on segments III to VI, rarely with cicatrices in small numbers on segments II and/or VII;

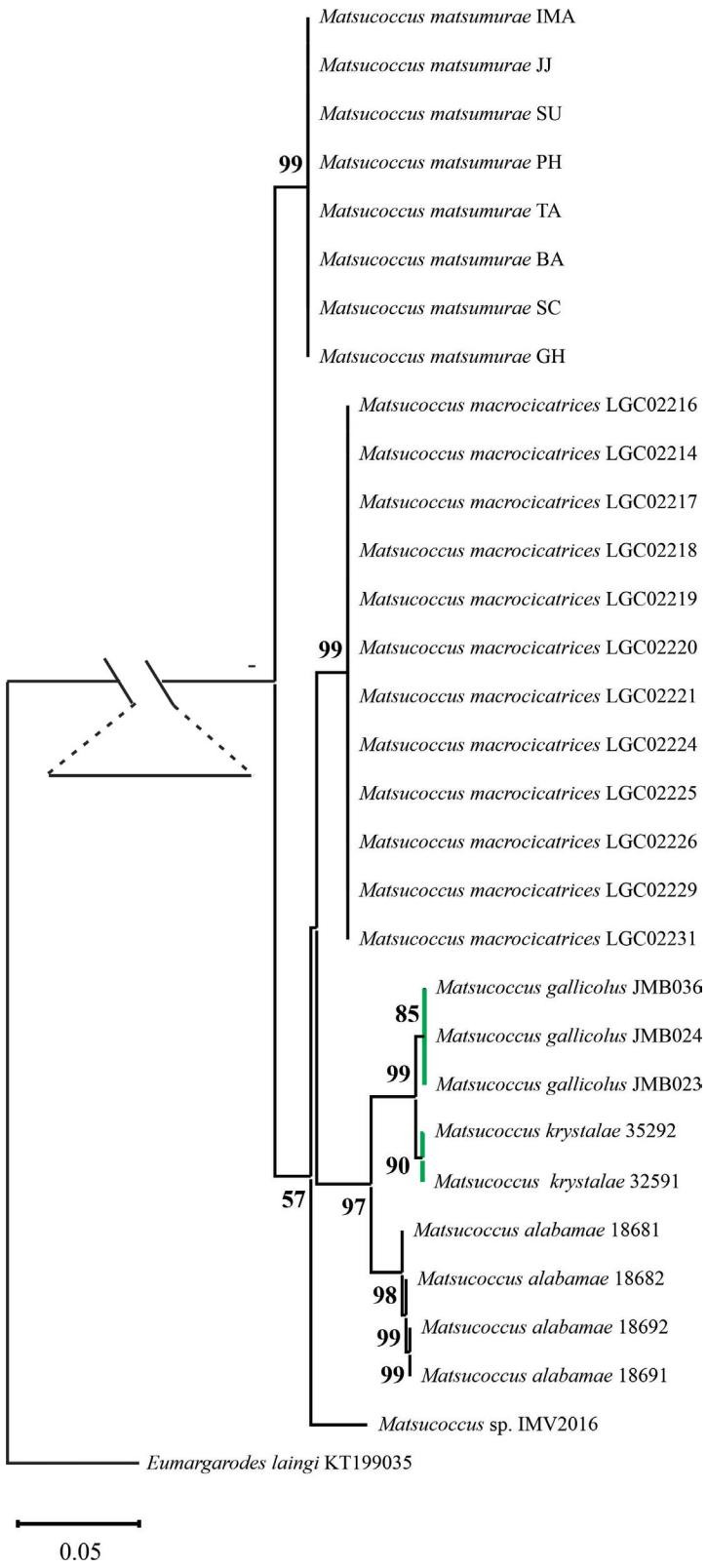

Figure 3. Neighbor-joining trees based on Kimura 2-Parameter genetic distance based on 18S rDNA. Bootstrap support values are shown at the branch points and are based on 1,000 replications. Support values labeled with "-" have support lower than 50%.

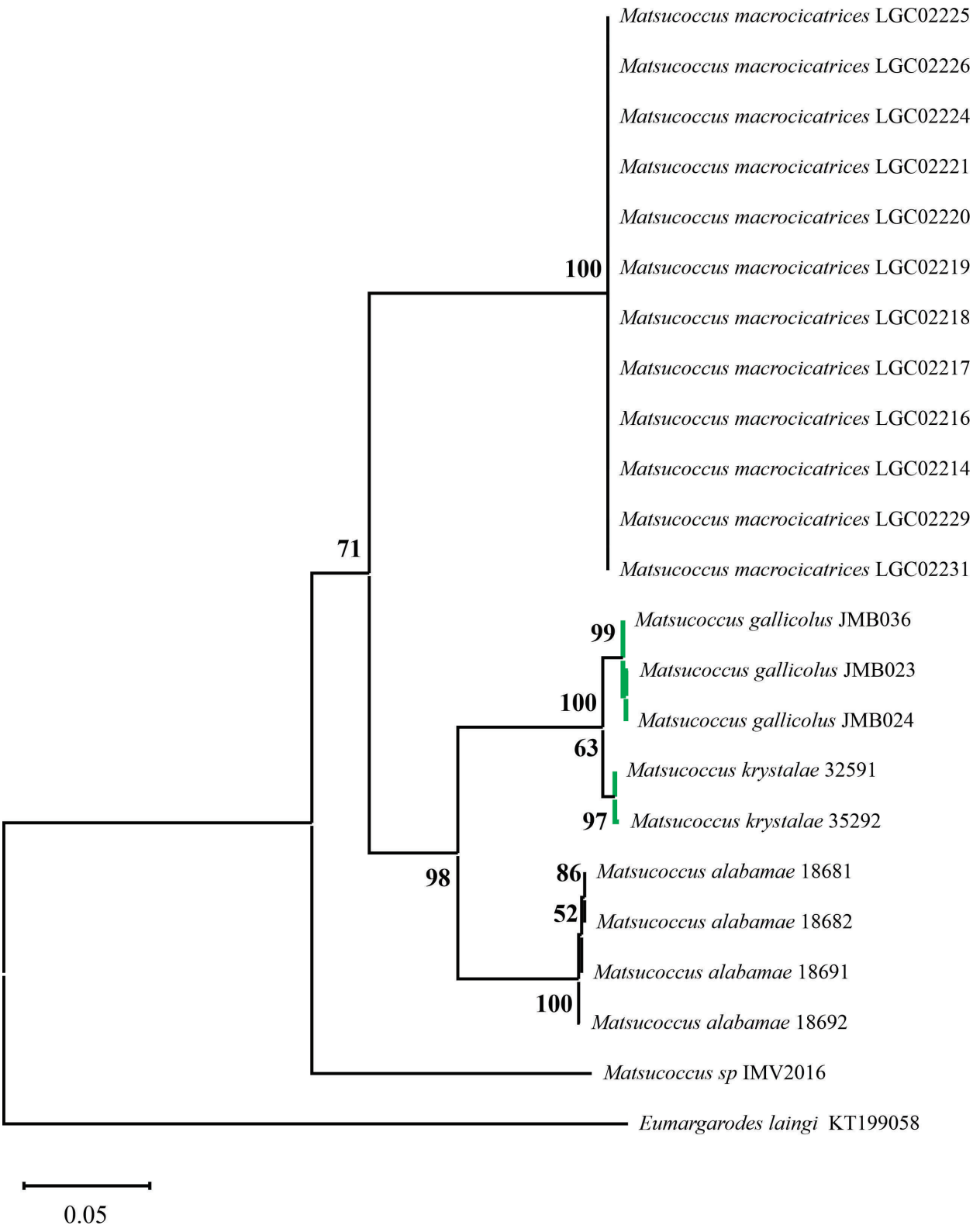

Figure 4. Neighbor-joining trees based on Kimura 2-Parameter genetic distance based on 28S D2/D3 rDNA. Bootstrap support values are shown at the branch points and are based on 1,000 replications.

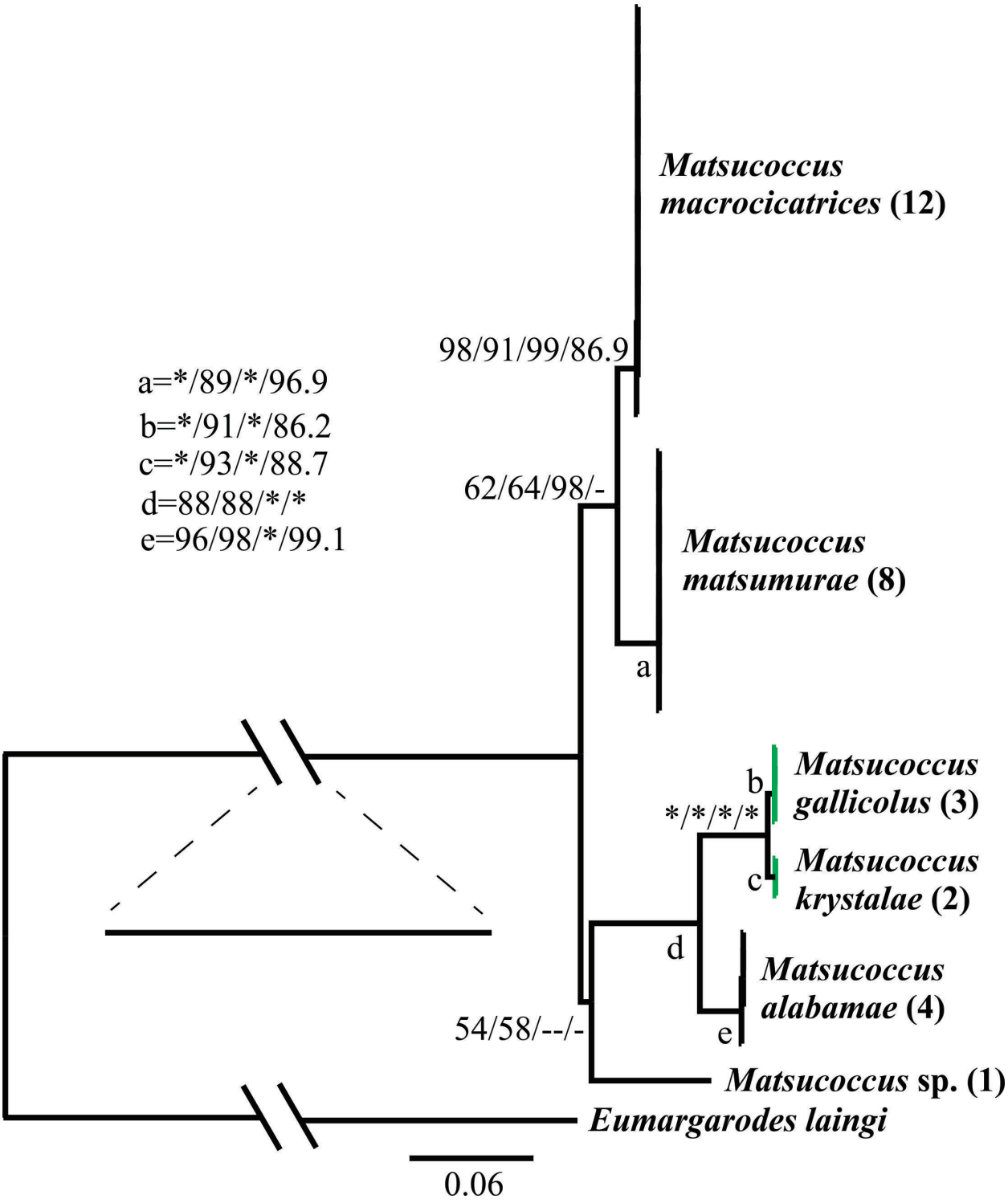

Figure 5. Bootstrap consensus tree from W-IQ-TREE analysis. Node support values from left to right are standard ML bootstrap, ultrafast ML bootstrap, ultrafast MP bootstrap, and SH-aLRT. Support values labeled with a "*" have 100% support. Support values labeled with "-" have support lower than 50%. Support values with a "--" indicate that node was not recovered by an analysis. Numbers in parentheses indicate the number of individuals.

