Emerald Ash Borer Biological Control
RECOVERY GUIDELINES 2026

Published by the **United States Department of Agriculture, Animal and Plant Health Inspection Service.**

Major contributions provided by USDA Agricultural Research Service, USDA Forest Service, and cooperating State Departments of Agriculture.

This is a SUBSECTION of the Emerald Ash Borer Biological Control Release and Recovery Guidelines, intended to instruct users on PARASITOID RECOVERY PROTOCOLS.
To access the full document, follow this link:
https://www.aphis.usda.gov/sites/default/files/eab-field-release-guidelines.pdf

Prepared by:

Christine Dodge, Biological Scientist
christine.dodge@usda.gov
USDA APHIS PPQ S&T Forest Pest Methods Laboratory
508-563-0942

Theresa Booth (*Murphy*), Biological Technician
USDA APHIS PPQ S&T Forest Pest Methods Laboratory
theresa.c.booth@usda.gov
508-563-0944

Elisabeth Darling, Supervisory Entomologist
USDA APHIS PPQ EAB Biocontrol Rearing Facility
elisabeth.darling@usda.gov
810-844-2704

Andrea Anulewicz, Biological Technician
USDA APHIS PPQ EAB Biocontrol Rearing Facility
andrea.anulewicz@usda.gov
810-844-2711

ACKNOWLEDGEMENTS

Juli Gould, Nicole Sawallich, Toby Petrice, Jian Duan, Benjamin Slager, and Leah Bauer prepared earlier versions of the guidelines and provided significant contributions. Substantial contributions to methods for rearing parasitoids and conducting field sampling were provided by: Benjamin Slager, Tracy Ayer, Houping Liu, Debbie Miller, Phil Taylor, Kristopher Abell, Jason Hansen, and Michael Ulyshen. The following personnel provided critical input in the development of this document: Joe Beckwith, James Buck, Russ Bulluck, Sharon Lucik, Michael Winks, Rhonda Santos, and Ron Weeks.

Cover photographs by: Yellow pan trap (left): Theresa Booth, USDA APHIS PPQ; Peeled log revealing *Tetrastichus planipennisi* larvae (right): Everett Booth, USDA APHIS PPQ

Cite this report as follows:

USDA–APHIS/ARS/FS. 2026. Emerald Ash Borer Biological Control Release and Recovery Guidelines. USDA–APHIS–ARS–FS, Riverdale, Maryland.

CONTENTS

List of Appendices:

EAB PROGRAM CONTACTS

PARASITOID RELEASES

USDA APHIS PPQ EAB Biocontrol Rearing Facility

5936 Ford Ct, Ste. 200

Brighton, MI 48116

Email: EAB.Biocontrol.Program@usda.gov

Lab Number: 810-844-2711

The deadline to submit site requests for program parasitoid releases is **December 1**.

Scott A. Whitehead, Release Coordinator

Email: scott.a.whitehead@usda.gov

Elisabeth Darling, Supervisory Entomologist

Email: elisabeth.darling@usda.gov

PARASITOID RECOVERY

Send adult specimens from yellow pan traps to:

Andrea Anulewicz
5936 Ford Ct, Ste. 200
Brighton, MI 48116
Email: andrea.anulewicz@usda.gov

Send EAB eggs/*Oobius agrili* from bark sifting or bark rearing to:

Toby Petrice
2601 Coolidge Rd, Ste. 203
East Lansing, MI 48823
Email: toby.petrice@usda.gov

If you have larval/cocoon specimens from tree peeling, certain specimens can be identified morphologically while others need to be DNA sequenced. Please email Theresa Booth (theresa.c.booth@usda.gov) for where to send them for identification.

OTHER PROGRAM CONTACTS

Chrissy Dodge, Biological Scientist
USDA APHIS PPQ S&T Forest Pest Methods Laboratory
1398 W. Truck Rd
Buzzards Bay, MA 02542
Email: christine.dodge@usda.gov

Theresa Booth, Biological Science Laboratory Technician
USDA APHIS PPQ S&T Forest Pest Methods Laboratory
1398 W. Truck Rd
Buzzards Bay, MA 02542
Email: theresa.c.booth@usda.gov

Craig Kellogg, Michigan State Plant Health Director
USDA APHIS PPQ FO
11200 Metro Airport Center Dr, Ste. 140
Romulus, MI 48174
Email: craig.kellogg@usda.gov

Russ Bulluck, National Operations Manager
USDA APHIS PPQ FO
920 Main Campus Dr, Ste. 200
Raleigh, NC 27606
Email: russ.bulluck@usda.gov

Ron Weeks, National Policy Manager
USDA APHIS PPQ EDP
920 Main Campus Dr, Ste. 200
Raleigh, NC 27606
Email: ron.d.weeks@usda.gov

ADDITIONAL TRAINING RESOURCES

Every two years, the program hosts an in-person EAB Biocontrol Release and Recovery Workshop for interested program participants, including representation from federal, state, university, and tribal affiliates. Contact Russ Bulluck (russ.bulluck@usda.gov) for more information.

We also offer a comprehensive series of three pre-recorded video training sessions. The video sessions review the necessary tools and resources to choose an EAB parasitoid release site, conduct releases, and perform recoveries. The videos will enhance your understanding of the guidelines and the science used to develop them. To access the training videos, visit the USDA APHIS EAB webpage (USDA APHIS | Emerald Ash Borer). The videos are listed under **Related Resources > Information for Cooperators > Training Videos.** Links to the videos are provided below for your convenience.

Module 1 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 7, 2022 - YouTube

Introduction, background, and lifecycle of EAB, how to identify EAB and possible look-a-likes, history of biological control of EAB, how to identify ash and possible look-a-likes, impacts of EAB parasitoids. **(1 hour 40 minutes)**

Module 2 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 14, 2022 - YouTube

Mass rearing of EAB biological control agents for release, choosing sites and considerations for releasing parasitoids, conducting parasitoid releases, and an introduction to MapBioControl. **(1 hour 47 minutes)**

Module 3 Emerald Ash Borer Release and Recovery Guidelines – April 21, 2022 - YouTube

Evaluating parasitoid establishment: debarking for *Oobius agrili*, peeling trees for larval parasitoids, and yellow pan traps. **(2 hours 21 minutes)**

RELEASE SITE SUBMISSION PROCESS

PLEASE NOTE: If your site is selected for biocontrol releases, you are committing to four consecutive years of program participation. You will be expected to do two years of releases, followed by two years of recovery work.

Step 1: Create a MapBioControl.org account.

Step 2: Locate a potential release site and record the following information:

Site Basics

1. Status (Proposed)
2. State
3. Date
4. Site Name
5. Site Location
6. Latitude and longitude (dd.dddddd)
7. Type (Program or Research)

General Details

1. Site size (acres)
2. Percent of ash present
3. Top three dominant tree species
4. EAB density (low, medium, or high)

Other physical details are optional.

Step 3: Enter this information into MapBioControl and record your new site ID.

Step 4: Email the program contacts (EAB.Biocontrol.Program@usda.gov) with your new site ID. They will provide you with a spreadsheet requesting your contact and address information if your site is selected for releases. **Fill this out and return it by December 1 of the year before you intend to release.**

Step 5: Apply for any required local land use permits.

Step 6: Site selections will typically be released to cooperators in early March. **Please check to see if your site made the selected list and reply to confirm you will be conducting releases!**

FAQs

1. ***How are site selections determined?***
   The Rearing Facility Release Coordinator and Supervisory Entomologist work together to determine which sites have the highest priority for releases that year. Sites are given higher priority if they are located in counties or states where releases have not been conducted yet.

2. ***When do releases typically begin?***
   Parasitoid release season is typically from April to September. Release dates are calculated based on growing degree days (GDD50F), so dates vary greatly by location and parasitoid species.

3. ***My site wasn't selected for releases. Can I be considered in the future?***
   Absolutely! Each year we do our best to try and accommodate as many sites as production allows. You are welcome to resubmit the site the following year if it was not selected.

4. ***Do I need a permit to receive parasitoids?***
   The Rearing Facility holds permits to ship to all EAB-infested states in the U.S., so you will not need a permit to receive them. A copy of this permit will be included inside of your shipment. However, you will need to ensure that you have the proper permits or land use approvals for local releases.

5. ***Can I release on my private land?***
   No, we do not accommodate releases on private land. Releases must be conducted on federal, state, county, tribal, or other public lands.

PARASITOID RECOVERY GUIDELINES

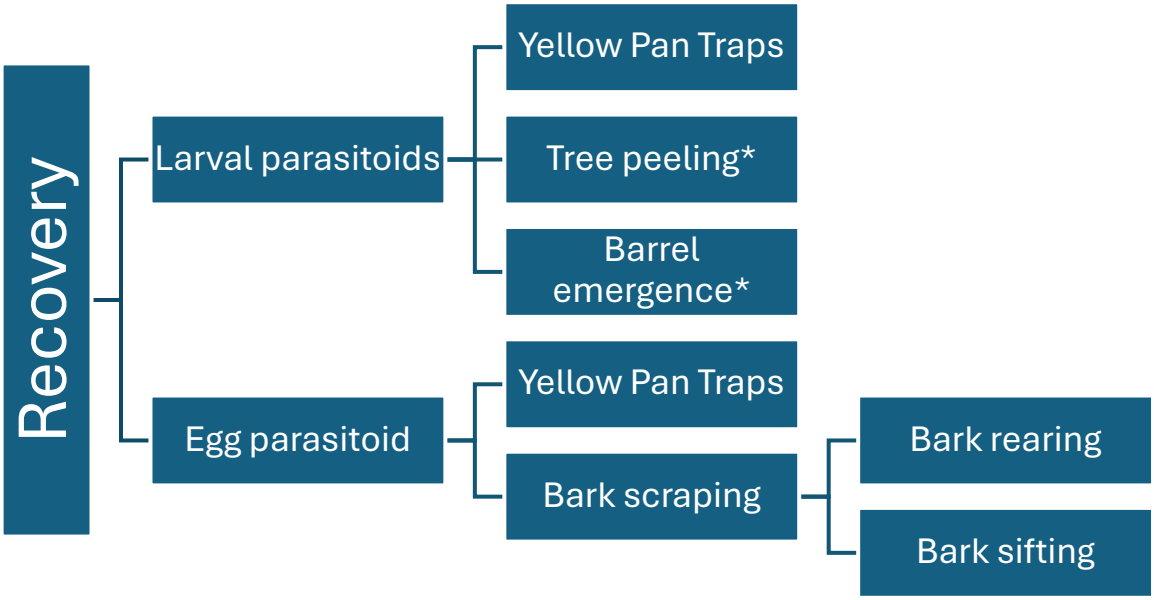
Program Site Release and Recovery Timeline
Years 1 and 2: Perform parasitoid releases.
Years 3 and 4: Perform parasitoid recovery efforts.