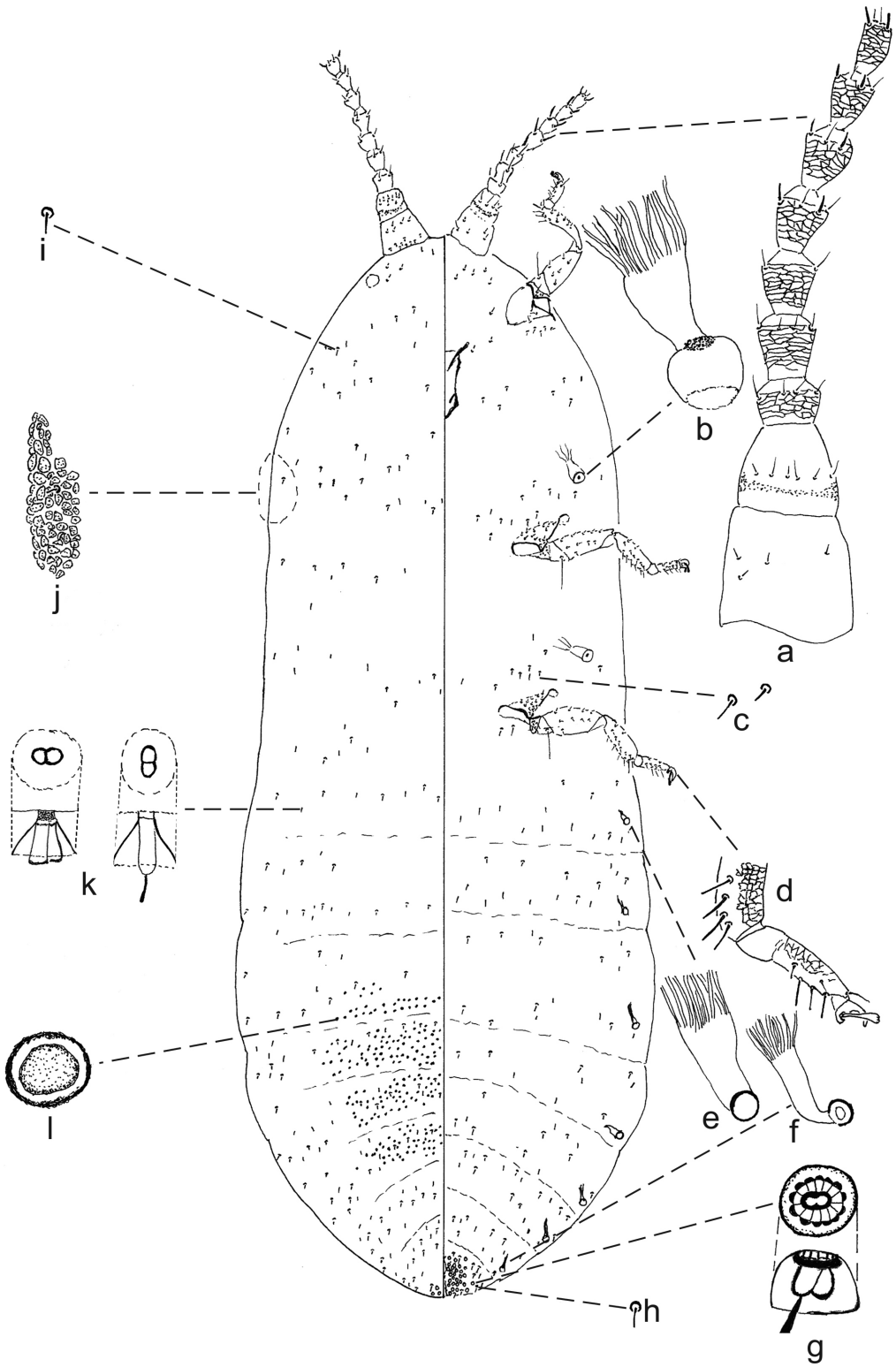

Figure 6. *Matsucoccus alabamae* Morrison. Adult female. Duval Co., Florida 9A, West of Yellow Bluff Fort Historic State Park, Jacksonville, Florida, November 1, 2018, on *Pinus* sp., M. Lara. **A)** Antenna. **B)** Thoracic spiracle. **C and H)** Short ventral filamentous seta. **D)** Hind tibia. tarsus, claw. **E)** First abdominal spiracle. **F)** last abdominal spiracle. **G)** Multilocular pore. **I)** Dorsal filamentous seta. **J)** Dermal pattern. **K)** Bilocular tubular ducts. **L)** Cicatrix.

anterior row on segment III usually about half as wide as other rows; with 223–370 (95–511) cicatrices; largest 8–11 (9–14) μm in diameter.

Venter. Setae uncommon, arranged in rows, of one size 8–10 (7–11) μm long, over most of surface; longest seta anterior of hind coxae 10–16 μm long. Bilocular tubular ducts arranged segmentally, uncommon on head, most abundant on margin; similar in size and shape as on dorsum. Multilocular pores in cluster surrounding vulva at, or near, abdominal apex; with 42–64 (44–88) pores; diameter of largest pore 8–11(11–15) μm. Thoracic spiracles without definite sclerotized rim on derm; posterior spiracle with largest diameter of atrium 32–49 (33–48) μm; with seven pairs of abdominal spiracles, normally with dermal orifice sclerotized, all equally sclerotized and about same size, diameter of sclerotized dermal orifice of first abdominal spiracle 13–20 (15–22) μm, of seventh spiracle 17–20 (14–17) μm. Legs large and conspicuous, with scale-like pattern on all segments except first tarsal segment; hind trochanter with one (rarely two) long seta, 7–11 (10–12) sensoria; femur 228–290 (274–309) μm long, with 12–18 (8–22) setae; tibia 220–255 (242–321) μm long, with 31–38 (25–41) setae; tarsus (both segments) 150–172 μm long, tarsal digitule undifferentiated; claw without denticle, with two conspicuous digitules, equal in size. Antennae nine segmented, 0.7–0.8 (0.7–0.8) mm long; apical four segments each with two enlarged setae; first segment 135–165 (131–168) μm long, 165–210 (148–226) μm wide, with 2–4 setae on ventral surface, 16–24 setae on dorsal surface; second segment 115–125 (111–136) μm long, 100–130 μm wide, with 4–9 setae on ventral surface, 6–10 setae on dorsal surface. Anterior apex of vulva lying under anterior apex of abdominal segment VIII on dorsum.

Remarks. For a comparison of this species with *M. gallicolus* see the notes section of the latter. This species is very similar to *M. banksianae* Ray and Williams, but the latter differs (characters in parentheses are of *M. alabamae*) by having 3, 4, or 5 rows of cicatrices (four rows), and occurs on *Pinus banksiana* Lamb. in Minnesota (occurs on *Pinus elliottii* Engelm., *P. taeda* L., and *P. rigida* Mill. in the southeastern U.S.). The above description is based on 14 specimens from seven localities.

Third-instar Male
(Fig. 7)

Diagnosis. One size of seta on venter, setae anterior of metacoxae 8–11 μm long; with eight or fewer bilocular tubular ducts on dorsum of abdominal segment III; developing genitalia at posterior end of abdomen; abdominal spiracles usually similar in size; fleshy setae present on distal four antennal segments.

Description. Body elongate, parallel sided, 2.5–2.9 mm long, 0.9–1.2 mm wide. Dorsum: Setae uncommon, inconspicuous, arranged in rows, of one size, 8–12 μm long. Bilocular tubular ducts scattered over surface, in segmental rows, associated with setal rows, arranged in longitudinal lines with lines present submedially, mediolaterally, and laterally, with six or seven ducts on abdominal segment III, tubes sometimes slightly divergent.

Venter. Setae uncommon, arranged in rows, of one size 8–10 μm long, over most of surface; longest seta anterior of hind coxae 10–16 μm long. Bilocular tubular ducts uncommon on head and medial areas of thorax, most abundant on margin, arranged in longitudinal lines, present submedially and laterally; similar in size and shape as on dorsum. Thoracic spiracles without definite sclerotized rim on derm; posterior thoracic spiracle with largest diameter of atrium 24–29 μm; with seven pairs of abdominal spiracles, normally with dermal orifice sclerotized, all equally sclerotized and about same size, diameter of sclerotized rim of dermal orifice of first abdominal spiracle 10–15 μm, of seventh spiracle 10–11 μm. Legs large and conspicuous, with scale-like pattern on all segments except first tarsal segment; hind leg with trochanter with one long seta, 8–13 sensoria; femur 170–195 μm long, with 16–21 setae; tibia 170–205 μm long, with 27–41 setae; tarsus (both segments) 100–120 μm long, tarsal digitule undifferentiated; claw without denticle, with two conspicuous digitules, equal in size. Antennae nine segmented, 0.5–0.8 mm long; apical four segments each with two enlarged setae; first segment 110–120 μm long, 110–160 μm wide, with 0–4 setae on ventral surface, 12–23 setae on dorsal surface; second segment 75–90 μm long, 95–125 μm wide, with 4–6 setae on ventral surface, 3–6 setae on dorsal surface, three or four sensilla on dorsal surface. Developing genitalia near posterior end of body.

Type material. Type data: USA: Alabama, Calhoun, on *Pinus* sp., March 16 and 31, April 21, 1902; collected by A.M. Troyer. Syntypes, female and first instar. Because Morrison (1939) did not specifically mention a holotype in the original description, the lectotype, **designated here**, is the single mounted adult female from the syntype

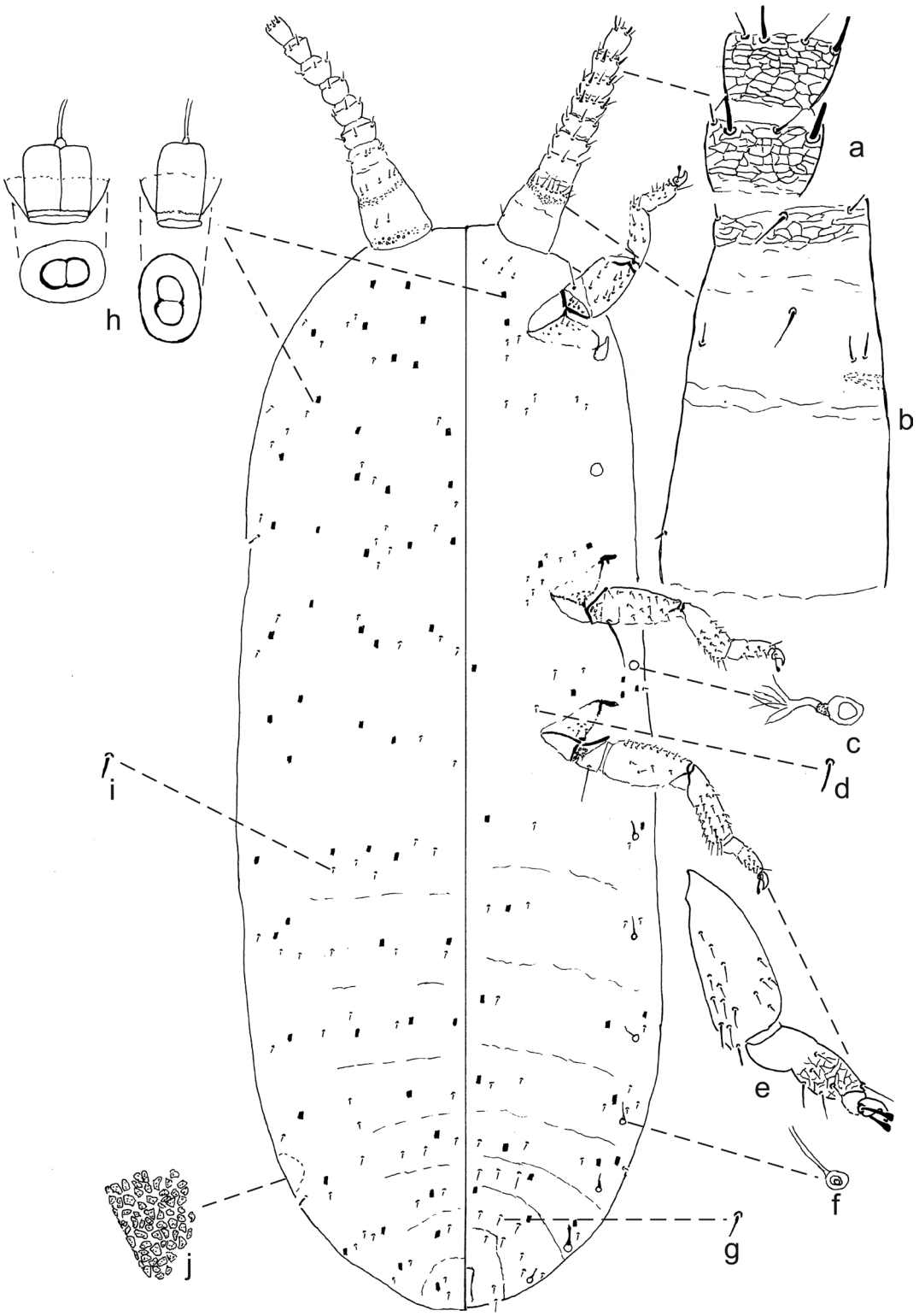

Figure 7. *Matsucoccus alabamae* Morrison. Third-instar male. Lee Co., Auburn, Alabama, March 1, 1979, on *Pinus echinata,* C.H. Ray, Jr. and I. Daniels. **A)** Seventh and eighth antennal segments. **B)** Basal antennal segments. **C)** Thoracic spiracle. **D and G)** Short ventral filamentous seta. **E)** Hind tibia, tarsus, claw. **F)** Abdominal spiracle. **H)** Bilocular tubular ducts. **I)** Dorsal filamentous seta. **J)** Dermal pattern.

series mounted on a slide and labeled as follows: left label: "9582 Matsucoccus/ alabamae/ Holotype/ On pine/ Calhoun, Ala./ March 16, 1902" right label "*Matsucoccus*/ *alabamae*/ Morrison/ lectotype/ desig. by Ahmed et al. 2020" (deposited at USNM). In addition, there are 14 paralectotypes. Type depositories: AUMNH, UCDC, USNM. We have examined the lectotype and several of the paralectotypes deposited in AUMNH and USNM.

Remarks. For a comparison of *M. alabamae* with *M. krystalae* see the notes section of the latter. The above description is based on seven third-instar males from the following locality: Lee County, Auburn, Alabama, March 1, 1979, on *Pinus* sp., C.H. Ray and I. Daniels, deposited in AUMNH.

Life history. The following information is from Ray (1982) based on observations made in Auburn, Alabama from November 1978 to April 1981. This species overwinters in the second-instar cyst beneath bark scales on the trunk of the host. In March, the third-instar males emerge from cysts, settle beneath bark scales and produce a waxy sac in which they molt to the fourth-instar pupa. Adult males emerge and begin to actively seek out females. Adult females emerge from the cyst, move to the outer surfaces of the tree trunk or between the edges of bark scales and extend the posterior end of their abdomen as far from the trunk as possible. Adult males run rapidly up and down the trunk and orient to the posterior apex of the female and copulate. After copulation is complete, adult females crawl beneath loose bark flakes, near a scar, or at the base of a branch and produce a waxy ovisac in which 501–860 eggs are laid. Copulation is necessary for oviposition: confined females do not produce eggs. As females deposit eggs, their bodies contract and fold so that when all eggs are deposited, the females are reduced to less than ⅓ of their original size.

Material examined. Alachua Co., Gainesville, behind Division of Plant Industry, (Long. 29.634987, Lat. −82.371138) September 6, 2018, on *Pinus elliottii,* M. Ahmed (dead mummy) (2018-4724) 3 ad. ♀; Broward Co., Ft. Lauderdale (Lat. 26.20512, Long. −80.1698), April 26, 2018, on *Pinus* sp., (Lindgren trap), J. Farnum (2018-2743) 2 ad. ♀; Duval Co., Jacksonville, Port of Jacksonville, Florida 9A, (Lat. 30.39461, Long. −81.58198), December 7, 2017, on *Pinus* sp., (Lindgren trap), M. Lara, (2018-1002) 1 ad. ♀; Jacksonville, Port of Jacksonville, November 1, 2017, on *Pinus* sp., (Lindgren trap), M. Lara, (2018-609) 1 ad. ♀ [we are unable to authenticate the location of these records; the official GPS coordinates of the closest trap are Lat. 30.394678, Long. −81.562037]; Franklin Co., Lanark Village, January 31, 2018, on *Pinus* sp., S. Halbert, M. Ahmed, D. Miller (dead mummy) (2018-343) 5 ad. ♀; Highlands Co., Archbald Research Station, April 28, 1975, on *Pinus elliottii*, R. Denno, J. Davidson, D. Miller 5 ad. ♀ (USNM); Liberty Co., US 27 N, January 31, 2018, on *Pinus* sp., S. Halbert, M. Ahmed, D. Miller (dead mummy) (2018-346) 1 ad. ♀; Miami-Dade Co., Miami, December 25, 1978, on *Pinus elliottii*, C. Ray 6ad. ♀ (AUMNH); Miami-Dade Co., Miami, July 20, 1980, on *Pinus elliottii*, C. Ray 2 ad. ♀ (AUMNH); Okaloosa Co., Niceville, February 17, 1979, on *Pinus elliottii*, C. Ray 8 ad. ♀ (AUMNH); Palm Beach Co., Magnolia Park (Lat. 26.76042, Long. −80.07526), November 19, 2018, on *Pinus* sp., (Lindgren trap), J. Farnum (2019-181) 1 ad. ♀; Wakulla Co., Apalachicola National Forest, January 16, 1997, on *Pinus palustris,* W. Tschinkel (97-0195) 8 second instar cysts (Fig. 8).

Distribution outside of Florida. *Matsucoccus alabamae* was known only from Alabama but Ray (1982) reported it from Florida, Georgia, South Carolina, and Tennessee in his unpublished dissertation. Recently Ahmed and Miller (2020) reported it from Florida and confirm the Florida occurrence data herein. A new record for Virginia is provided by DRM follows: Bayville Farms Park, Virginia Beach, Virginia December 24, 2019, under bark of *Pinus taeda*, D. R. Miller (2020-25).

Damage. This species has not been implicated in causing economic damage.

Matsucoccus gallicolus Morrison, 1939

(Fig. 9–10)

Matsucoccus matsumurae (Kuwana); Herbert 1921: 15. misidentification (Morrison 1939: 9–13) (in part; also see *M. alabamae*).

Matsucoccus sp.; Parr 1939: 624–630.

Matsucoccus gallicolus Morrison 1939: 9. Accepted valid name.

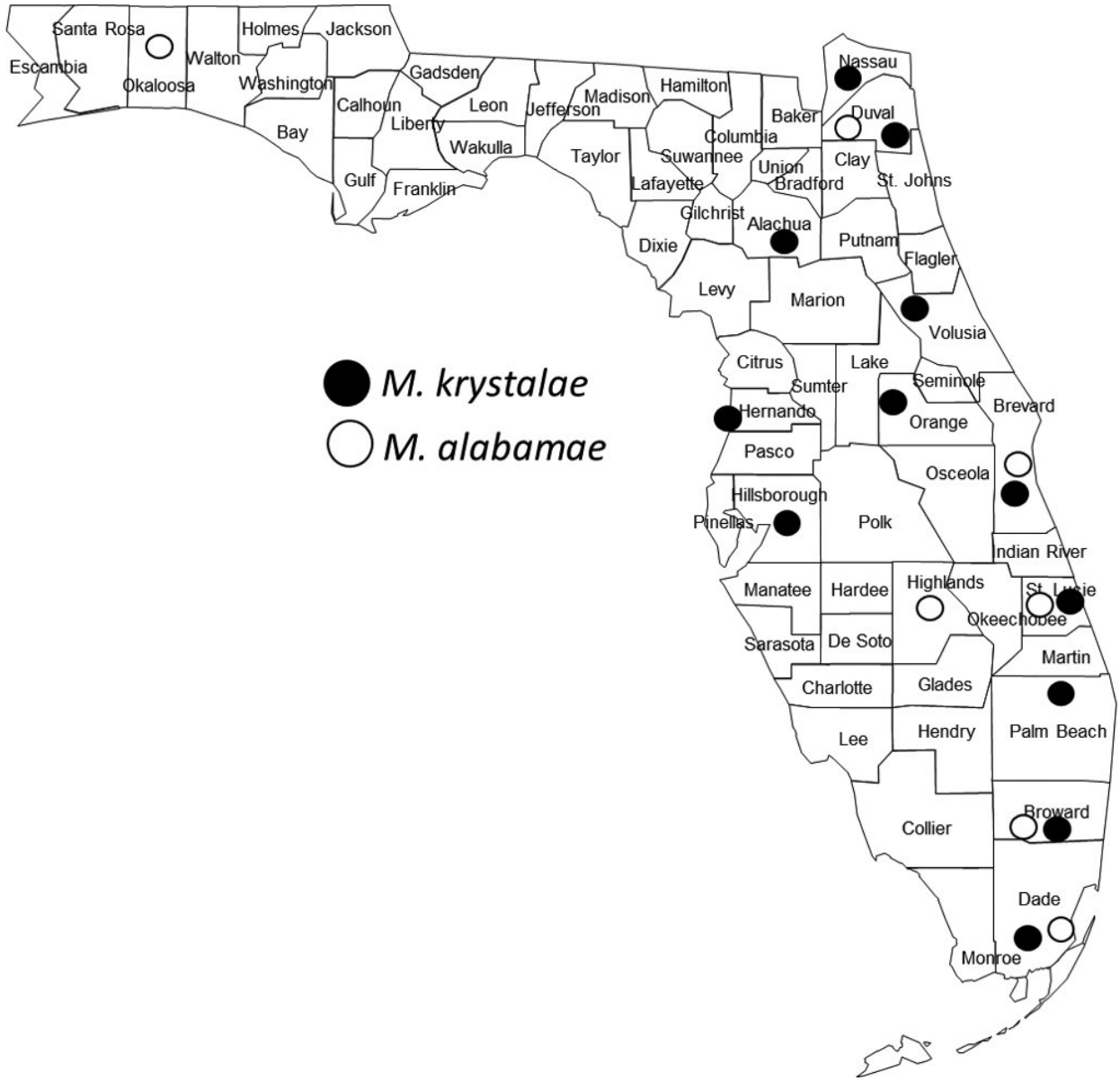

Figure 8. Florida distribution of *Matsucoccus krystalae* (solid circle) and *M. alabamae* (open circle).

Adult Female

(Fig. 9)

Diagnosis. Cicatrices numbering less than 310, present on segment I to segment VI; cicatrices absent from near body margin; with fewer than 30 multilocular pores; body setae of two distinct sizes; fleshy setae present on distal four antennal segments.

Description. Body elongate, parallel sided, 2.2–4.5 mm long, 1.0–2.7 mm wide. Dorsum: Setae uncommon, inconspicuous, arranged in rows, of one size, 5–8 μm long. Bilocular tubular ducts scattered over surface, uncommon on head and within rows of cicatrices; tubes usually slightly divergent. Cicatrices on segment I–VI; cicatrices present in medial and mediolateral areas, absent near body margin; some anterior segments with separate rows on anterior and posterior sections, anterior rows usually with fewer cicatrices; with 170–378 cicatrices; largest 6–12 μm in diameter.

Venter. Setae uncommon, arranged in rows, of two sizes, smaller setae 5–12 µm long, over most of surface; longest seta anterior of hind coxae 40–55 µm long, longer setae also present in medial areas of abdominal segments. Bilocular tubular ducts arranged segmentally, uncommon on head, most abundant on margin; similar in size and shape as on dorsum or slightly smaller. Multilocular pores in cluster around vulva, most specimens with ventral vulva located at or near abdominal apex; with 20–32 multilocular pores; diameter of largest pore 9–15 µm. Thoracic spiracles without definite sclerotized rim on derm or with narrow band of sclerotization; posterior spiracle with largest diameter of atrium 22–30 µm; with seven pairs of abdominal spiracles, normally with dermal orifice sclerotized, more weakly sclerotized posteriorly, sometimes decreasing in size posteriorly, diameter of dermal orifice of first abdominal spiracle 12–18 µm, of seventh spiracle 8–20 µm. Legs large and conspicuous, with scale-like pattern on all segments except first tarsal segment; hind leg with trochanter with one long seta, 7–10 sensoria; femur 190–260 µm long, with 8–18 setae; tibia 172–310 µm long, with 22–40 setae; tarsus (both segments) 115–170 µm long, tarsal digitule undifferentiated; claw without denticle, with two conspicuous digitules, equal in size. Antennae nine segmented, 0.4–0.9 mm long; apical four segments each with two enlarged setae; first segment 100–155 µm long, 90–160 µm wide, with 5–8 setae on ventral surface, 18–31 setae on dorsal surface; second segment 62–100 µm long, 75–100 µm wide, with 5–8 setae on ventral surface, 5–8 setae on dorsal surface.