RECOVERY OVERVIEW

Recovery efforts should be started the year after releases are finished. You only need to sample one release site per county, although more is always better. **Two years of recovery work are standard** following your releases so that you can assess whether parasitoids are establishing. We define **establishment** as consistently recovering parasitoids at least two years after releases have finished.

Several methods have been developed that can successfully recover the four parasitoids released for EAB biocontrol. Unfortunately, none of the methods is consistently more effective than the others, and there are circumstances where parasitoids are recovered using certain methods but not others. Yellow pan traps are inexpensive and easy to sample, but they do not provide any measure of EAB parasitism. Collecting EAB eggs and larvae from trees allows us to calculate percent parasitism, but this method requires active sampling and may require felling trees for an adequate sample of EAB larvae. If resources permit, the best option is to use a variety of methods to ensure that if parasitoids are present, some will be recovered.

The graphic below illustrates which methods are used by the program to recover parasitoids. If you have released larval and egg parasitoids, please make sure you are making efforts to recover both. Yellow pan traps are the only method that can be used to sample for all parasitoids at once, but tree peeling and barrel emergence can be coupled with bark scraping so that you can recover both larval and egg parasitoids with those combined activities.



***You may scrape bark from trees you peel or logs you put in barrels for larval parasitoid recovery to also look for egg parasitoids.**

RECOVERY METHOD: YELLOW PAN TRAPS (YPTs)

Summary:

- Hang YPTs from ash trees showing current symptoms of EAB infestation.
- Deploy 15 YPTs per site.
- Deploy traps in the spring, summer, or early fall, depending on the parasitoid(s) you released, for at least 6 weeks.
- Change trap liquid every 5-7 days depending on the weather.
- Freeze samples that will not be processed right away, but do not refreeze thawed samples.

Many insects are attracted to the color yellow. Parasitoid recovery studies have shown that YPTs are effective at trapping all four of the introduced EAB parasitoids. Other known EAB larval parasitoids (e.g. *Atanycolus* spp., native *Spathius* spp., *Phasgonophora sulcata*, and *Balcha indica*; see Appendix F) are also frequently trapped, along with many other species of insects.

Yellow Pan Trap Deployment Considerations

Choosing promising trees

Successful parasitoid recovery in YPTs seems to depend on where they are placed, and some trees are better for recovering parasitoids than other trees. Often, we find the same trees will catch parasitoids week after week, while nearby trees will recover nothing. We are not sure of all of the conditions that make an ideal tree, which is why we recommend placing multiple traps. **The trees you select must show some symptoms of current EAB infestation (e.g. fresh woodpecker feeding, epicormic shoots) with moderate to major signs of canopy dieback.** Do not put the traps on dead or healthy ash trees, since parasitoids will not be foraging on these trees.

YPTs seem to have the greatest success under the following conditions:

- On trees with visible signs of recent EAB damage (e.g. fresh woodpecker holes on the lower half of the tree) and live phloem.
- In sites with moderate to high canopy dieback (10-80%).
- In sites with ash along a forest edge (e.g. greenways, bike paths, roads).
- On trees that get a lot of sunlight, or on the sunny south/west facing sides of trees.

Due to the dynamics of our forested areas, it is not always possible to find "ideal" trees, and a given tree will only be suitable for hanging YPTs for a few years. Each year, do the best you can to find live but infested trees on which to hang the traps. If possible, select a variety of sizes of ash on which to place the traps. *Tetrastichus planipennisi* cannot parasitize EAB in ash larger than ~6 inches DBH, *Oobius agrili* are more likely to be found on trees with flaky bark (as in larger trees), and *Spathius* species can be found on trees of varying sizes. Distribute YPTs on ash trees throughout the release areas and, if possible, place some traps at or near the release trees.

How many YPTs should I deploy?

Deploy a total of 15 YPTs (one trap per tree) at your release site. Please know that trapping can generate a substantial sample load that will need to be processed. Samples can be frozen until able to be processed. If staff hours are limited, you may want to consider fewer sample dates or fewer sites. **WE DO NOT RECOMMEND PUTTING OUT FEWER THAN 15 YELLOW PAN TRAPS WITHIN A SITE.**

When should I deploy YPTs in the field?

Deploy YPTs at release sites in the year after your final parasitoid releases. For example, if you finish releases in August, you can start YPT recovery the following spring.

The timing of possible parasitoid captures in YPTs will depend on the climate in your area and the adult flight period of each species. Growing degree day base 50°F (GDD_{50F}) estimates are based on EAB phenology models and may not be accurate for geographic regions where we have less data available. See below for more specific guidance by species.

When to deploy YPTs for Tetrastichus planipennisi and/or Spathius galinae

If you can do intensive sampling, we recommend deploying YPTs by 200 GDD_{50F} and sampling throughout the spring, summer, and early fall until 2900 GDD_{50F}. This requires visiting sites weekly, collecting the samples, and replacing the liquid in the bowl for the next week of sampling. A few studies have found the highest captures for larval parasitoids *T. planipennisi* and *S. galinae* occurred in the spring so if you can't do intensive sampling, then we recommend deploying YPTs by 200 GDD_{50F} and sampling for at least 6 weeks.

When to deploy YPTs for Spathius agrili

If you are trapping for *Spathius agrili* in the southern U.S., deploy the YPTs when 3rd-instar larvae become available. This could be as early as mid-June (in southern states such as Arkansas), or mid-July in more central states (such as Tennessee).

When to deploy YPTs for Oobius agrili

To capture *Oobius agrili* we recommend deploying the YPTs by 720 GDD_{50F} and trapping until 2200 GDD_{50F}. Petrice et al. (2021) found peak *Oobius agrili* captures at 1444-1598 GDD_{50F}. If you can't do intensive sampling, then we recommend having the traps out during this peak window.

How long should I leave YPTs in the field?

YPT samples should be collected and the liquid replaced every 5-7 days. Samples left too long in the field will decay or dry up. Seven days is ideal because the longer the traps remain in the field, the more likely they will trap one of the target parasitoids. If you anticipate heavy rain, you might want to consider collecting the samples early. In limited cases, cooperators have found that if they increased concentrations of propylene glycol to 50%, samples could go up to 2 weeks before collecting. Care must be taken to test that this method will work at your site. If you find samples drying out or decaying, restart weekly collections. When servicing traps, collection bowls must be filled to the overfill hole to prevent them from drying out. In addition, if you get a heavy rain event, empty traps before or soon after because rain will both displace the solution from the bowl and dilute the remaining solution, which can lead to rapid decay of insect specimens.

Assembling and Deploying Yellow Pan Traps

Materials needed to build YPTs:

- Two 12-oz, 7–8-inch bright yellow plastic bowls
- One 8-inch (8 x 6 or 8 x 10) right-angled shelf-bracket
- Three 1.25-inch-long wood screws
- Two mini binder clips for securing the collection bowl to the holding bowl
- A one-hole punch and a utility knife for altering the bowls
- Fine-mesh screening (e.g. organza or no-see-um mesh)
- A hot glue gun
- Weather-proof marking pen (e.g. Sharpie) or grease pencil (needed if bowls are wet)
- Three 6-inch zip-ties (thin enough to fit through the holes on the shelf bracket)
- 20% solution of **clear** (not pink or green) **food grade** and/or **USP grade** propylene glycol (non-toxic antifreeze) diluted with water. It is easiest to buy pure propylene glycol and dilute it yourself
- Rechargeable portable electric screwdriver with bit and extra battery pack
- Unscented clear dish detergent

To request a kit for making YPTs or detailed instructions on how to construct them, please email Theresa.c.booth@usda.gov.

How do I set up the YPTs?

1. Using an electric screwdriver, attach the shelf bracket to the tree trunk approx. 5 feet above the ground, with three wood screws. Make sure the top bracket is level or the bowl will not sit properly. You may have to leave the top screws partially unscrewed so that the bracket remains level.
2. Attach the bottom yellow "holding bowl" to the top side of the bracket with zip ties threaded through the three shelf bracket holes. Do not pull zip-ties too tightly to avoid distorting the holding bowl.
3. Set the top collection bowl in the holding bowl. Fill the collection bowl ~¾-full with 20% propylene glycol solution.
4. Add one drop of unscented dish detergent to the solution to break the surface tension, causing insects to become entrapped in the liquid.
5. Secure the bowls together using two binder clips to prevent the bowl from getting blown out by the wind, which can happen as the liquid level lowers during drier weeks.

6. Label each YPT holding bowl with a unique ID number using a weather-proof pen (e.g. Sharpie) or grease pencil if the bowl is wet. On a data sheet, record the YPT ID number and date.
7. Record the GPS coordinates of the YPT. This will help you find the trap later to recover the sample, and it will let researchers know where the parasitoids were recovered.

A collection bowl being put into the holding bowl of a yellow pan trap. Photo by J. Hansen.

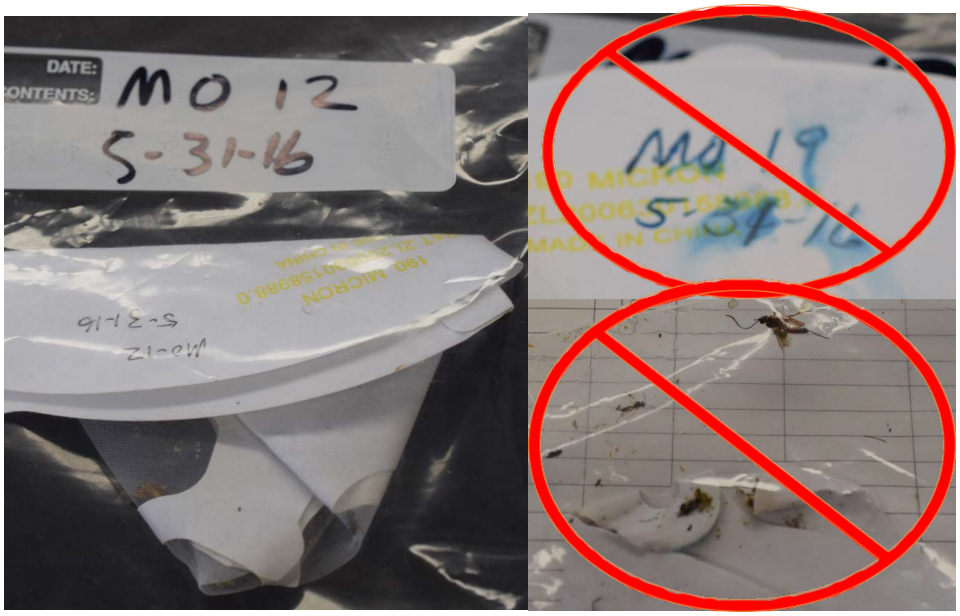
Servicing Yellow Pan Traps

Materials needed to collect YPT samples:

- 190-micron fine mesh paint filters
- Zipper top or whirl-pak plastic bags
- Pencils **(not pens)**
- 20% solution of **clear** (not pink or green) **food grade** and/or **USP grade** propylene glycol (non-toxic antifreeze) diluted with water. It is easiest to buy pure propylene glycol and dilute it yourself
- A squirt bottle with water for dislodging insects from the bowl or from leaves/slugs/debris
- A cooler to store samples in during transit

How do I collect insect samples from YPTs?

1. After locating the YPT in the field, label your paint filter with the site, date (including year), and YPT ID # in pencil (pen or marker may wash out).
2. If there are many leaves in the sample, swish them in the liquid to dislodge any insects and discard them. Consider doing this with slugs as well as their slime can make the sample gummy and hard to process. Pour the contents of the trap (insects plus liquid) through a paint filter. A squirt bottle with water is recommended for dislodging insects from the bottom of the bowl or from leaves/slugs. The propylene glycol is not toxic and can be poured on the ground.
3. Fold over the open end of the paint filter and place each one separately into a zippered bag. If the filter is labelled in pencil, you do not need to label the bag. If you cannot label the filter paper (because it is too wet, for example) then label the bag instead with Sharpie.
4. If it is hot, bring a cooler with ice packs while collecting samples to help preserve insects before they are processed. A few hours in a hot car can cause insects to start to disintegrate and make it harder to ID samples later.
5. Store samples in the refrigerator and process them within one or two days. If processing is delayed, store the samples in the freezer.



Label the filter paper in pencil (left) to avoid ink smearing (right). Fold the filter paper over (left) to avoid insects spilling out prior to processing (right).

Processing Yellow Pan Trap Samples

Processing YPT samples should be done locally, and suspect parasitoids you think may be EAB parasitoids must be sent for positive identification. To assist you in sorting the insects in the YPT samples, the Rearing Facility will send reference specimens of each EAB parasitoid species included in YPT kits. Until you are very comfortable identifying the parasitoids, it is a good idea to look through your reference specimens right before processing your pan traps to familiarize yourself with the look and size of the insects you are searching for. While you may be looking at your reference parasitoids under high magnification for detailed identification, also remember to look at your reference parasitoids under the lowest magnification that you will be using as you look through your samples to develop a 'search image'.

Materials needed to sort through samples:

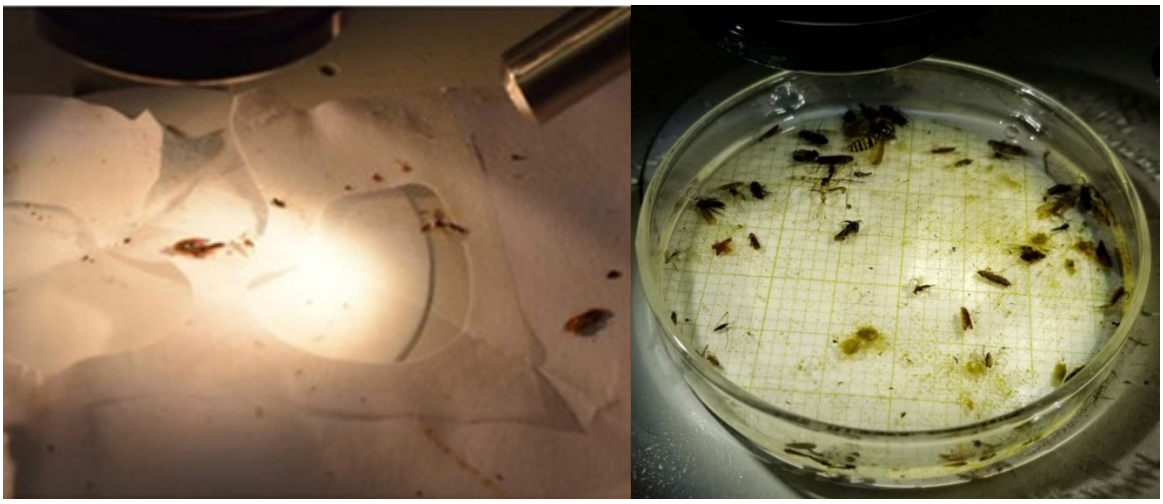
- Dissecting microscope
- Two pairs of fine forceps
- A fine 0, 00, or 000 paintbrush
- 95% ethanol (for preserving specimens)
- Small screw top vials (for specimens that need confirmation ID)
- A glass or plastic Petri dish to hold insect samples during the sorting process
- *Optional* 1% saltwater or 50% ethanol (for going through samples)
- *Optional* A small dropper to help add or remove specimens from the vials
- Datasheet (https://www.aphis.usda.gov/sites/default/files/ypt-recovery-datasheet.xlsx)

Step 1. Prepare your samples for processing.

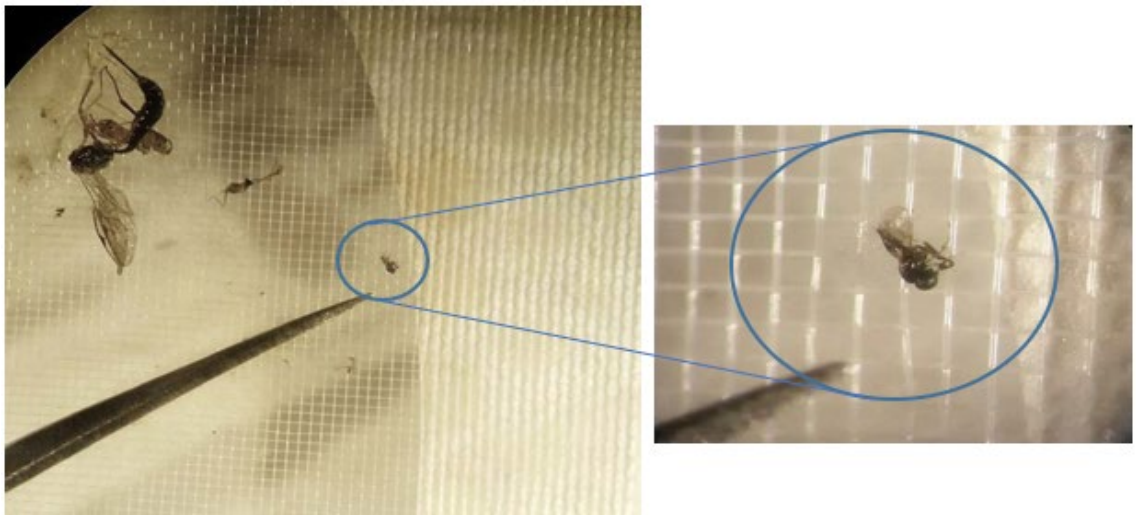
- If you stored your samples in the freezer, give them time to thaw before processing them. Try not to refreeze samples once thawed to help preserve the insects. If you have already thawed them, you can leave the samples in the refrigerator for a day or two while you process them.
- To start, open the zippered bag and record the location, date of sample collection (including year), and YPT ID number on your data sheet. Carefully unfold the filter paper, pull the filter apart at the seam, and dump the contents into a Petri dish. Check the bag and outside of the filter to make sure insects have not slipped out.

Step 2. Sort through insect specimens.

- Systematically sort through the insects caught on the filter under the microscope at the lowest magnification, looking for anything that looks like the EAB parasitoid species (see section below, "Overview of identifying EAB parasitoids in YPTs"). You can use your paintbrush and/or forceps to help manipulate and check the sample. If you find something, look at it under a higher magnification and refer to the section "Overview of identifying EAB parasitoids in YPTs," Appendix C, and your reference specimens from the Rearing Facility.
- Once you finish checking the filter paper, check the Petri dish containing all the larger insects that you dumped out. Systematically go through the sample, sweeping top to bottom and then gradually left to right until you look through the entire sample. We find it helpful to touch each insect and move it aside after determining that it is not a suspect EAB parasitoid. It may be possible for smaller insects to stick to a larger insect or to some of the leaves or debris in your sample, so be sure to flip over insects and leaves and spread out debris to make sure you are not missing any small insects. Be sure to check the legs and tarsi of larger insects as parasitoids often become tangled and trapped there. This commonly occurs with *O. agrili* due to its tiny size.
- You may find it helpful to add a 1% salt solution or 50% ethanol solution to help break up the sample, so everything is not clumped together. Some processors find it helpful to add a grid to the bottom of the dish to help sort through the sample systematically without missing anything. Finally, make sure that you have labelled and filled out your datasheet for that sample, toss away your insect and filter paper, and move onto the next sample.



Left: A filter paper spread out and ready for viewing under the microscope. Right: A sample that has been poured onto a glass Petri dish ready for viewing under the microscope. This sample has alcohol added to help separate the insects and grid paper underneath the dish to help provide reference points for sorting through the sample.



Under lower magnification the insect near the forceps may be a specimen of interest. Readjusting to higher magnification makes it clear that the body shape does not match that of *Oobius agrili*.

Step 3. Preserve suspect EAB parasitoids.

- If you think you have recovered an EAB parasitoid, place it in a vial with 95% ethanol. Please use a vial with a capacity of at least 1 dram. Please do not use vials with very tiny openings since identifiers will need to insert forceps to remove the insects. Do not label the outside of the vial with marker or pen, which will wash off if exposed to ethanol. Include inside the vial a paper label with all the following info written in pencil:

1. MapBioControl Site ID#
2. Collection Date
3. Recovery ID # (This is generated in MapBioControl after you enter a possible recovery on the website. See Step 4 below)
4. Town and State

Step 4. Enter the data into MapBioControl.