Material examined. Alabama, near Birmingham, April 13, 1929, on *Pinus virginiana,* E.J. Palmer (35332) 1 adult ♀ (paratype). Connecticut: New Haven, received October 26, 1937, on *Pinus rigida,* R.C. Brown, 3 adult ♀. Georgia: St. Mary's River, May 1, 1921, on *Pinus glabra,* unknown collector (39 1510), 3 cysts, 1 adult ♀ (paratype). Maryland: Beltsville, received September 23, 1946, on *Pinus rigida,* St. George (Hopkins 34109), 3 adult ♀; Berlin, September 20, 1970, on *Pinus taeda*, D.R. Miller, 5 adult ♀; Cambridge, March 14, 1971, on *Pinus taeda,* D.R. Miller, 3 adult ♀. Massachusetts: Amherst, June 9, 1946, on *Pinus rigida*, W.B. Becker, (46 795) 4 adult ♀; Cape Cod, November 5, 1935, on *Pinus rigida,* W. Becker, 1 adult ♀ (paratype); Sandwich, June 9, 1916, on *Pinus rigida,* Fernald and Hun, 4 adult ♀ (paratype). Missouri: near Tecumseh, N. fork of White River, Ozark Co., October 7, 1927, on *Pinus echinata,* E.J. Palmer (30893) 1 adult ♀, 1 cyst (paratype). New Hampshire: Nashua, May 27, 1937, on *Pinus rigida,* T. Parr, 3 adult ♀, 3 cysts, 1 first-instar nymph (paratype). New Jersey: between Tom's River and Tuckerton, February 18, 1937, on *Pinus rigida* ?, Mr. May, 2 adult ♀; Barnegat, July 22, 1936, on *Pinus rigida,* R.R. Whitten, 2 adult ♀. New York: Centerport, December 1, 1919, on *Pinus rigida,* J.T. Morton, (Hopkins 16404A) 1 adult ♀ (paratype); Centerport, August 25, 1919, on *Pinus rigida,* L.C. Griffith, 1 adult ♀, 2 cysts (paratype); Great River, September 25, 1953, on *Pinus rigida,* G.V. Johnson (T.C. # 116) 2 adult ♀, 6 cysts. North Carolina: Ashville, May 8, 1937, on *Pinus rigida* or *P. echinata,* B.H. Wilford, (39 1506) 2 adult ♀ (paratype). Ohio: Delaware, received for identification December 2, 1970, on *Pinus rigida,* J.B. Hanson (70-23862), 4 adult ♀; Pennsylvania: State Forest Park, Mont Alto, September 1935, on *Pinus rigida,* J. C. Kase, 1 ad ♀, 3 first-instar exuviae, 3 cysts (paratype); Mont Alto, September 18, 1935, on *Pinus rigida,* J. C. Kase, 1 adult ♀ (paratype); Mont Alto, Januaruy 30, 1937, on *Pinus echinata,* collector unknown, 5 adult ♀, 1 3rd ♂ (paratype); Mont Alto, Guilford Turnpike, March 1937, on *Pinus rigida,* J. C. Kase, 1 adult ♀ (paratype); South Carolina: Cooper River, Charleston, April 4, 1909, on *Pinus taeda,* J.G. Sanders, 1 3rd ♂. Virginia: Falls Church, Franklin Park, April 20, 1936, on *Pinus virginiana,* I. Weckerly, 2 adult ♀, 2 cysts (paratype); Glouchester, unknown location, January 5, 1968, on *Pinus virginiana* (witches broom) C.L. Morris, 1 adult female; Gouchland County, locality unknown, on pine, collector unknown 1 3rd ♂; Princess Anne County, May 1898, on *Pinus taeda,* T.H. Kearney, Jr., (USNM 355800), 1 adult ♀, 1 cyst (paratype); Rockbridge County, October 31, 1941, on *Pinus rigida,* M.C. Howard (41 2603), 5 adult ♀, 3 cysts. Washington, D.C., Good Hope Hill, March 19, 1905, on *Pinus virginiana,* J.G. Sanders, 1 adult ♀; Good Hope Hill, May 11, 1936 & March 19, 1905, on *Pinus virginiana,* L.M. Russell (39 1498), 7 adult ♀.

Remarks. Adult females of *M. gallicolus* can be separated from adult females of *M. krystalae* (characters in parentheses are of *M. krystalae*) as follows: With 170–378 cicatrices (437–900); cicatrices absent from marginal areas (marginal cicatrices present on one or more of segments IV–VI); with 20–32 multilocular pores (36–72); vulva and multilocular pores usually at or near posterior apex of abdomen (many specimens with vulva and multilocular pores located under dorsum of segment IV or V).

The above description is based on 52 specimens from 23 localities.

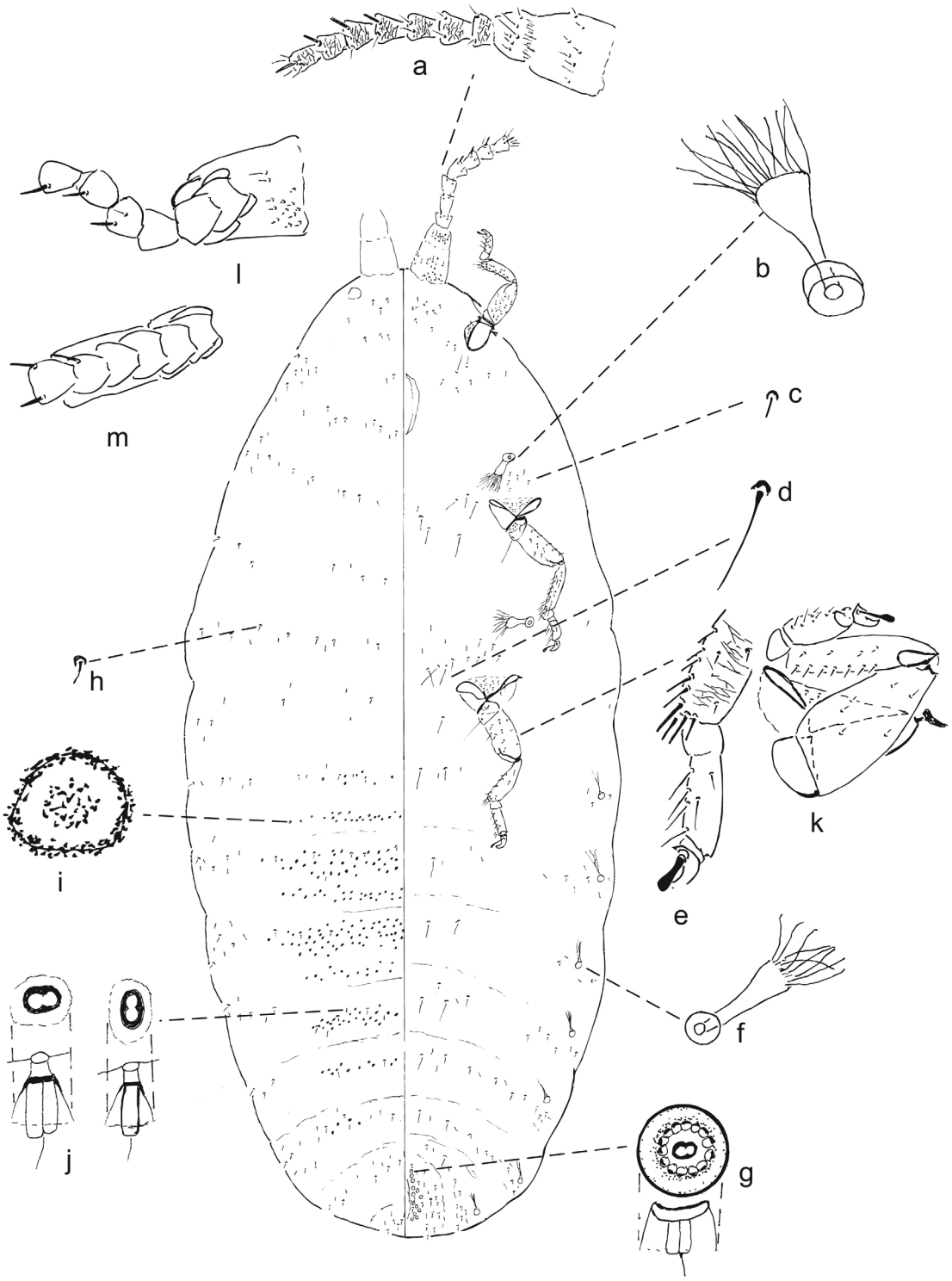

Figure 9. *Matsucoccus gallicolus* Morrison. Adult female. Prince Georges Co., Beltsville, Maryland, September 23, 1946, on *Pinus sp.*, Hopkins No., 34109. **A)** Antenna. **B)** Thoracic spiracle. **C)** Short ventral filamentous seta. **D)** Long ventral filamentous seta. **E)** Hind tibia, tarsus, claw. **F)** Abdominal spiracle. **G)** Multilocular pore. **H)** Dorsal filamentous seta. **I)** Cicatrix. **J)** Bilocular tubular ducts. **K)** Middle leg folded after emergence through the exit hole in the gall, **L–M)** Antennae at two stages of elongation after emergence through the exit hole in the gall.

Third-instar Male
(Fig. 10)

Diagnosis. Two sizes of setae on venter, setae anterior of metacoxae about 20 µm long, marginal setae about 5 µm long; with less than eight bilocular tubular ducts on dorsum of abdominal segment III; abdominal spiracles usually decreasing in size posteriorly; fleshy setae present on distal four antennal segments.

Description. Body elongate, 1.1–1.9 mm long, 0.7–1.1 mm wide. Dorsum: Setae uncommon, inconspicuous, arranged in rows, of one size, about 5 µm long. Bilocular tubular ducts scattered over surface, in segmental rows, associated with setal rows, not arranged in longitudinal lines; with less than eight ducts on abdominal segment III, tubes fused.

Venter. Setae uncommon, arranged in rows, of two sizes, larger 18–20 µm long, smaller about five µm long; longest seta anterior of hind coxae about 20 µm long. Bilocular tubular ducts uncommon on head and medial areas of thorax, most abundant on margin, arranged in longitudinal lines, present submedially and laterally; similar in size and shape as on dorsum, of two variable sizes. Thoracic spiracles without definite sclerotized rim on derm; posterior thoracic spiracle with largest diameter of atrium 15–25 µm; with seven pairs of abdominal spiracles, normally with dermal orifice sclerotized, all equally sclerotized, normally decreasing in size posteriorly, diameter of sclerotized rim of dermal orifice of first abdominal spiracle 12–17 µm, of seventh spiracle 6–10 µm. Legs large and conspicuous, with scale-like pattern on all segments except first tarsal segment; hind leg with trochanter with one long seta, sensoria hidden by bend; femur 125–195 µm long, with 4–7 setae; tibia 92–177 µm long, with 20–32 setae; tarsus (both segments) 77–102 µm long, tarsal digitule undifferentiated; claw without denticle, with two conspicuous digitules, equal in size. Antennae nine segmented, 0.4 mm long; apical four segments each with two enlarged setae; first segment 100 µm long, 88 µm wide, with two setae on ventral surface, 12 setae on dorsal surface; second segment 50 µm long, 67 µm wide, with three setae on ventral surface, three setae on dorsal surface, four sensilla on dorsal surface. Ventral developing genitalia located near posterior apex of abdomen.

Type material. Type data: USA: Pennsylvania, Stroudsburg, farm of Mrs. E. Bethel, on *Pinus rigida*, April 1936, collected E.C. Pyle. Holotype, female, Type depository: Washington: United States National Entomological Collection, U.S. National Museum of Natural History, District of Columbia, USA (USNM). Accepted valid name. We have examined the holotype and several of the paratypes. There is a long series of paratypes from many eastern states.

Remarks. Third-instar males of *M. gallicolus* can be separated from *M. krystalae* (characters in parentheses are of *M. gallicolus*) as follows: 10 or more bilocular tubular ducts on dorsum of abdominal segment III (fewer than 8). Third-instar males of *M. gallicolus* can be separated from *M. alabamae* (characters in parentheses are of *M. gallicolus*) as follows: setae anterior of hind coxa and in medial area of abdomen about same size as other body setae (setae in these areas conspicuously longer than other body setae).

The above description is based on three third-instar males from three localities. The specimens used for this description are in poor condition; some still have their antennae and legs compacted in dermal pockets causing many of the characters to be difficult to discern.