(Refer to section *ENTER RECOVERY DATA INTO MAPBIOCONTROL*.)

- The Recovery ID# is generated when you create a recovery entry in MapBioControl. **BEFORE you send your samples, please create your recovery entries in MapBioControl and write the Recovery ID# on the paper in the vial with the insects**.
- Data for **ALL YPTs** at **ONE SITE** for the **SAME COLLECTION DATE** should be consolidated into **ONE** Recovery entry. Trap specific information can be included in the comments section if desired.
- Because you can have more than one vial on a given date at a site, these vials will have the same Recovery ID#. Include the MapBioControl Site ID# with each sample sent for cross reference.

Step 5. Ship your suspect EAB parasitoids for ID confirmation.

- Once you have several specimens for identification, ship them overnight to:

Andrea Anulewicz
USDA APHIS PPQ
5936 Ford Ct, Ste. 200
Brighton, MI 48116
Andrea.Anulewicz@usda.gov

- Ethanol in vials must be poured off prior to shipping due to shipping restrictions, but alcohol will be added back to the vials by the receiver.

Overview of Identifying EAB Parasitoids in YPT Samples

This section provides a basic description of identifying characteristics to look for for each parasitoid. Please see the attached **Appendix C** for detailed check lists that can help you identify each parasitoid. For a more detailed discussion of the parasitoids and how to identify them, please see Module 3 Emerald Ash Borer Release and Recovery Guidelines virtual training. This training also includes a quiz that can be used to test your knowledge.

***Tetrastichus planipennisi* (Eulophidae)**

Tetrastichus planipennisi female. Photo by T. Booth USDA.

Morphological characteristics of *T. planipennisi* include:

- Dark bodied
- Red to dark red eyes
- Light-colored legs with dark femurs
- Smooth thorax with two lateral grooves on middle thoracic segment

Female *T. planipennisi* have the following diagnostic characters:

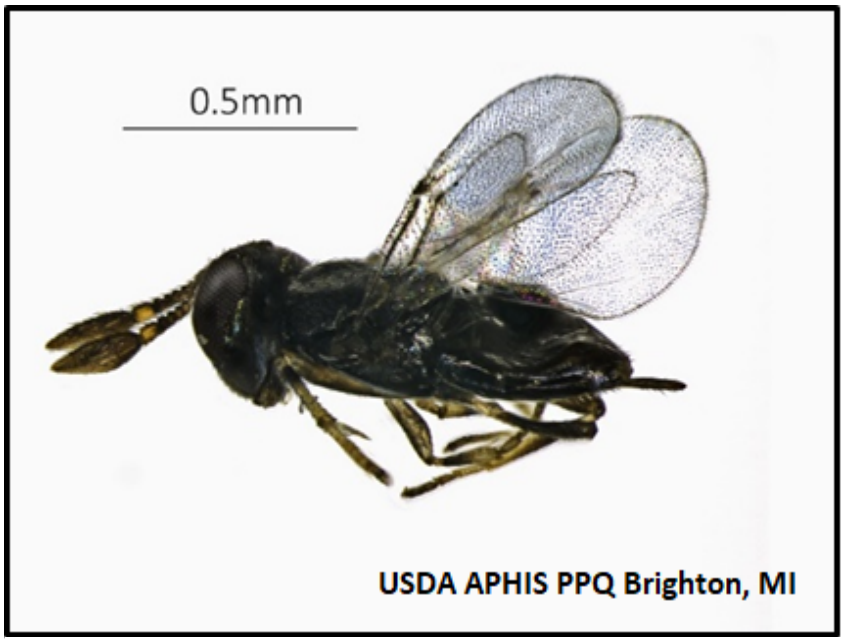
1. 6-segmented antennae
2. Abdomen longer than thorax
3. Last segment of abdomen is 3-4x as long as it is wide at the base

Male *T. planipennisi* have the following diagnostic characters:

1. 7-segmented antennae with clusters of longer hairs that look like ‘eye lashes’
2. Last segment of abdomen not elongated

***Oobius agrili* (Encyrtidae)**

Oobius agrili released for EAB biocontrol are all females. (This species is parthenogenic, i.e. they do not need to mate, and they only produce daughters.)



An adult female *Oobius agrili*.

Morphological characteristics of *O. agrili* include:

- Tiny size (~1 mm long)
- Compact, stout body with no waist
- Visible, short ovipositor
- Dark bodied with blue-green sheen

Oobius agrili can be identified by the following diagnostic characters:

1. Second-to-last antennal segment is light-colored; all other segments are dark
2. Clava (antennal "club") is tapered upwards to a blunt point when the insect is viewed in profile

***Spathius agrili* and *Spathius galinae* (Braconidae)**

Left: *Spathius galinae*. Right: *Spathius agrili*. Photos by T. Booth USDA.

There are many native species of *Spathius* in the United States that can easily be confused for the two released species. The following morphological characteristics will help you distinguish between our introduced parasitoids and native species.

Morphological characteristics of S. *agrili* and *S. galinae* include:

- Reddish brown bodies with dark brown or black abdomen
- Spherical head with spherical eyes
- Back of head smooth, without wrinkles
- Wings with brown veins
- Very long antennae with >26 segments
- Short pedicel (narrow waist connecting thorax and abdomen)
- Long ovipositor (in females)

Spathius galinae can be identified by the following diagnostic characters:

1. Brown-tinged forewings with obvious clear spots
2. Base of all tibia yellowish; 3/5ths base of hind tibia yellow

Spathius agrili looks very similar to *S. galinae*, except the forewings have clear bands rather than clear spots, and the legs and body are generally darker in color.

RECOVERY METHOD: TREE PEELING AND DEBARKING

OVERVIEW:

A. **Sampling for larval parasitoids (*T. planipennisi* and *Spathius* spp.):**
   - Felling trees and peeling logs
   - Peeling standing trees

B. **Sampling for egg parasitoid (*O. agrili*):**
   - Bark scraping cut logs
   - Bark scraping live trees
     - Sheet method
     - Tote method
   - Processing bark samples
     - Rearing bark samples in tubes
     - Sifting bark samples

Peeling and debarking trees to determine parasitoid establishment can be done in the fall, winter, or early spring, at least one year after the final release at a given site. We recommend felling trees for peeling and/or debarking between November and March.

Sampling for Larval Parasitoids (*T. planipennisi* and *Spathius* spp.)

Felling and Peeling Logs to Recover Larval Parasitoids

The USDA has a 5-minute video called “Debarking Ash Tree Logs to Look for Emerald Ash Borer” available on YouTube at: https://www.youtube.com/watch?v=sMV-1r5lnvs&t=9s

If felling trees, select at least four trees within or near where parasitoid releases occurred that are alive (based on bark peeling and confirmation of live phloem), show signs of fresh damage due to EAB (woodpecker holes, bark splits, epicormic shoots), and are less than 10-inches DBH. At least two trees should be “small” trees under 4-inches DBH to capture *T. plannipennisi*. Give each tree a unique ID number and record its DBH, location (GPS coordinates), and the date the tree was felled. We generally cut these trees into 1-meter logs to make them easier to handle and peel.

Below is a guide to peeling ash logs to look for EAB. If you have questions about tree peeling or want some additional visual resources, please contact Theresa.C.Booth@usda.gov .

An ash log with the bark peeled back, revealing EAB galleries.

Materials needed for tree peeling:

- Safety glasses
- Cut-proof gloves
- 9" drawknives
- 5" small drawknives
- Small knives and/or chisels
- Hammer to be used with a chisel to help dig into the wood for J-larvae (optional)
- Face shield or bandana for dust (optional)
- Peeling stand/table (good for large logs)
- Shave mule/jawhorse/C-clamps (good for smaller logs or branches)
- *Optional* Datasheet (https://www.aphis.usda.gov/sites/default/files/tree-peeling-datasheet.xlsx)

Step 1. Peel the ash logs.

- Logs are easiest to peel if debarked soon after felling.
- If you need to store the logs, try to store them in a way that reduces moisture loss.

You can keep them in a cold chamber and/or store them in a barrel of water or seal the ends (ex. with Anchorseal®) to reduce moisture loss.

- It is easiest to peel using a large 9-15” drawknife.
- If the bark is too thick, you can remove the outer bark with a drawknife first. If you find an EAB gallery, carefully remove the phloem in that specific area. For more precision, use a sharp chisel, small knife, or small 5” drawknife.
- The phloem will easily separate from the outer sapwood when the ash logs are fairly fresh. Phloem and layers of outer sapwood will be harder to remove in areas where the tree is dead and dried out, but EAB and larval parasitoids may still be present in these areas.

(Left) A peeling table. (Right) A peeling stand.

Step 2. Inspect all EAB galleries.

Examine the end of each gallery for EAB larvae or signs of parasitism. (See Appendix B for photos.) You may see the following fates:

- **EAB larva parasitized by *Tetrastichus*, which may:**
  1. look healthy.*
  2. appear lumpy, like a “braided rope.”
  3. be replaced by a mass of small grub-like larvae (white), pupae (color ranges white to bluish black) and/or adults (dark metallic blue).
  4. have emerged from the EAB gallery, leaving only the head and tail of the EAB larva and small black spots in the gallery called meconium (the spots are waste excreted by *T. planipennisi* larvae before pupation is complete). The EAB larva head and tail may not always be visible.

**Tetrastichus planipennisi* larvae remain inside the EAB larva for at least a week before emerging. If you have access to a laboratory, you may find more parasitized larvae if you place EAB late-instar and J-larvae in a petri dish and dissect them. If parasitized by *T. planipennisi*, many small eggs and/or larvae can be seen within in the EAB hemolymph. Note: EAB larvae can be saved in labeled petri dishes, stored in the fridge, and dissected weeks later.

- **EAB larva parasitized by *Spathius*.** All *Spathius* life stages live on the outside of the host. *Spathius* eggs and small larvae are difficult to see with the naked eye. By late fall, most will be large larvae or will have spun multiple silken cocoons that are fairly easy to see within the EAB galleries.
- **EAB larva parasitized by a single solitary larva or cocoon.** An EAB parasitized by a single larva or cocoon is probably *Atanycolus* (native parasitoid) or *Balcha indica* (naturalized parasitoid) (Appendix F). You do not need to ship these parasitoids.
- **A healthy EAB larva.** If you find a healthy EAB larva that is a small instar (1st or 2nd) it does not need to be dissected. If you find a healthy EAB larva that is a larger instar (3rd, 4th, or J-larva) you can dissect it for signs of internal parasitism. A J-larva is not going to be visible until you chisel into the wood of the tree.
- **An empty gallery where a woodpecker has taken the larva.** Use a Sharpie to mark woodpecker holes before peeling so you know why the gallery you've peeled is empty. Recording data on woodpeckered EAB is not required.
- **A dead EAB larva.** EAB can die for a variety of reasons including tree defenses, fungi, viruses, or extreme cold.

Step 3. Extract and ship suspected parasitoids.

- If you find a potentially parasitized EAB or parasitoid clutch, carefully remove the EAB larva and any parasitoid eggs, larvae, and pupae/cocoons, and place them in a small petri dish with a friction-fitting lid (Fisher Scientific 50 x 9-mm dishes – catalog no. 08-757-105 is a good choice) or a vial with a capacity of at least 1 dram. Please do not use vials with very tiny openings since identifiers will need to insert forceps to remove the insects.
- Save each clutch of parasitoids and/or each parasitized EAB larva in a separate vial or petri dish.
- Once you enter the data into MapBioControl, it will generate a Recovery ID #. **Please include that Recovery ID # on shipments you send.** This number will be used by the identifier to enter the parasitoid ID into MapBioControl.
- **Using a pencil**, label a small piece of paper with the state, Recovery ID #, Site ID #, tree number, and date (Use Day-Month-Year in format: DD-MMM-YYYY; ex.10-Jul-2023) and place the paper into the vial or petri dish. Pencil is preferred as pen and

Sharpie can be washed away by ethanol.

- Ship the specimens for identification within 48 hours to:

  Andrea Anulewicz
  USDA APHIS PPQ
  5936 Ford Ct, Ste. 200
  Brighton, MI 48116
  Andrea.Anulewicz@usda.gov

  If you need to keep samples longer before shipment, add 95% ethanol to preserve the specimens and then pour off the ethanol before shipment.
- You may also wish to take photos of each parasitoid clutch and/or any suspect signs of *T. planipennisi* meconium and email those photos to Andrea Anulewicz.

Step 4. Enter the data into MapBioControl.

(Refer to section *ENTER RECOVERY DATA INTO MAPBIOCONTROL*.)

- Once you enter the data into MapBioControl, it will generate a Recovery ID #. **Please include that Recovery ID # on shipments you send.**
- Peeling 4 trees at one release site will count as **one** recovery entry.
- Record your peeling event and if you have found potential parasitoids. **Please make a record on MapBioControl even if you do not find parasitoids**. Negative data is just as important as positives. If you found parasitoids, please note in the comments how many potentially parasitized clutches you are shipping.

***Note: Count the number of EAB larvae that were parasitized, not the number of parasitoids in each gallery. Each group of parasitoids in a single EAB gallery counts as ONE clutch. Please ship each clutch in a separate vial or petri dish.**

Peeling Standing Trees for Larval Parasitoids

This is a modified version of the previous section for if you want to peel trees without felling them. You will need the same materials and follow the same methods as above, but peel only on the sections you can reach. If peeling standing trees, debark the lower 2 m of the trees. **Sample at least four trees** but consider sampling more since you are only peeling the lower part of the tree, especially if your initial four trees do not yield parasitoids.

Sampling for Egg Parasitoid (*Oobius agrili*)

Choosing Promising Trees

Bark sampling can recover EAB eggs that may be parasitized by *O. agrili*. It is not worth sampling for EAB eggs if the log shows no signs of fresh EAB infestation. If you are unsure about a tree or log, you can peel a small section to see how many galleries are underneath the bark.

- You are more likely to find EAB eggs by sampling a part of the tree trunk that shows signs of recent EAB infestation and is still alive (i.e. the bark is still attached to the sapwood). Fresh woodpecker holes or blonding and/or live epicormic shoots are a great indicator of recent EAB infestation. If scraping a standing tree, it is recommended that trees have at least 5-10 fresh woodpecker holes within the first 3-4 meters above the ground (but the more the better).
- Choose an area with furrowed or flaky bark (smooth bark will not have many eggs).

To help reduce the burden of sifting bark samples, some collaborators have noted that they avoid peeling bark areas covered in moss/lichen because this adds a ton of debris that makes sorting those samples take significantly longer.

Bark Scraping Cut Logs

Bark samples can be taken from ash trees felled for other recovery work, but we recommend sampling at least 10 trees per site. So, if you are already felling four trees for larval parasitoid recovery, we recommend taking bark samples from additional trees that you do not fell, to reach 10 total. We suggest taking one to two bark samples per tree from a section of the tree that is likely to have EAB eggs (see above section, "Choosing Promising Trees").

From each log section:

1. Mark off a vertical area of bark 10 x 100 cm.
2. Lay the log down and use a draw knife to carefully shave the outer layer of bark into the tray. A non-plastic tray is best to avoid static that can affect bark and EAB eggs, but a plastic tray can be used if treated with anti-static spray or a dryer sheet.
3. Pour each bark sample into a large paper lunch bag (Masking tape the bottom of the lunch bag to prevent bark/eggs from spilling out).

4. Label the bag with the date, site, state, log number, and DBH.
5. Rear and/or sift the bark samples as described below.

Bark scraping a cut log for *Oobius agrili*. Photo by W. Davidson, NH Department of Forest and Lands.

Bark Scraping Live Trees

You can also take bark samples from living, EAB-infested ash trees in the vicinity of the original release site in the field (see above section, “Choosing promising trees”).

To sample trees in the field, mark off a vertical area of bark 10 x 100 cm on the south, southwest, or west side on the lower trunk (about 1 m above ground) on at least 10 trees.

To collect the bark samples, use one of the following methods:

Sheet method (requires two people):

1. Wrap a piece of heavy plastic sheeting around the base of the tree and secure it with duct tape. Have one person hold the edges of the sheeting up in the shape of an inverted cone.
2. Using a drawknife, the other person shears off a thin layer of outer bark within the delineated area, and the bark debris will fall into the inverted plastic cone.

3. Remove the duct tape and, using the plastic sheeting, funnel the bark sample into a paper lunch bag (reinforce the bottom of the lunch bag with masking tape to prevent bark/eggs from spilling out).
4. Label the sample with the date, site, state, tree number, and DBH.

Tote method:

1. Modify a 12-to-18-gallon plastic tote:
   a. Cut the lip on one side of the tote and put duct tape over the cut edge. This will allow the tote to sit flush against the tree with no gap.
   b. Add extra duct tape around the corners to reinforce them. The corners are the points most likely to weaken and break from pushing against the tree.
   c. Cut two small holes on either side of the tote. Attach a rope to hold the tote around your neck while scraping.
2. In the field, press the tote into a suitable tree so that it sits flush against tree.
3. Using a drawknife, shear off a thin layer of outer bark within the delineated area. Slice off multiple shallow layers of bark until the entire area has been debarked.
4. Once a sample has been collected, dump it into a paper bag (reinforce the bottom of the lunch bag with masking tape to prevent bark/eggs from spilling out). Brush any residual bark from the tote into the paper bag.
5. Label the sample with the date, site, state, tree number, and DBH.

Bark sampling using the sheet method.

Bark sampling using the tote method.

Optional: Toby Petrice has designed a drawknife shield (green item in picture above) to help with bark scraping. If you're interested in this design, you can contact Toby Petrice for more details at toby.petrice@usda.gov.

Processing Bark Samples

Once you collect your bark samples, you have two options for finding *Oobius agrili* eggs:

- Keep samples in tubes to rear out any overwintering *O. agrili* adults that have not yet emerged.
- Sift samples to look for all EAB eggs, including any that were parasitized by *O. agrili*.

Only a small number of parasitized EAB eggs will contain overwintering *O. agrili* that haven't yet emerged, so a larger number of bark samples should be collected if you do not plan to sift the bark after rearing. **If you are only rearing bark samples and not sifting, we recommend taking 30 bark samples per site.** If you are sifting, we recommend taking 10 bark samples per site.

Rearing Bark Samples

Oobius agrili overwinters inside EAB eggs as mature larvae and emerges in the spring. Bark samples can be reared in containers (i.e. rearing tubes or barrels) to collect adult *O. agrili* that may be overwintering in EAB eggs. These rearing containers can be constructed from cardboard poster tubes with tight-fitting plastic plugs.

Materials needed for bark rearing tubes:

- 4" diameter poster tubes cut 10-12" long with tight fitting plastic plugs for each end
- Black spray paint designed to adhere to plastic
- 4.5 oz. clear specimen cup with lid
- Small clear funnel (large end 2" and funnel end 0.25" in diameter)
- Hot glue gun
- 1.5" diameter hole saw bit for drill. Alternatively, a sharp knife and extreme care.

Construction and collection from bark rearing tubes

1. The outer surface of plugs should be spray painted black. This will reduce light transmitted and direct emerging adult parasitoids to the opening.

2. Cut a 1.5”-diameter hole in the center of one of the plugs.
3. Cut a 1.5”-diameter hole in the cap of a 4.5 oz. specimen cup.
4. Glue the larger end of the funnel to the bottom side of the specimen cup lid (side with threads) using hot glue.
5. Glue the top of the specimen cup lid to the outside surface of the poster tube plug, centering the holes that were drilled. Make sure there are no tiny gaps between the plug surface and cap surface that would allow these small parasitoids to escape.
6. Screw the specimen cup onto the cap.
7. Check cups at least every other day for adult emergence. If parasitoids are present, you can squirt them with ethanol to prevent them from escaping and use a small brush to place them in vials with ethanol.
8. Hold bark in rearing containers for at least 6 weeks at room temperature to allow all parasitoids to emerge.

Sifting Bark Samples

1. Dry the sample for at least one month at room temperature. (If the bark was already processed for rearing *O. agrili* it will already be dry.)
2. Place small portions of bark in a No. 14 covered soil sieve and shake vigorously for several minutes to sieve EAB eggs, small insects, and other small debris from the bark sample into a white ceramic pan. (Ceramic is recommended because it eliminates static electricity that makes working with eggs difficult.)
3. Using a dissecting microscope to sort through each bark sample, we recommend following the method described by the Minnesota Department of Agriculture. Their presentation, which includes many photos of parasitized EAB eggs and look-alikes, can be found at: Biological Control of Emerald Ash Borer: Bark Sifting for Oobius agrili.