Life history. The following information is from Parr (1939). The species overwinters as eggs in the ovisac which are usually located under the bark on branches of the pine host. Hatching occurs in late April in Connecticut and Pennsylvania when the new growth on the pine terminals is one to three inches long. Immediately after hatching the dark yellow crawlers move to the new growth, insert their mouthparts, and begin feeding. After continued feeding, the body of the crawler gradually turns dark brownish black and the integument around the mouthparts begins to swell. There is concomitant degradation of plant tissue around the base of the stylets and the ventral surface of the crawler sinks into the cavity in the plant. There also is slight swelling of the plant tissue around the outside of the crawler that continues until the body of the crawler is almost completely enclosed. After six to nine weeks from hatching, the crawler molts to the second-instar cyst. The shed skin of the crawler is usually trapped in the hole in the epidermis of the plant and the cyst is totally encapsulated in plant tissue except for the hole where the shed skin of the crawler is placed. The brownish black cyst is nearly round, legless, with large spiracular openings, and mouthparts. The second molt occurs about a month later. Emergent adult females have legs, but

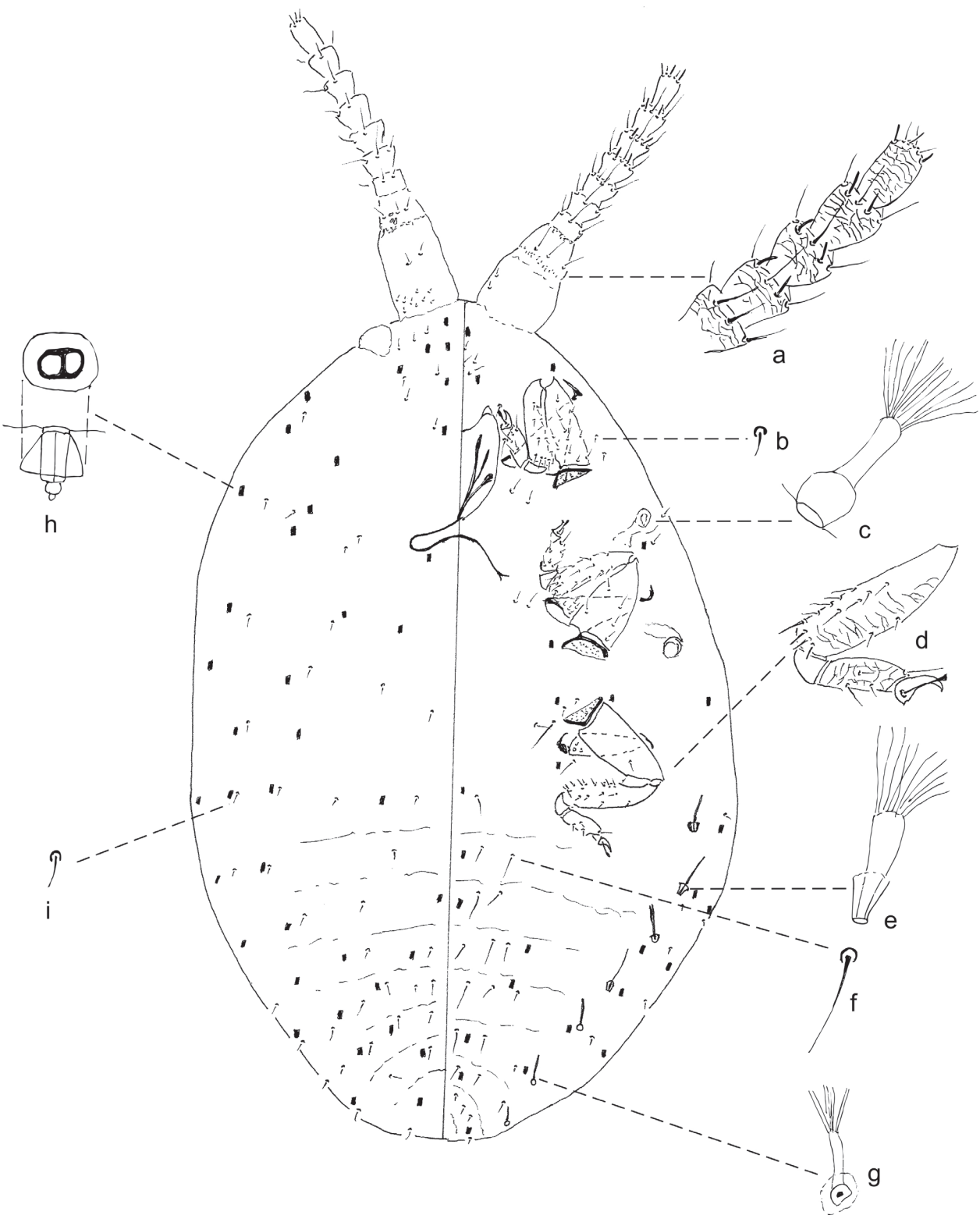

Figure 10. *Matsucoccus gallicolus* Morrison. Third-instar male. Cooper River, Charleston, South Carolina, April 4, 1909, on *Pinus taeda,* S.G. Sanders. **A)** Apical four antennal segments. **B)** short filamentous seta. **C)** Thoracic spiracle. **D)** Hind tibia, tarsus, and claw. **E)** 1st abdominal spiracle. **F)** Long filamentous seta. **G)** 6th abdominal spiracle. **H)** Bilocular tubular ducts. **I)** Short dorsal filamentous seta.

mouthparts are lacking or nonfunctional. Immediately after emerging from the cyst, the adult female integument is very flexible, and the legs and antennae are telescoped inside the body (Fig. 9). The adult female pushes the shed skin of the crawler aside and squeezes through the small opening of the gall. Adult females settle under bark flakes on the main branches or trunk of the host, produce an oval flattened pad of wax that is covered by white wax threads, and lay 150–500 lemon yellow eggs. There is no discussion of males in any stage in Parr (1939) but Morrison (1939) discussed the existence of second and third-instar males when he described the species.

It is not entirely clear that this species has the same life history as that suggested by Parr (1939) in other parts of its distribution. We have examined the dates of collection of all adult females deposited in the U. S. National Museum scale-insect collection in Beltsville, Maryland, and find specimens from every month of the year except August (Table 4). We excluded any specimens that appeared to have been mummies when they were collected.

As indicated above, Parr (1939) suggested that the legs and antennae are telescoped inside the body while pushing through the hole in the host. It appears to us that they are external but are in pockets in the derm. In either case, they are folded in specific ways. Each antenna is oriented posteriorly and is folded at segment three with the distal segments resting under or within the much longer two basal segments. The third segment is bent when the antenna is folded (Figure 9). Each leg is oriented with the claw and tarsus anterior of the tibia and femur. The trochanter is bent in the middle and is attached to the coxa which lies under the rest of the leg (claw, tarsus, tibia, femur and part of the trochanter) (Figure 9). There is a definite division in the middle of the trochanter in fully extended legs, which suggests it is folded during emergence.

Florida distribution. We have examined the specimens reported by Morrison (1939) from Florida that are deposited in the USNM. They are either cysts or first instars and we are unable to verify their identity without examination of associated adult females or third-instar males. The cysts of *M. gallicolus* are different from all other species treated by Morrison in 1939 based on the protrusion on the dorsum used by the adult female and third-instar male to escape the gall, but we suspect that *M. krystalae* has the same characteristics. Because identifiable specimens of *M. gallicolus* have not been collected in Florida, we suspect that the Florida records are actually of *M. krystalae*.

Distribution outside of Florida. *Matsucoccus gallicolus* Morrison has been reported from Connecticut, District of Columbia, Georgia, Kentucky, Maine, Maryland, Massachusetts, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, Tennessee, and Virginia. We examined a specimen from Alabama and Ray (1982) reported it from South Carolina.

Table 4. Collection month, number and US states and territories of adult females of *Matsucoccus gallicolus* in Smithsonian National Museum of Natural History collection. Connecticut – CT, Delaware – DE, District of Columbia – DC, Georgia – GA, Maryland – MD, Massachusetts – MA, Missouri – MO, New Hampshire – NH, New Jersey – NJ, New York – NY, North Carolina – NC, Pennsylvania – PA, Tennessee – TN, Virginia – VA.

Month	Total	CT	DC	DE	GA	MA	MD	MO	NC	NH	NJ	NY	PA	TN	VA
January	2												x		x
February	2										x			x	
March	3		x				x						x		
April	3		x										x		x
May	6		x		x	x			x	x					x
June	2					x							x		
July	2										x		x		
August	0														
September	3						x					x	x		
October	4	x						x				x			x
November	1					x									
December	2			x								x			

Damage. Parr (1939) did a careful analysis of the effect of the feeding of *M. gallicolus* on host tissue in Connecticut and Pennsylvania. About two weeks after a crawler begins feeding, a slight yellowing of the twig surface develops around the insect body where it is in contact with the host. Normal epidermal and collenchyma cells outside of the feeding area have heavy walls but those in the area of feeding become thin walled, empty and stop growing. This causes the formation of a depression under the insect and this combined with abnormal growth of the epidermal and collenchyma cells surrounding the body of the crawler causes gall formation. By the end of June, the cells under the insect have lignified and this degradation continues until late August when twig death may occur. On pitch pine an infestation of 1.2 insects per square millimeter will cause twig death by the middle or end of July but an infestation of 0.5 insects per square millimeter will cause death of the twig by late August or September. Parr was able to demonstrate that the saliva from the feeding insect was the source of gall induction by the plant.

According to Aughanbaugh (1949) a quarter acre plot was set up in a pitch pine (*Pinus rigida* Mill.) plantation in Pond Bank, Pennsylvania in 1931. At the time the trees seemed healthy containing about 40 cubic feet of wood per acre. The following table is a synopsis of the tables presented by Aughanbaugh (1949) of the damage sustained by trees in the plot for the next several years (Table 5).

Aughanbaugh (1949) suggested that the annual increment accumulated in 1942 of trees at the early age of 23 years demonstrated the serious damage sustained by the trees. Only 652 trees were alive in 1949 (48%) and it seemed doubtful that they would ever attain timber size. Damage can be recognized by trees with dead leaders or branches as the season progresses.

Matsucoccus krystalae Ahmed and Miller, new species

(Fig. 11–12)

Matsucoccus gallicolus Morrison 1939: 12 (in part). Possible misidentification of paratype specimens taken from herbarium specimens from *Pinus glabra* Walter, Chattahoochee, Florida, November, unknown date, A. H. Curtiss.

Adult Female

(Fig. 11)

Diagnosis. Cicatrices numbering more than 400, normally present on metathorax to segment VI, occasionally absent from metathorax; cicatrices present near body margin on one or more posterior abdominal segments; with more than 35 multilocular pores; body setae of two distinct sizes; fleshy setae present on distal four antennal segments.

Description. Body elongate, parallel sided, 3.1–5.9 mm long, 1.4–2.2 mm wide. Dorsum: Setae uncommon, inconspicuous, arranged in rows, of one size, 8–11 μm long. Bilocular tubular ducts scattered over surface, uncommon on head and within rows of cicatrices; tubes usually slightly divergent. Cicatrices on metathorax (some specimens without or with only one or two) or segment I to segment VI; some segments with cicatrices present near body margin; some anterior segments with separate rows on anterior and posterior sections, anterior rows usually with fewer cicatrices; totaling 437–900; largest 6–12 μm in diameter.

Venter. Setae uncommon, arranged in rows, of two sizes, smaller setae 8–11 μm long, over most of surface; longest seta anterior of hind coxae 30–55 μm long, longer setae also present in medial areas of abdominal segments. Bilocular tubular ducts arranged segmentally, uncommon on head, most abundant on margin; similar in size and shape as on dorsum or slightly smaller. Multilocular pores in cluster around vulva, many specimens with

Table 5. Damage sustained by trees in an experimental plot presented by Aughanbaugh et al. (1949). NA=not available.

Year	Twigs killed	Leaders killed	% dead trees	Growth cu. ft./acre/year
1938	2393	56	0	39.5
1939	3262	61	3	58.1
1942	3824	75	16	69.9
1948	NA	NA	28	57.4