(Left) A No. 14 covered soil sieve for sorting bark. (Right) A white ceramic baking pan with sifted bark ready for viewing under a dissecting microscope.

If you find EAB eggs or egg parasitoids:

- Collect all EAB eggs into a small Petri dish with a friction-fitted lid (Fisher Scientific 50 X 9-mm dishes – catalog no. 08-757-105 is a good option).
- Collect adult parasitoids into a vial with 95% ethanol (ethanol needs to be poured off before shipping).
- Label petri dishes with state, Recovery ID #, Site ID #, and date of collection (DD-MMM-YYYY, ex. 05-Feb-2024) using a fine-tip permanent marker. For ethanol vials, include information on a small slip of paper inside the vial (written in pencil to avoid smearing).
- Ship the EAB eggs and parasitoids collected from bark sifting or reared from bark or logs to:

Toby Petrice
US Forest Service
2601 Coolidge Rd, Ste. 203
East Lansing, MI 48823

RECOVERY METHOD: BARREL EMERGING FELLED LOGS OR BRANCHES*

Peeling logs or trees is a great way to directly measure EAB density and parasitoid presence/absence but takes a significant amount of time to complete. For a passive recovery method that is less time-intensive, you can emerge parasitoids from cut logs or branches in barrels. This method can theoretically be used for all parasitoids, but has had the best success with larval parasitoids. Consider bark scraping after barrel emergence to look for *Oobius agrili* if you do not find any during the emergence period.

**This method is still in development. If you've had success with barrel emergence, please consider sending feedback to Theresa.c.booth@usda.gov for incorporation into these recovery guidelines.*

Choosing Promising Trees

- Trees must be **alive** but infested with EAB. The higher the larval density, the better. Try to select trees around **4-6 inches DBH.**
- Smaller trees are more likely to host *Tetrastichus planipennisi* and are easier to fell, transport, and fit into barrels.
- Look for signs of infestation (fresh woodpecker feeding holes in winter or crown dieback, epicormic shoots, and fresh woodpecker feeding holes in spring/summer/fall).
- Make sure the tree is alive. You can **use a drawknife to peel back bark** to confirm the tree is alive and/or to assess infestation levels.
- You only need to take sections of the tree that show fresh signs of EAB infestation. If infestation is near the crown but the base is clean, skip collecting the lower logs. If the top is dead but the lower trunk is heavily infested, leave the dead portion and harvest only the live infested material.

Note: You may have to scout multiple parts of your site to locate suitable trees. The extra effort to find suitable trees yields better parasitoid recovery.

How to Build Barrels

- You can use any large barrel (25 to 55 gallons). Larger barrels can store more logs.

- Attached a small, transparent container with a lid (like a Mason jar) to the barrel lid to collect parasitoids.
    - To attach the collection container, cut a hole through the container lid so that the parasitoids can enter the container. Then secure the container lid to the barrel so the container can be removed to collect the parasitoids. See above section, "Rearing bark samples for *Oobius agrili*" for some suggested materials and methods.

Examples of how collection containers have been attached to barrel lids to collect emerging parasitoids.

Barrels being stored in a rack.

Storage Conditions

- Leave the logs laid out for a few weeks to allow them to dry and prevent mold proliferation in the barrel. Make sure logs are labelled with the date, site information, and tree information for tracking parasitoid emergence.
- Place logs into barrels, giving enough space that parasitoids can still emerge between logs. Avoid tightly packing the barrel.
- Barrels can be stored and stacked horizontally to take up less space. You can buy a commercial barrel or drum rack or build one.
- Ideal ambient temperatures for emergence are 75-80˚F with 65-75% humidity. Room temperatures between 70-72˚F will allow parasitoid emergence, but at a slower rate and reduced number.

Labeling and Monitoring

- Label barrels with the date, site information, and tree information for tracking parasitoid emergence.
- Check barrels at least once per week for parasitoid emergence.
- Monitor emergence for at least 6 weeks at room temperature or through the next field season (until the end of August) if kept at outdoor temperatures.
- Collect the contents of the barrels after you remove the logs to ensure you get everything that didn't make its way into the collection jar. Shake the barrel out onto a large metal tray, then transfer loose material to a jar for sorting through.
- Optional: Logs can be peeled 3-4 weeks after attempting emergence to look for parasitoids stuck under the bark.

Collecting Parasitoids

- Collect parasitoids and ship at least a few adults from each emergence to the Rearing Facility for ID. The rest can be re-released if desired.
- Label the adults with state, Recovery ID #, Site ID #, and date of collection (DD-MMM-YYYY, ex. 05-Feb-2024). The collection date should be the date the trees were recovered from the field.
- Specimens should be sent to:

Andrea Anulewicz
USDA APHIS PPQ
5936 Ford Ct, Ste. 200
Brighton, MI 48116
Andrea.Anulewicz@usda.gov

ENTER RECOVERY DATA INTO MAPBIOCONTROL

Enter all recovery data, INCLUDING ALL NEGATIVE DATA ('zero' entries where EAB parasitoids were not recovered). 'Zero' data is extremely important because we need to know if parasitoids are not establishing at release sites.

Once you have completed surveys to detect established parasitoids, enter the data on the MapBioControl website or using the mobile app **before shipping samples for identification.** Once you make a recovery entry, MapBioControl will give that entry a Recovery ID #. It is the first number on the Parasitoid Recovery page, under "ID." **Please include this Recovery ID # in any samples you ship so that we can match it up to the appropriate record.**

To enter data on the website, go to www.mapbiocontrol.org and click on RECOVERY in the blue banner at the top. Click the 'New' button to enter new data. You will be asked to enter the following data **(* indicates required information):**

Identification Information

- **Site ID:** Enter the MapBiControl Site ID for your site. If you find parasitoids not connected with a particular site, leave this blank.
- **Trap ID:** If you wish to record which trap collected the insects for your own information, you can enter the trap number here.

Location Information

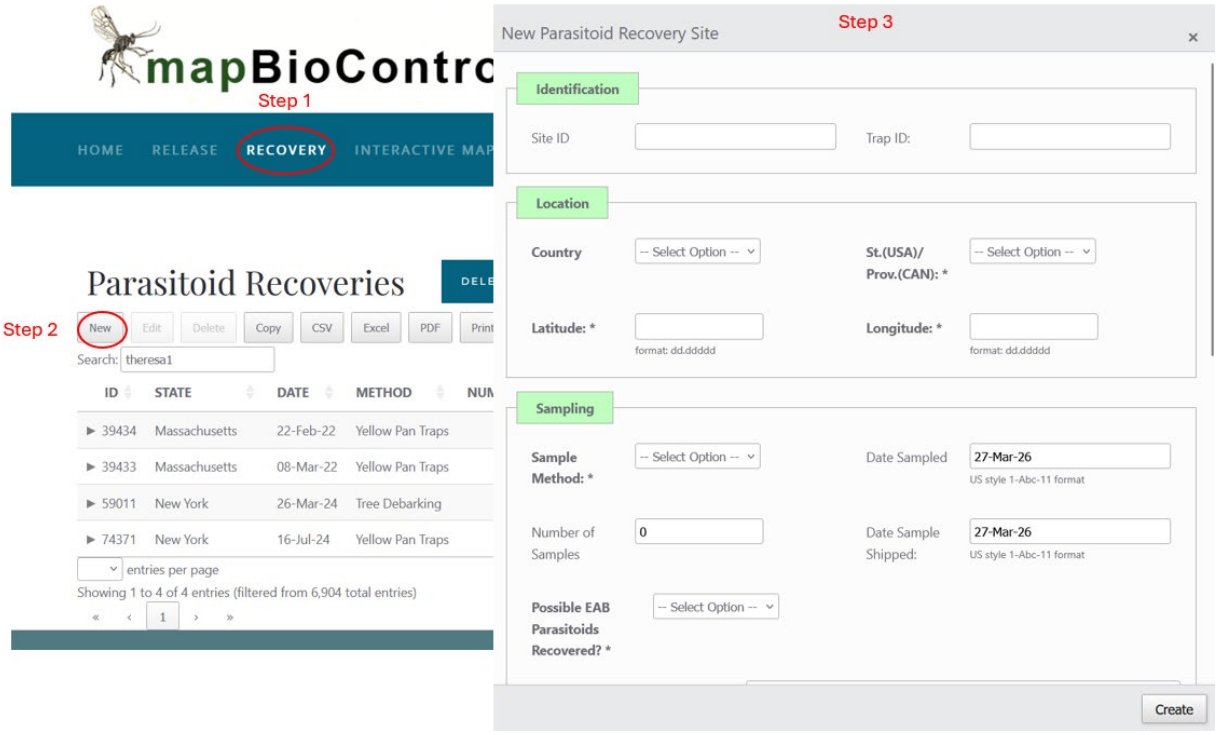
- **Country***
- **State(USA)/Province(Canada):***
- **Latitude ***(dd.ddddd): You may enter the LAT/LONG from your Site ID if you don't have specific recovery LAT/LONG.
- ***Longitude** (dd.dddd): You may enter the LAT/LONG of your Site ID if you don't have specific recovery LAT/LONG.

Sampling Information

- **Date sampled:** In format DD-MMM-YY, Ex. 20-JUN-23
- **Sampling Method*:** (Bark in Tubes, Bark Sifting, Burlap Trap, Egg Collection, Logs in Tubes, Sentinel Eggs, Sentinel Larvae, Tree Debarking, Yellow Pan Traps, Other)

- **Number of Samples:** If 'Yellow Pan Traps', enter the number of YPTs you sampled that week at that site (usually 15). If 'Tree Debarking', 'Logs in Tubes', 'Bark in Tubes', 'Egg Collection', or 'Bark Sifting' enter the number of trees you sampled.
- **Date Sample Shipped**: In format DD-MMM-YY, Ex. 05-FEB-24. Leave blank if no parasitoids were recovered.
- **Samples Shipped to**: Who you shipped your suspect parasitoid samples to. This will most commonly be **Andrea Anulewicz**. Leave blank if no parasitoids were recovered.
- **Possible EAB Parasitoids Recovered?* '**Yes' if shipping suspected samples. 'No' if no suspect parasitoids were found/shipped. If needed, this field will be updated by the identifier after processing samples.
- **Comments:** It is very helpful if you write which parasitoids you thought you recovered in this field. Ex. "Suspect 2 *T. planipennisi* and 3 *O. agrili*". This will help the identifier determine what they are looking for in the vial.

NOTE: The actual number of parasitoids recovered can only be entered into the database by program identifiers, but you can write what you suspect in the comments.



PUBLICATIONS ON RECOVERY METHODS

Petrice, T.R., Bauer, L.S., Miller, D.L., Stanovick, J.S., Poland, T.M. and Ravlin, F.W., 2021. Monitoring field establishment of the emerald ash borer biocontrol agent *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae): Sampling methods, sample size, and phenology. *Biological Control*, *156*, p.104535. https://doi.org/10.1016/j.biocontrol.2021.104535

Petrice, T.R., Poland, T.M., Bauer, L.S., Strazanac, J.S., Duan, J.J., Schmude, J.M. and Ravlin, F.W., 2023. Factors affecting yellow pan trap captures of the emerald ash borer biocontrol agents *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae): implications for monitoring establishment and seasonal abundance. *Biological Control*, *184*, p.105272. https://doi.org/10.1016/j.biocontrol.2023.105272.

Rutledge, C.E., Van Driesche, R.G. and Duan, J.J., 2021. Comparative efficacy of three techniques for monitoring the establishment and spread of larval parasitoids recently introduced for biological control of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). *Biological Control*, *161*, p.104704. https://doi.org/10.1016/j.biocontrol.2021.104704

Parisio, M. S., Gould, J. R., Vandenberg, J. D., Bauer, L. S., & Fierke, M. K. (2017). Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer. Biological Control, 106, 45-53. https://doi.org/10.1016/j.biocontrol.2016.12.009

Jennings, D. E., Duan, J. J., & Shrewsbury, P. M. (2018). Comparing Methods for Monitoring Establishment of the Emerald Ash Borer (*Agrilus planipennis*, Coleoptera: Buprestidae) Egg Parasitoid *Oobius agrili* (Hymenoptera: Encyrtidae) in Maryland, USA. Forests, 9(10). https://doi.org/10.3390/f9100659

Abell, K. J., Bauer, L. S., Duan, J. J., & Van Driesche, R. (2014). Long-term monitoring of the introduced emerald ash borer (Coleoptera: Buprestidae) egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae), in Michigan, USA and evaluation of a newly developed monitoring technique. Biological Control, 79, 36-42. https://doi.org/10.1016/j.biocontrol.2014.08.002

Spathius agrili

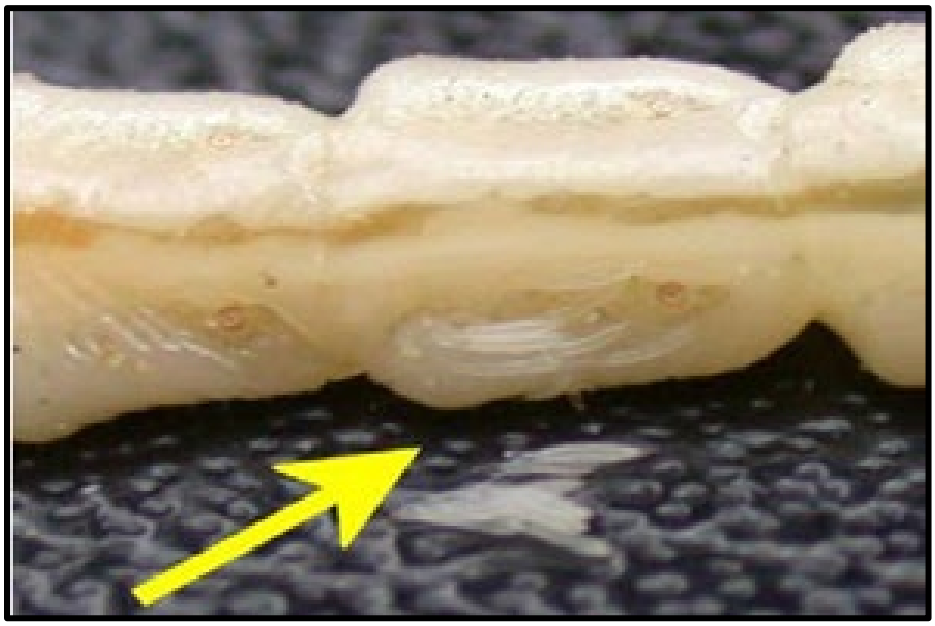


Figure B1. *Spathius agrili* eggs, indicated by an arrow, laid on the surface of an EAB larva.

Figure B2. Larvae of *Spathius agrili* feeding externally on an EAB larva.

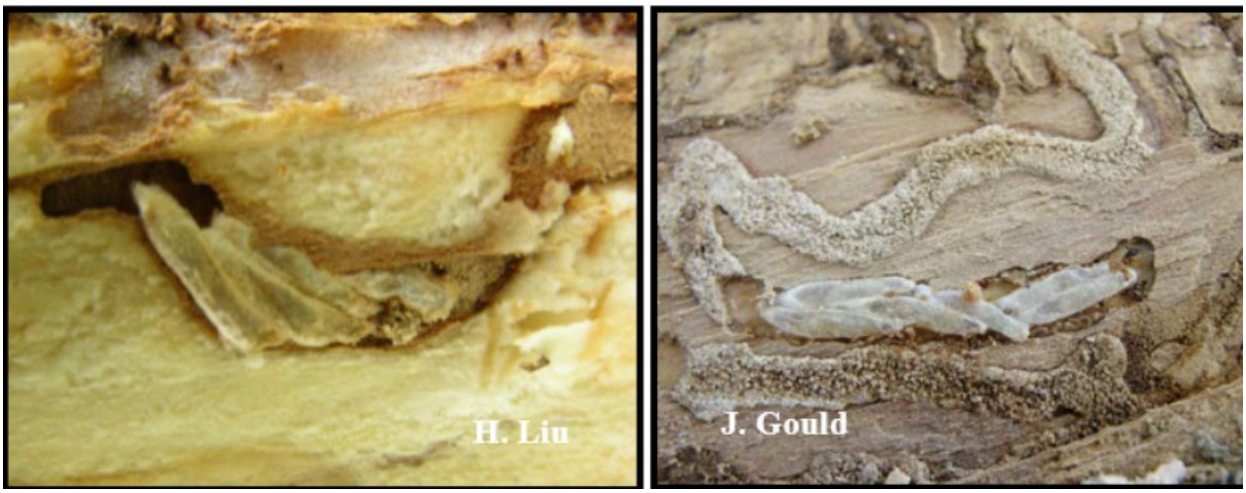


Figure B3. Two photos of silken cocoons of *Spathius agrili* in the host gallery of EAB. The cocoons contain mature larvae or pupae of *S. agrili*.

Figure B4. Female *Spathius agrili* laying eggs through ash bark onto an EAB larva.

Spathius galinae

Figure B5. *Spathius galinae* cocoons in one gallery at the top of the photo and *S. galinae* larvae in another gallery at the bottom of the photo.

Figure B6. *Spathius galinae* adult female.

Tetrastichus planipennisi

Figure B7. Immature *Tetrastichus planipennisi* larvae inside an EAB larva.

Figure B8. Mature *Tetrastichus planipennisi* larvae inside an EAB larva.

ha

Figure B9. *Tetrastichus planipennisi* larvae that have emerged from the host. These larvae remain and pupate in the EAB gallery.

Figure B10. *Tetrastichus planipennisi* larvae and pupae in an EAB gallery. Larvae of *T. planipennisi* develop asynchronously, and larvae and pupae are often found together inside one EAB gallery.

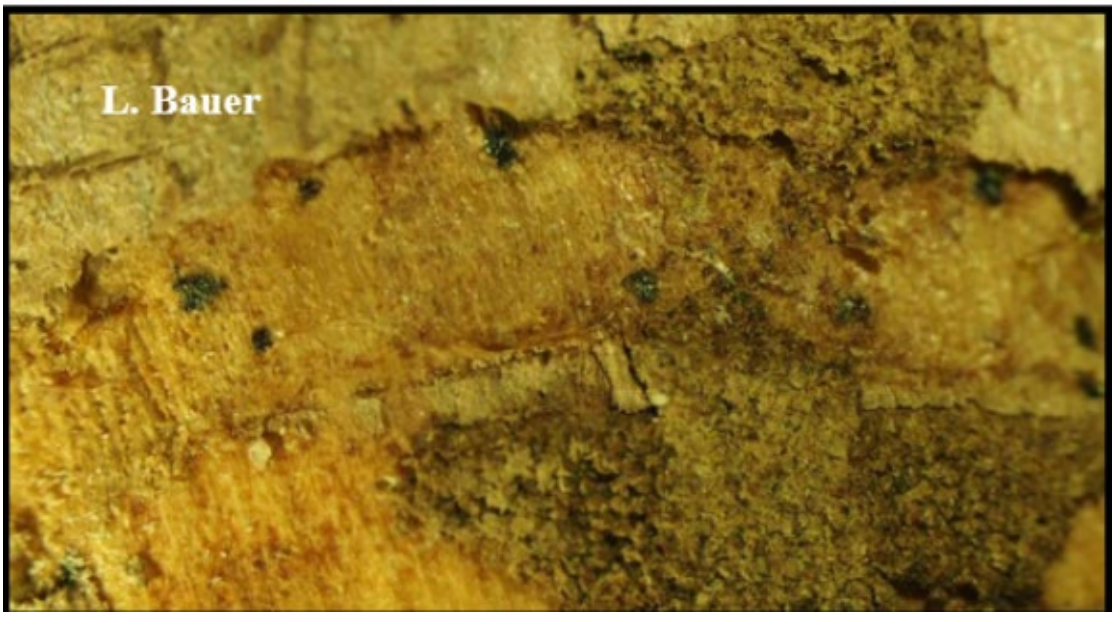


Figure B11. *Tetrastichus planipennisi* meconia (waste) in an EAB gallery. The meconia appear as black spots in the empty EAB gallery after adult emergence is complete.

Figure B12. *Tetrastichus planipennisi* female lays eggs in an EAB larva through ash bark.