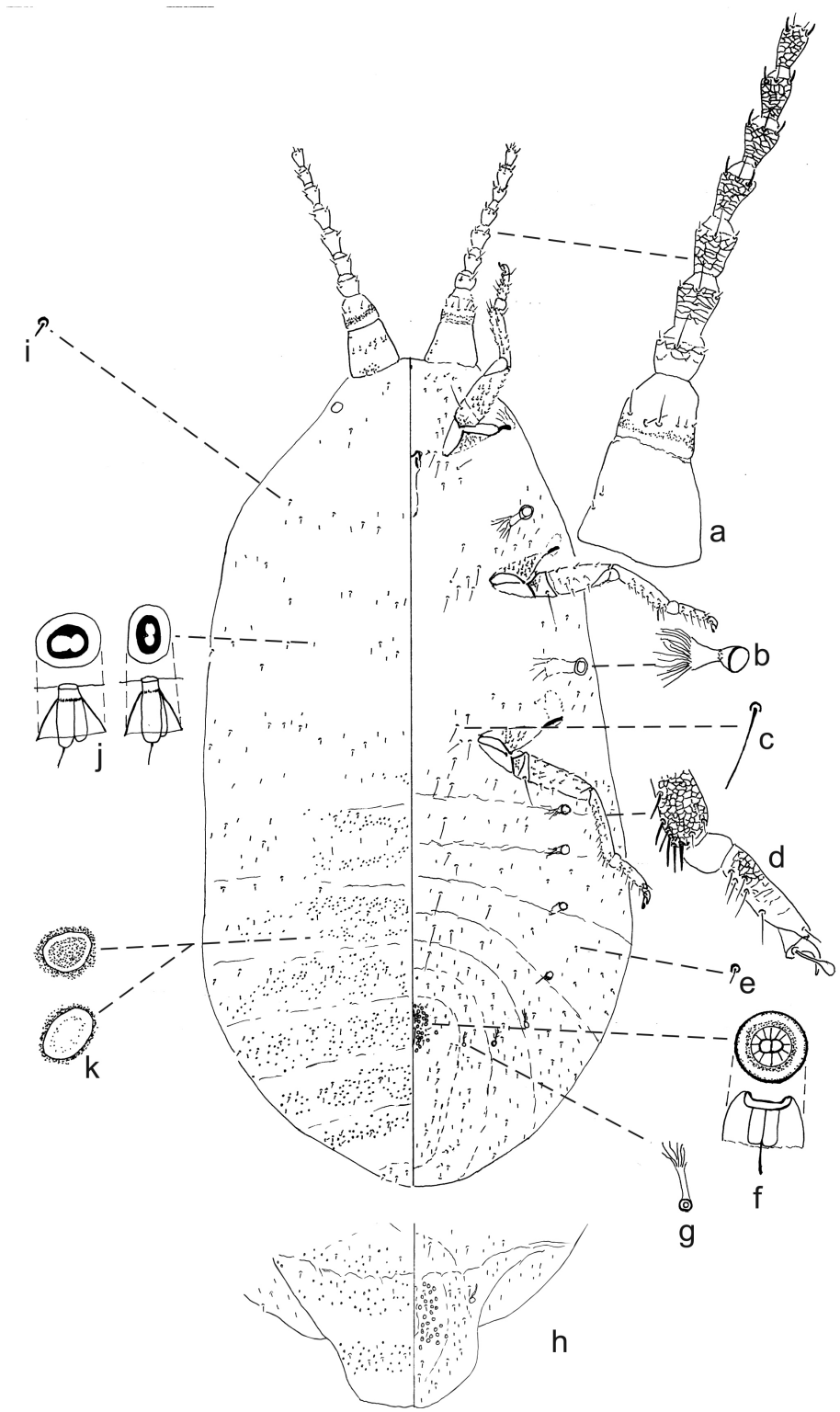

Figure 11. *Matsucoccus krystalae* Ahmed and Miller, n.sp. Adult female. Broward Co., Proglis Warehouse, Pompano Beach, Florida, March 23, 2018, on *Pinus ellottii*, J. Farnum. **A)** Antenna. **B)** Thoracic spiracle. **C)** Long filamentous setae. **D)** Hind tibia, tarsus, claw. **E)** Short filamentous seta. **F)** Multilocular pore. **G)** Seventh abdominal spiracle. **H)** Abdomen bent under body shifting abdominal apex under segments IV, V, or VI. **I)** Dorsal filamentous seta. **J)** Bilocular tubular ducts. **K)** Cicatrices.

ventral vulva located beneath segments IV, V or VI; with 36–72 multilocular pores; diameter of largest pore 10–12 μm. Thoracic spiracles without definite sclerotized rim on derm or with narrow band of sclerotization; posterior spiracle with largest diameter of atrium 28–50 μm; with seven pairs of abdominal spiracles, normally with dermal orifice sclerotized, more weakly sclerotized posteriorly, sometimes decreasing in size posteriorly, diameter of dermal orifice of first abdominal spiracle 17–25 μm, of seventh spiracle 8–12 μm. Legs large and conspicuous, with scale-like pattern on all segments except first tarsal segment; hind leg with trochanter with one long seta, 9–14 sensoria; femur 228–365 μm long, with 16–29 setae; tibia 228–285 μm long, with 21–47 setae; tarsus (both segments) 155–180 μm long, tarsal digitule undifferentiated; claw without denticle, with two conspicuous digitules, equal in size. Antennae nine segmented, 0.6–0.8 mm long; apical four segments each with two enlarged setae; first segment 145–205 μm long, 160–200 μm wide, with 7–10 setae on ventral surface, 19–26 setae on dorsal surface; second segment 85–130 μm long, 110–130 μm wide, with 5–8 setae on ventral surface, 6–7 setae on dorsal surface.

Remarks. Adult females of *M. krystalae* can be separated from *M. gallicolus* (characters in parentheses are of *M. gallicolus*) as follows: 437–900 cicatrices (170–378 cicatrices); cicatrices often present on metathorax (not on metathorax); cicatrices present near body margin on one or more posterior abdominal segments (not present near body margin); with 36–72 multilocular pores (20–32 multilocular pores). Adult females of *M. krystalae* can be separated from *M. alabamae* (characters in parentheses are of *M. krystalae*) as follows: Cicatrices present from abdominal segment III to VI (metathorax or segment I to segment VI); setae anterior of hind coxae 10–16 μm long (18–55 μm long); abdominal spiracles approximately same size (7th abdominal spiracle normally smaller than 1st abdominal spiracle); 2–4 setae on venter of first antennal segment (5–9); 7–18 setae on hind femur (16–29). Adult females of *M. krystalae* also are similar to *M. apachecae* Ray and Williams from Arizona but differ from the latter (characters in parentheses are of *M. krystalae*) by having from 21–45 cicatrices (437–900 cicatrices) present in the medial area of the dorsum (medial, mediolateral, and lateral areas).

The above description is based on 28 adult females from seven localities.

Third-instar Male

(Fig. 12)

Diagnosis. Two sizes of setae on venter, setae anterior of metacoxae about 30 μm long, marginal setae about 12 μm long; with 10 or more bilocular tubular ducts on dorsum of abdominal segment III; abdominal spiracles usually decreasing in size posteriorly; fleshy setae present on distal four antennal segments.

Description. Body elongate, parallel sided, 2.1–2.8 mm long, 1.0–1.2 mm wide. Dorsum: Setae uncommon, inconspicuous, arranged in rows, of one size, 9–11 μm long. Bilocular tubular ducts scattered over surface, in segmental rows, associated with setal rows, not arranged in longitudinal lines; with 10 or more ducts on abdominal segment III, tubes sometimes slightly divergent.

Venter. Setae uncommon, arranged in rows, of two sizes, larger 20–30 μm long, smaller 6–12 μm long; longest seta anterior of hind coxae 20–36 μm long. Bilocular tubular ducts uncommon on head and medial areas of thorax, most abundant on margin, arranged in longitudinal lines, present submedially and laterally; similar in size and shape as on dorsum. Thoracic spiracles without definite sclerotized rim on derm; posterior thoracic spiracle with largest diameter of atrium 25–30 μm; with seven pairs of abdominal spiracles, normally with dermal orifice sclerotized, all equally sclerotized, normally decreasing in size posteriorly, diameter of sclerotized rim of dermal orifice of first abdominal spiracle 14–18 μm, of seventh spiracle 8–11 μm. Legs large and conspicuous, with scale-like pattern on all segments except first tarsal segment; hind leg with trochanter with one long seta, 8–12 sensoria; femur 185–260 μm long, with 18–24 setae; tibia 185–240 μm long, with 37–58 setae; tarsus (both segments) 125–135 μm long, tarsal digitule undifferentiated; claw without denticle, with two conspicuous digitules, equal in size. Antennae nine segmented, 0.6–0.8 mm long; apical four segments each with two enlarged setae; first segment 120–195 μm long, 125–175 μm wide, with 1–8 setae on ventral surface, 12–18 setae on dorsal surface; second segment 85–95 μm long, 100–120 μm wide, with 4–7 setae on ventral surface, 4–6 setae on dorsal surface, 2–7 sensilla on dorsal surface. Ventral developing genitalia located under dorsal segment IV, V, VI, or VII.

Remarks. Third-instar males of *M. krystalae* can be separated from *M. alabamae* (characters in parentheses are of *M. krystalae*) as follows: setae anterior of hind coxae 10–16 μm long (20–36 μm long); abdominal spiracles

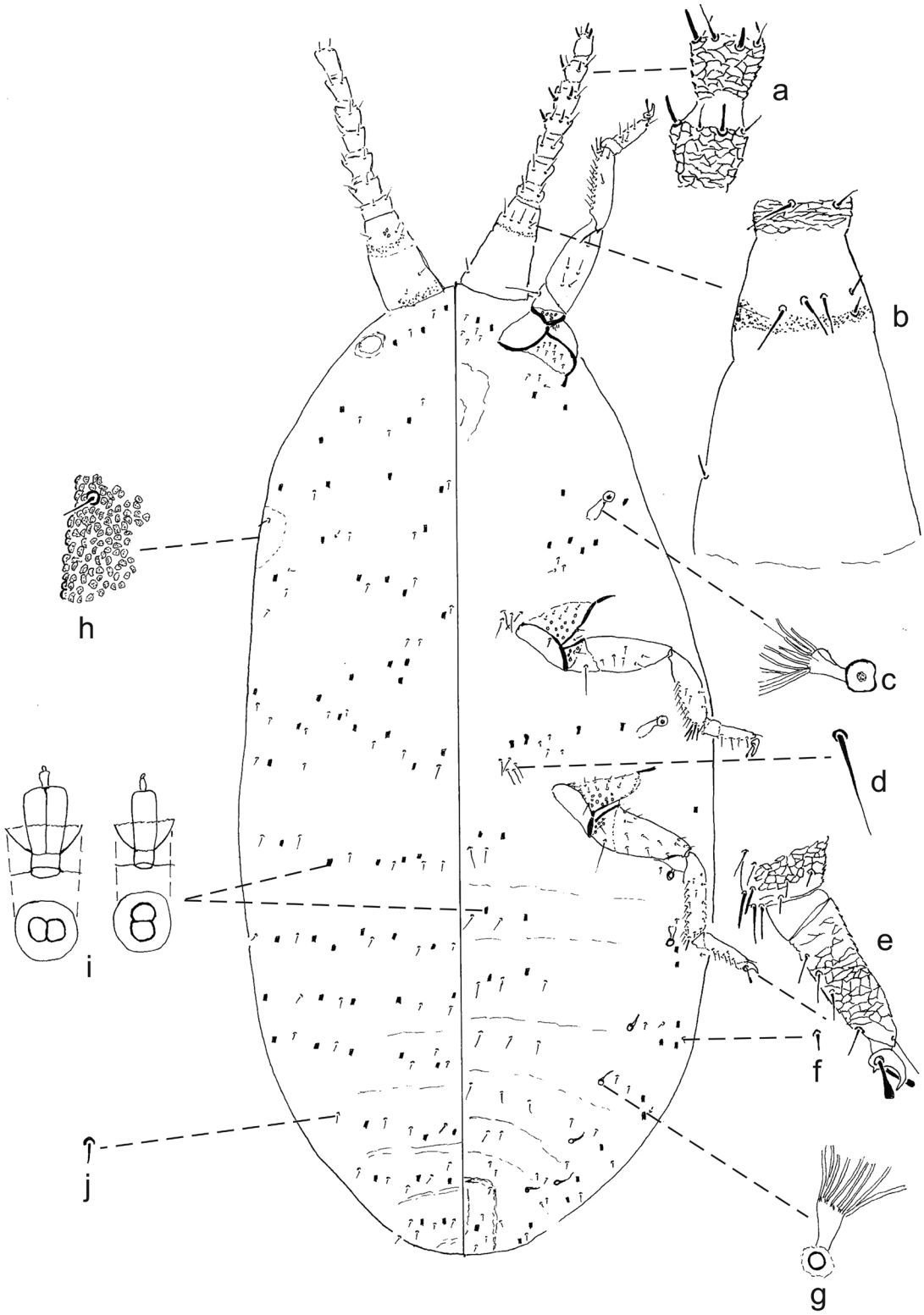

Figure 12. *Matsucoccus krystalae* Ahmed and Miller, n.sp. Third-instar male. St. Lucie Co., Ft. Pierce, Florida, March 20, 2018, on *Pinus ellottii,* B. Saunders. **A)** Seventh and eighth antennal segments. **B)** Basal antennal segments. **C)** Thoracic spiracle. **D)** Long filamentous seta. **E)** Hind tibia, tarsus, claw. **F)** Short filamentous seta. **G)** Abdominal spiracle. **H)** Dermal pattern. **I)** Bilocular tubular ducts. **J)** Dorsal filamentous seta.

approximately same size (7th abdominal spiracle normally smaller than 1st abdominal spiracle); eight or fewer bilocular tubular ducts on dorsum of abdominal segment III (10 or more).

The above description is based on 118 third-instar males from 14 localities.

Type material. Holotype adult female mounted singly on a slide; left label "Holotype/ Hemiptera/ Matsucoccidae / *Matsucoccus krystalae*/ Ahmed and Miller/Det. MZAhmed, 2018/ E-2018-1367" right label "USA, Florida: St Lucie Co./Fort Pierce/ 2001 Rock Rd/ 28 FEB 2018/ E-2018-1367-1" Deposited in FSCA. In addition, there are 145 paratypes deposited in the following collections: AUMNH, CSCA, NHMUK, UCDC, USNM.

Etymology. The species epithet *krystalae* is named in honor of Krystal Ashman who first noticed *Matsucoccus* specimens in Lindgren funnel trap samples and who has continued to collect specimens, providing clues to the life history of the species. We are most grateful for her efforts.

Material examined. All specimens are considered paratypes. Except the two slides from Chattahoochee. Alachua Co., Leveda Brown Environmental Park, Gainesville, October 16, 2018 (Lat. 29.70818, Long. −82.26424), on *Pinus* sp., (Lindgren trap, baited with ethanol and Alpha-Pinene lures), R. Leahy and B. Alford (2018-6381) 1 ad. ♀; Brevard Co., Melbourne, October 17, 2017, on *Pinus ellottii,* (Lindgren trap), B. Saunders (2018-231) 1 ad. ♀; Titusville, February 26 and March 30, 2018, April 30, 2018 (Lat. 28.49972, Long. −80.78294), on *Quercus* sp., (Lindgren trap baited with ethanol, Alpha-Pinene, and Monochamol lures), B. Saunders (2018−877, 2018-2279, 2018-3118) 8 3rd ♂ [the exact location and host are questionable since there is a discrepancy in the correct GPS coordinates; the official location was Lat. 28.500785, Long. −80.781750]; Broward Co.: Ft. Lauderdale (Lat. 26.20512, Long. −80.1698), March 23, 2018 and April 26, 2018, on *Pinus* sp., (Lindgren trap), J. Farnum (2018-1688, 2018-2743) 6 3rd ♂; Ft. Lauderdale, Port Everglades Airport, (Lat. 26.07176, Long. −80.13222) May 29, 2019, on *Pinus* sp., (Lindgren trap), J. Farnum (2019-4471) 1 3rd ♂; Pompano Beach, (Lat. 26.26933, Long. −80.15826) March 23, 2018, April 26, 2018, November 19, 2018, December 18, 2018, April 30, 2019, March 28, 2019, May 29, 2019, April 24, 2020, on *Pinus elliottii* (Lindgren trap), J. Farnum (2018-1659, 2018-2744, 2019-139, 2019-401, 2019-3259, 2019-3878 and 2020-1594) 55 3rd ♂, 14 ad. ♀; Duval Co., Baldwin, Highway 217, Yellow Water Rd. (Lat. 30.27885, Long. −81.97732), December 4, 2018, on *Pinus* sp., (Lindgren trap, baited with ethanol and Alpha-Pinene lures), M. Byron and R. Leahy, (2019-249), 1 3rd ♂; Jacksonville (Lat. 30.31203, Long. −81.86378), October 16, 2018, on *Pinus* sp., (Lindgren trap, baited with ethanol, Alpha-Pinene, and Monochamol lures), M. Echols, (2018-6376), 1 3rd ♂; Gadsen Co., Chattahoochee, November, day and year unknown sometime before 1939, on *Pinus glabra,* A.H. Curtis (USNH # 941030 & HNYGB # 2656) 8 cysts, collected from pressed herbarium specimens by Louise Russell (USNM); Hillsborough Co., MacDill Air Force Base, Tampa, (Lat. 27.8383, Long. −82.5026), October 4, 2017, October 2, 2018, October 16, 2018, October 27, 2018, on *Pinus* sp. P. Barker, (Lindgren trap) (2018-324, 2018-6211, 2018-1961, 2018-6378) 8 3rd ♂, 2 ad. ♀; Miami-Dade Co., Medley, (Lat. 25.85173, Long. −80.32365) February 23, 2018, March 19, 2018, April 27, 2018, April 30, 2018, November 20, 2018, December 18, 2018, on *Pinus elliotti* (Lindgren trap), J. Farnum (2018-1311, 2018-1587, 2018-2720, 2019-182, 2019-402) 15 3rd ♂, 1 ad. ♀; Nassau Co., Fort Clinch State Park (Lat.30.6942, Long. −81.4389), March 28, 2018, September 28, 2018, March 12, 2019, on *Pinus* sp., (Lindgren trap, baited with ethanol and Alapha-Pinene lures), R. Leahy (2018-1689, 2018-6018, 2019-2553) 4 3rd ♂; Orange Co., Moss Park, Orlando (Lat. 28.37468, Long. −81.18946), October 3, 2011, on *Pinus elliotti,* M. Weiss (Lindgren trap) (2011-9369) 3 3rd ♂; Palm Beach Co., Jupiter (Lat. 26.92833, Long. −80.18029), February 25, 2019, on *Pinus* sp., (Lindgren trap), J. Farnum (2019-1993) 1 3rd ♂; Palm Beach Co., Jupiter, Jupiter Country Club (Lat. 26.645895, Long. −80.430269), April 5, 2018, on *Pinus elliottii*, J. Johnson, submitted by Lyle Buss, (2018-1988) 2 cysts; St. Lucie Co., Fort Pierce (Lat. 27.42909, Long. −80.40859), February 28, 2018, March 20 and 30, 2018, July 30, 2018, on *Pinus elliottii*., B. Saunders (Lindgren trap) (2018-1367, 2018-2275, 2018-3173, and 2018-4864), 14 3rd ♂, 7 ad. ♀; Volusia Co., Daytona Beach (Lat. 29.20743, Long. −81.01269), January 8, 2018 and May 10, 2018, on *Pinus* sp., P. Coffey (2018-471 and 2018-3047) 2 3rd ♂; Daytona Beach (Lat.29.207554, Long. −81.012311), June 27, 2018, on *Pinus* sp., (Lindgren trap), P. Coffey (2018-4028) 2 ad. ♀; Daytona Beach (Lat.29.22651, Long. −81.0242), October 10, 2018, on *Pinus* sp., (Lindgren trap), P. Coffey (2018-6210) 1 3rd ♂.

Life history. Little is known about the life cycle of this species. Most of the biological data were gathered from Lindgren funnel traps. Adult females were collected in traps from Brevard Co., Melbourne, October; Broward Co., Pompano Beach, April, November and December; Hillsborough Co., Tampa, October; in St. Lucie Co., Fort

Pierce, February, March, April and July; St. Lucie Co., in Daytona Beach, June; Alachua Co., Gainesville, October; Miami-Dade Co., Medley, March. Similar results were discovered with third-instar males: Brevard Co., Titusville, February, March and April; Broward Co., Ft. Lauderdale, March and April; Broward Co., Pompano Beach, March, April, November and December; Duval Co., Baldwin, December; Duval Co., Jacksonville, October; Hillsborough Co., MacDill Air Force Base, Tampa, October; Miami-Dade Co., Medley, February, April, November, December; Nassau Co., Fort Clinch State Park, March, September; Orange Co., Moss Park, Orlando October; Palm Beach Co., Jupiter, February: St. Lucie Co., Fort Pierce, February and March; Volusia Co., Daytona Beach January, May, June and October. Since specimens were active during the month when they were collected, and since active third-instar males and adult females were taken in every month but August, it appears that the species has more than a single generation each year. One collection of cysts was recorded in Palm Beach County, Jupiter, on the small branches of *Pinus elliottii,* April 5, 2018 (Figure 13). The cysts were deeply imbedded in the current year's growth but there was no indication of swelling or galling; the only indication that a cyst was present under the bark was a small hole with the shiny black integument of the cyst showing through (Figure 13). These pines were showing serious die back, but it appears that this was caused by a fungal disease and not the *Matusucoccus* infestation. However, because of the cryptic nature of this insect it is possible that they may have been a contributing factor.

Other Species

It is worth mentioning that *M. macrocicatrices* has been collected in eastern North America from Canada to Georgia and is often associated with *Septobasidium pinicola* Snell on *Pinus strobus* L. (Mech et al. 2013). It can be distinguished from the other species in Florida by having (characters of *M. alabamae, M. gallicolus,* and *M. krystalae* are given in parentheses) enlarged setae on the last five antennal segments (four antennal segments) and by cicatrices that are greater than 15 microns in diameter (less than 15 microns). Because *M. macrocicatrices* has

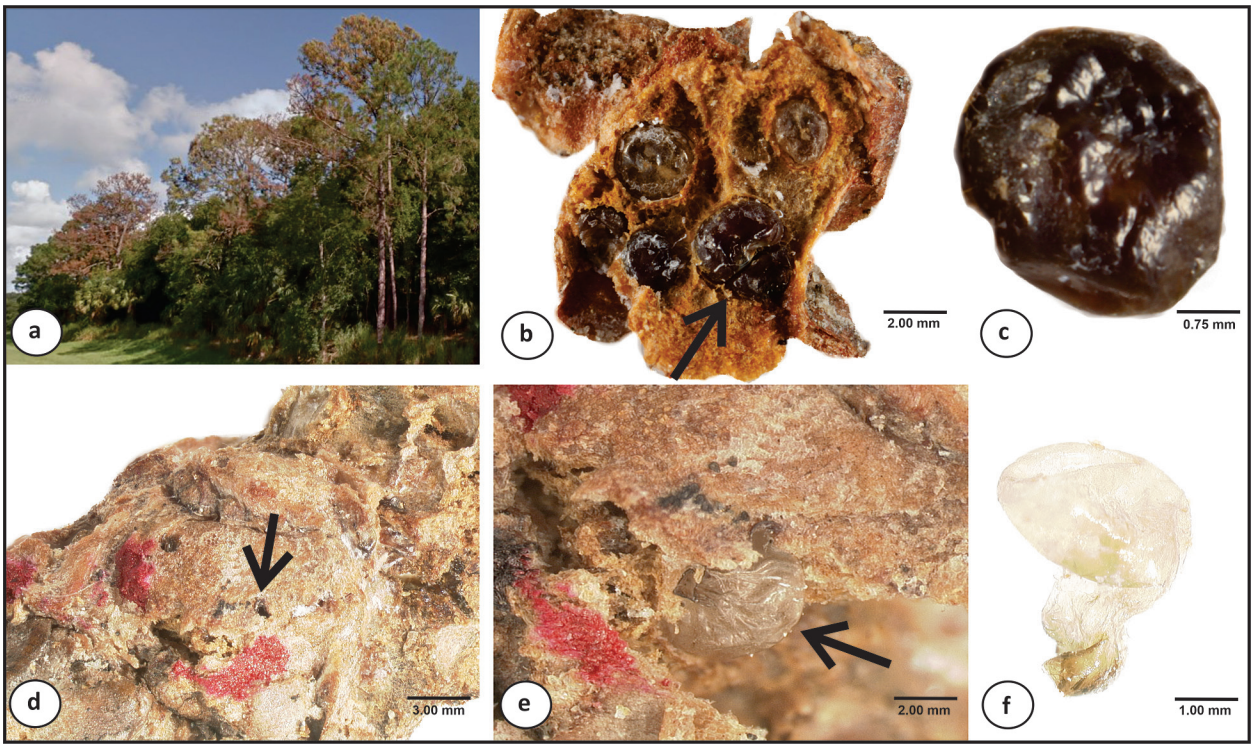

Figure 13. Damage likely caused by a pathogen, but trees also infested with *Matsucoccus krystalae*. **A)** Slight yellowing on pine trees. **B–C)** *Matsucoccus krystalae* cysts associated with damage. **D–F)** Cysts under the bark of pine twigs exposed by removing bark with exit holes.

only been reported on *P. strobus* and because Florida is outside the natural range of this host it is unlikely that this *Matsucoccus* species will be found in Florida unless the tree is planted in an ornamental setting.

Conclusions

Two species of *Matsucoccus* occur in Florida, *M. alabamae* and *M. krystalae*. Some of the characters that can be used to distinguish between these species in the adult female also are useful in diagnosing third-instar males, including the lengths of the setae on the venter and the relative sizes of the abdominal spiracles. Molecular phylogenetic analyses based on 18S, 28S D2/D3, 28S D10, and COI genes support morphological determinations. Though genetic distance between *M. krystalae* and *M. gallicolus* is low, their consistent morphological differences relative to the other species in the genus corroborate their independent placements in well-supported clusters in the phylogenetic trees. In addition, we did not find any overlap in their geographical ranges. This suggests that there are reproductive barriers at the interface that keep them separate. Additional sampling and more allelic data will help to understand the situation more precisely. However, we have attempted to collect new samples of *M. gallicolus* for more than two years without success. *Matsucoccus* species are difficult to find and work with in general. Lindgren funnel traps can be used as a means of collecting them. Lindgren funnel traps data have provided some interesting insight into the life history of these species. Based on the collections from 2018 and 2019, it appears that *M. krystalae* may have more than a single generation each year in Florida.

Acknowledgments

Thanks to Elijah Talamas, John Mcvay, Paul Skelley and Greg Hodges (FDACS-DPI), for constructive reviews. Krystal Ashman (Cooperative Agricultural Pest Survey, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida) helped us by discovering *Matsucoccus* specimens taken in the Lindgren funnel traps and for continuing to collect specimens of both species to give us a better understanding of the life history of these unusual insects in Florida. Ian Stocks (Animal and Plant Health Inspection Service, United States Department of Agriculture, Beltsville, Maryland) spent considerable time in the field searching for live specimens of *M. gallicolus.* We also thank Morgan Byron and Bradley Danner (Cooperative Agricultural Pest Survey, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida) for providing detailed information about the Lindgren funnel traps that contained *Matsucoccus* specimens and for the images of a trap in a tree. We also are grateful to Janie Booth (Seattle, Washington), Lyn Cook (School of Biological Sciences, University of Queensland, Brisbane, Australia) and Penny Gullan (College of Science, Australian National University, Canberra, Australia) for providing the sequence information for *Matsucoccus gallicolus.* The digital images of the cysts of *M. krystalae* were kindly provided by Lyle Buss (Entomology and Nematology Department, University of Florida, Gainesville, Florida). We also are grateful to Jeffrey Eickwort (Florida Forest Service, Florida Department of Agriculture and Consumer Services, Gainesville, Florida) for providing us samples and pointing out the problems on the Jupiter Country Club pines. We thank Alan Jeon and Holly Goodwin (Auburn University, Auburn, Alabama) for collecting live *Matsucoccus alabamae* specimens for molecular investigation. We thank technicians, Gabi Ouwinga, Jessica Awad, and Lily Deeter (FDACS-DPI) for their help in mounting slides during this study. DRM is grateful to the Division of Plant Industry, especially Paul Skelley and Greg Hodges, for providing space and resources to conduct this and other research. The contributions of MZA and MRM were supported by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

Literature Cited

Ahmed MZ, Miller DR. 2020. *Matsucoccus alabamae* Morrison, Alabama pine scale (Matsucoccidae: Coccomorpha: Hemiptera): a potential pest of Florida pines. Florida Department of Agriculture and Consumer Services, FDACS-P-02144, Entomology Circular 444: 1–5.

Amouroux P, Crochard D, Germain JF, Correa M, Ampuero J, Groussier G, Kreiter P, Malausa T, Zaviezo T. 2017.

Genetic diversity of armored scales (Hemiptera: Diaspididae) and soft scales (Hemiptera: Coccidae) in Chile. Scientific Reports 7: 2014.

Aughanbaugh J. 1949. The *Matsucoccus* scale (*Matsucoccus gallicolus*) in pitch pine plantations. Pennsylvania Forests and Waters 1(8): 182–186.

Booth JM, Gullan PJ. 2006. Synonymy of three pestiferous *Matsucoccus* scale insects (Hemiptera: Coccoidea: Matsucoccidae) based on morphological and molecular evidence. Proceedings of the Entomological Society of Washington 108: 749–760.

Boratynski KL. 1952. *Matsucoccus pini* (Green, 1925) (Homoptera, Coccoidea: Margarodidae). Bionomics and external anatomy with reference to the variability of some taxonomic characters. Transactions of the Royal Entomological Society of London 103: 285–326.

Chen P, Liu Q, Qiao X, Wang J, Zhang T. 2018. Identification and phylogenetic analysis of pear psyllids (Hemiptera: Psyllidae) in Chinese pear orchards. Journal of Economic Entomology 20: 1–6.

Collins RA, Boykin LM, Cruickshank RH, Armstrong K. 2012. Barcoding's next top model: an evaluation of nucleotide substitution models for specimen identification. Methods in Ecology and Evolution 3: 457–465.

Dietrich CH, Rakitov RA, Holmes JL, Black WC IV. 2001. Phylogeny and the major lineages of Membracoidea (Insecta: Hemiptera: Cicadamorpha) based on 28S rDNA sequences. Molecular Phylogenetics and Evolution 18: 293–305.

Dowton M, Austin AD. 1998. Relationships among the microgastroid wasps (Hymenoptera: Braconidae): combined analysis of 16s and 28s rDNA genes and morphological data. Molecular Phylogenetics and Evolution 10: 354–366.

Dunkelblum E, Mendel Z, Assael F, Harel M, Kerhoas L, Einhorn J. 1993. Identification of the female sex pheromone of the Israeli pine bast scale *Matsucoccus josephi*. Tetrahedron Letters 34: 2805–2808.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797.

Foldi I. 2005. The Matsucoccidae in the Mediterranean basin with a world list of species (Hemiptera: Sternorrhyncha: Coccoidea). Annales de la Société Entomologique de France 40(2) (2004): 145–168.

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

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59(3): 307–21.

Gwiazdowski RA, Foottit RG, Maw HEL, Hebert PDN. 2015. The Hemiptera (Insecta) of Canada: constructing a reference library of DNA barcodes. PLoS ONE 10(4): e0125635.

Herbert FB. 1919. A new species of *Matsucoccus* from pines in California (Hemip.-Homop.). Proceedings of the Entomological Society of Washington 21: 157–161.

Herbert FB. 1921. The genus *Matsucoccus* with a new species. Proceedings of the Entomological Society of Washington 23: 15–22.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences 101: 14812–14817.

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2017. UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522.

Hoang DT, Vinh LS, Flouri T, Stamatakis A, von Haeseler A, Minh BQ. 2018. MPBoot: fast phylogenetic maximum parsimony tree inference and bootstrap approximation. BMC Evolutionary Biology 18: 1–11.

Kalyaanamoorthy S., Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589.

Kang AR, Baek JY, Lee SH, Cho YS, Kim W, Han YS, Kim IS. 2010. Geographic homogeneity and high gene flow of the pear psylla, *Cacopsylla pyricola* (Hemiptera: Psyllidae), detected by mitochondrial COI gene and nuclear ribosomal internal transcribed spacer 2. Animal Cells and Systems 16: 145–153.

Kanturski M, Lee Y, Choi J, Lee S. 2018. DNA barcoding and a precise morphological comparison revealed a cryptic species in the *Nippolachnus piri* complex (Hemiptera: Aphididae: Lachninae). Scientific Reports 8: 8998.

Katoh H, Inoue H, Kuchiki F, Ide Y, Uechi N, Iwanami T. 2013. Identification of a distinct lineage of *Cacopsylla chinensis* (Hemiptera: Psyllidae) in Japan on the basis of two mitochondrial DNA sequences. Journal of Economic Entomology 106: 536–542.

Katoh H, Inoue H, Uechi N, Fujikawa T, Miyata S, Iwanami T. 2014. Analysis of the genetic population structure of *Cacopsylla chinensis* (Hemiptera: Psyllidae) with mitochondrial DNA markers in Saga and Yamaguchi prefectures, Japan. Japan Agricultural Research Quarterly 48: 413–417.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166.

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.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35(5): 1547–1549.

Lee HC, Yang MM, Yeh WB. 2008. Identification of two invasive *Cacopsylla chinensis* (Hemiptera: Psyllidae) lineages based on two mitochondrial sequences and restriction fragment length polymorphism of cytochrome oxidase I amplicon. Journal of Economic Entomology 101: 1152–1157.

Leite LAR. 2012. Mitochondrial pseudogenes in insect DNA barcoding: differing points of view on the same issue. Biota Neotropica 12: 301–308.

Lindgren BS. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). Canadian Entomologist 115: 299–302.

McCambridge WF, Pierce DA. 1964. Observations on the life history of the pinyon needle scale *Matsucoccus acalyptus* (Homoptera, Coccoidea, Margarodidae). Annals of the Entomological Society of America 57: 197–200.

McClure MS. 1981. A new (?) insect pest of red pine in Connecticut. Frontiers of Plant Science, Connecticut Agricultural Experiment Station 33: 4–6.

McKenzie HL. 1941. A new species of *Matsucoccus* attacking piñon pine in California (Homoptera; Coccoidea; Margarodidae). Microentomology 6: 2–5.

McKenzie HL. 1942. Seasonal history of the margarodid scale, *Matsucoccus bisetosus* Morrison, occurring on ponderosa and Jeffrey pines in California (Homoptera; Coccoidea; Margarodidae). (Contribution No. 31). Microentomology 7: 19–24.

McKenzie HL. 1943. The seasonal history of *Matsucoccus vexillorum* Morrison (Homoptera; Coccoidea; Margarodidae). (Contribution No. 39.) Microentomology 8: 42–52.

McKenzie HL, Gill LS, Ellis DE. 1948. The Prescott scale (*Matsucoccus vexillorum*) and associated organisms that cause flagging injury to ponderosa pine in the Southwest. Journal of Agricultural Research 76: 33–51.

Mech AM, Asaro C, Cram MM, Coyle DR, Gullan PJ, Cook LG, Gandhi KJK. 2013. *Matsucoccus macrocicatrices* (Hemiptera: Matsucoccidae): First report, distribution, and association with symptomatic eastern white pine in the southeastern United States. Journal of Economic Entomology 106(6): 2391–2398.

Mendel Z, Assael F, Saphir N, Zehavi A, Nestel D, Schiller G, Ne'eman G. 1997. Seedling mortality in regeneration of Aleppo pine following fire and attack by the scale insect *Matsucoccus josephi*. International Journal of Wildland Fire 7(4): 327–333.

Mendel Z, Assael F, Dunkelblum E. 2004. Kairomonal attraction of predatory bugs (Heteroptera: Anthocoridae) and brown lacewings (Neuroptera: Hemerobiidae) to sex pheromones of *Matsucoccus* species (Hemiptera: Matsucoccidae). Biological Control 30(2): 134–140.

Morrison H. 1928. A classification of the higher groups and genera of the coccid family Margarodidae. United States Department of Agriculture Technical Bulletin 52: 1–239.

Morrison H. 1939. Descriptions of new species of *Matsucoccus* (Hemiptera: Coccidae). Proceedings of the Entomological Society of Washington 41: 1–20.

Park DS, Suh SJ, Oh HW, Hebert PDN. 2010. Recovery of the mitochondrial COI barcode region in diverse Hexapoda through tRNA-based primers. BMC Genomics 11: 423.

Parr T. 1939. *Matsucoccus* sp. a scale insect injurious to certain pines in the northeast (Hemiptera-Homoptera). Journal of Economic Entomology 32: 624–630.

Qi JL, Wang YY. 1981. Studies on the Yunnan bast scale. Scientia Silvae Sinicae 17: 20–25.

Ray CH. 1982. Revision of the Genus *Matsucoccus* (Homoptera: Coccoidea: Margarodidae) in North America. Auburn University, Ph.D. thesis [unpublished]. Auburn, Alabama. 295 p.

Rieux R. 1976. *Matsucoccus pini* Green (1925) (Homoptera, Margarodidae) dans le Sud-Est de la France. Variations intraspécifiques. Comparaison avec des espèces les plus proches. Annales de Zoologie. Ecologie Animale 8: 231–263.

Riom J, Fabre JP. 1979. Décalage phenologique des éclosions des deux sexes chez Matsucoccus feytaudi Duc. (Homoptera, Coccoidea, Margarodidae). Comptes Rendus Hebdomadaires des Séances de l'Academie des Sciences, Serie D. Paris 288(1): 89–92.

Shin HC, Lee SM, Lee KS, Kim YK. 2003. Analysis on the recovering and damage caused by *Matsucoccus thunbergianae* in *Pinus thunbergii* stand. KFRI Journal of Forest Science 66: 1–10.

Shin S, Jung S, Lee H, Lee S. 2013. Molecular identification of dipteran pests (Diptera: Sciaroidea) from shiitake mushroom. Molecular Ecology Resources 13: 200–209.

Song H, Buhay JE, Whiting MF, Crandall KA. 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proceedings of the National Academy of Sciences 105: 13468–13491.

Song C, Lin XL, Wang Q, Wang XH. 2018. DNA barcodes successfully delimit morphospecies in a superdiverse insect genus. Zoological Scripta 47: 311–324.

Srivathsan A, Meier R. 2011. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics 28: 190–194.

Tautz D, Hancock JM, Webb DA, Tautz C, Dover GA. 1988. Complete sequences of the ribosomal rRNA genes of *Drosophila melanogaster*. Molecular Biology and Evolution 5: 366–376.

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44: W232–W235.

Unruh TR, Luck RF. 1987. Deme formation in scale insects: A test with pinyon pine scale and a review of other evidence. Ecological Entomology 12: 439–449.

Vea IM, Grimaldi DA. 2016. Putting scales into evolutionary time: the divergence of major scale insect lineages (Hemiptera) predates the radiation of modern angiosperm hosts. Scientific Reports 6: 23487.

von Dohlen CD, Moran NA. 1995. Molecular phylogeny of the Homoptera: a paraphyletic taxon. Journal of Molecular Evolution 41: 211–223.

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC. 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18s and 28s ribosomal DNA sequences and morphology. Systematic Biology 46: 1–68.

Wu Y, Trepanowski NF, Molongoski JJ, Reagel PF, Lingafelter SW, Nadel H, Myers SW, Ray AM. 2016. Identification of wood-boring beetles (Cerambycidae and Buprestidae) intercepted in trade-associated solid wood packaging material using DNA barcoding and morphology. Scientific Reports 7: 40316.

Yeh WB, Yang CT, Kang SC. 1997. Identification of two sibling species, *Ephemera formosana* and *E. sauteri* (Ephemeroptera: Ephemeridae), based on mitochondrial DNA sequence analysis. Chinese Journal of Entomology 17: 257–268.

Young BL, Hu JL, Ren ZY. 1976. Pine bast scales from China. Acta Entomologica Sinica 19: 199–204.

Young BL, Hu JL, Ren ZY. 1980. On pine needle scales. Acta Entomologica Sinica 23: 42–46.

Received August 15, 2020; accepted September 22, 2020.
Review editor Aline Barcellos.