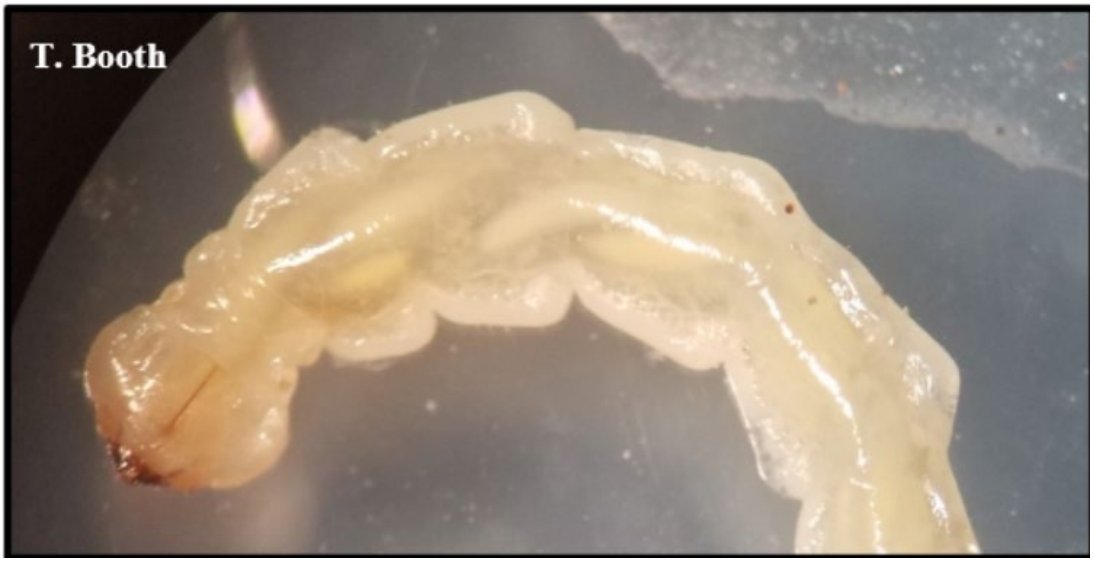


Figure B13. *Tetrastichus planipennisi* larvae are visible swimming within the EAB hemolymph under the microscope.

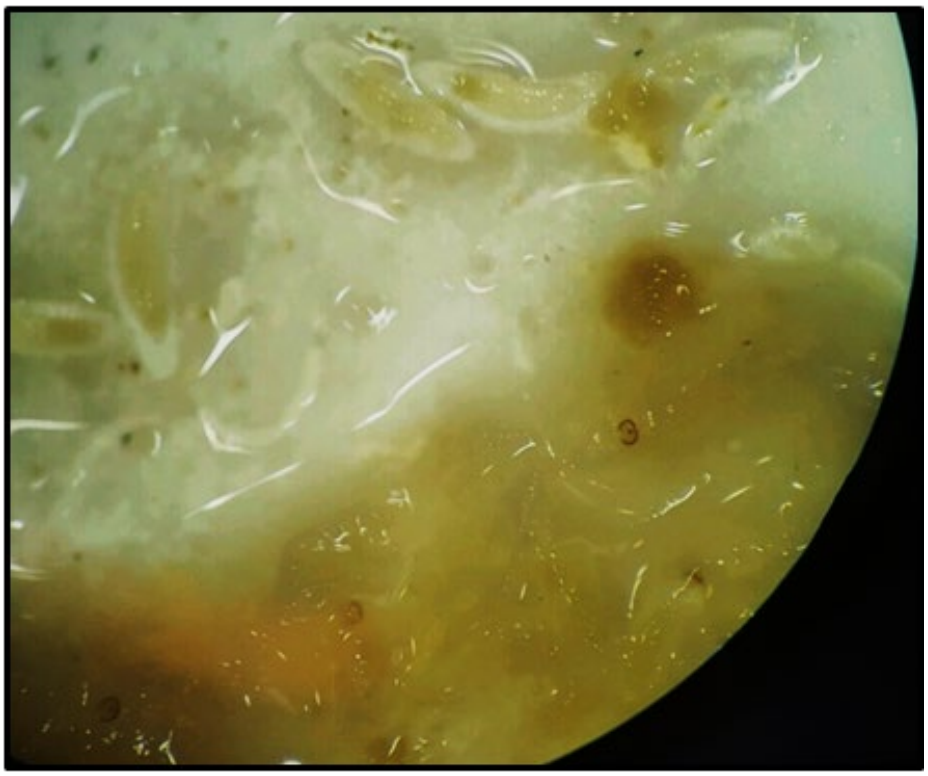


Figure B14. Dissected EAB larva with visible *Tetrastichus planipennisi* larvae under the scope. Use caution when searching for parasitoid larvae or eggs as dissected EAB larvae may have bits and particles that look superficially like parasitoid larvae or eggs.

Oobius agrili

Figure B15. EAB eggs often turn dark brown (left egg) or black (right egg) when parasitized by *Oobius agrili*; fresh EAB eggs will start off white and turn amber (center egg) in color as it develops if not parasitized by *O. agrili*.

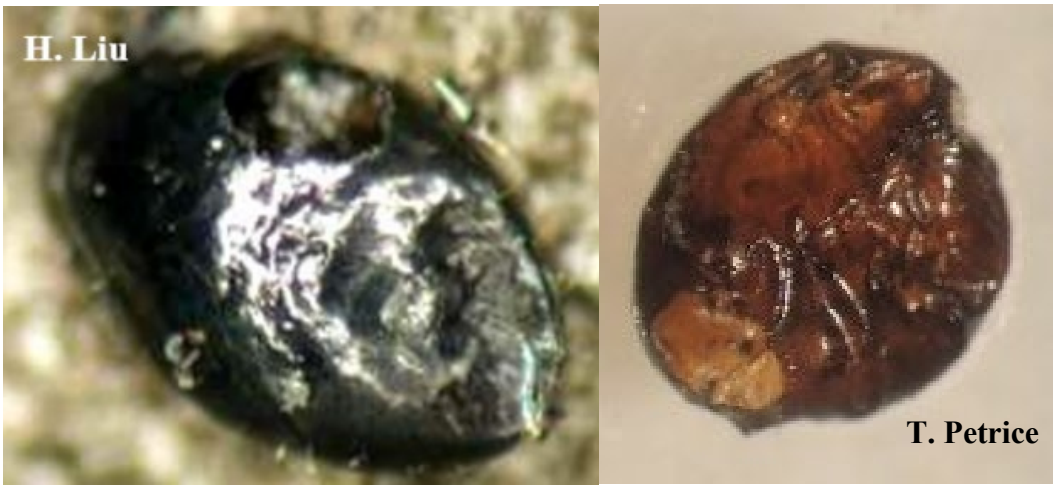


Figure B16. Two EAB eggs, each showing an *Oobius agrili* exit hole. Adult *O. agrili* chew a circular hole through the EAB eggshell and emerge.

Figure B17. *Oobius agrili* female parasitizing an EAB egg laid on ash bark.

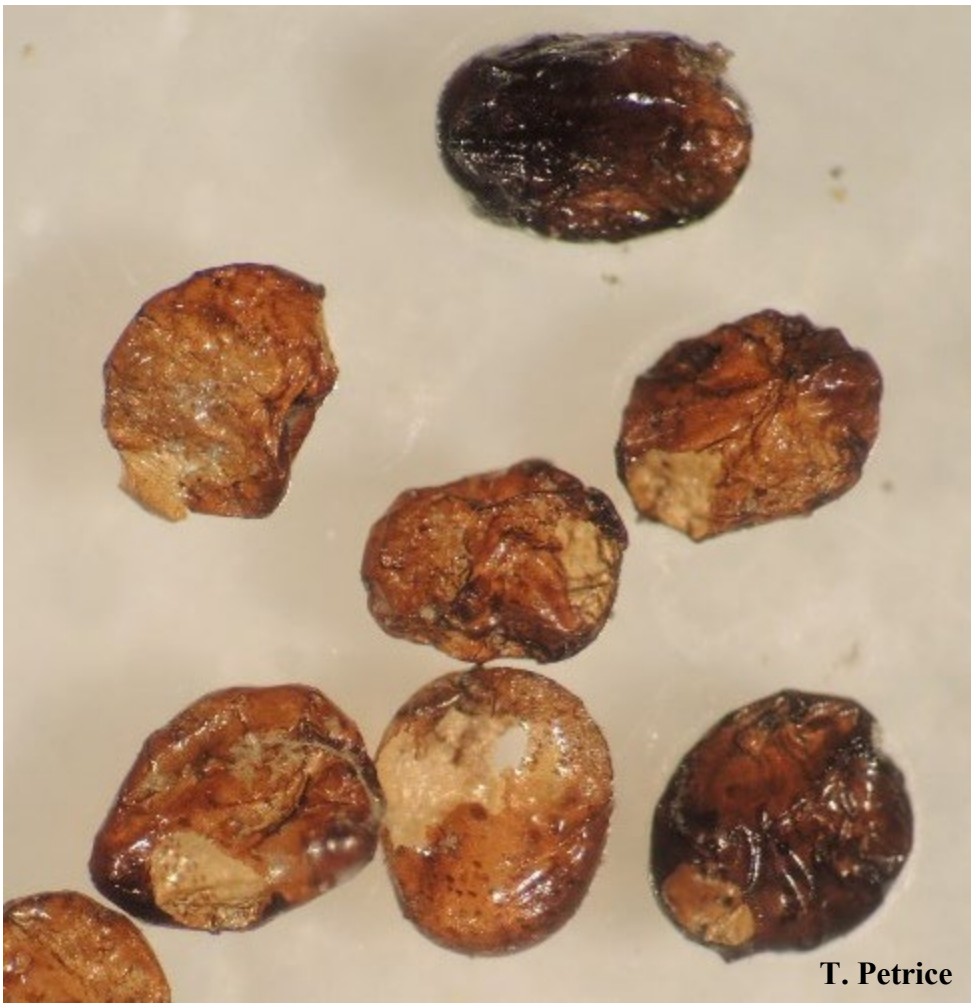
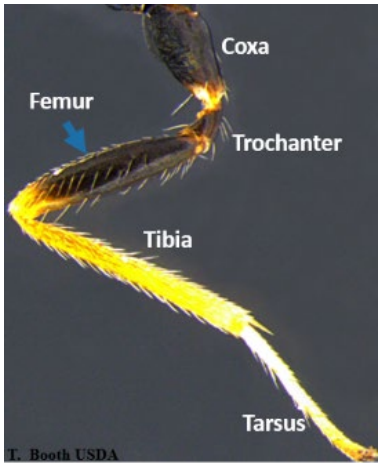


Figure B18. EAB eggs parasitized by *Oobius agrili* and collected from the field. Eggs collected from the field are often less dark in appearance than parasitized eggs produced under laboratory conditions.

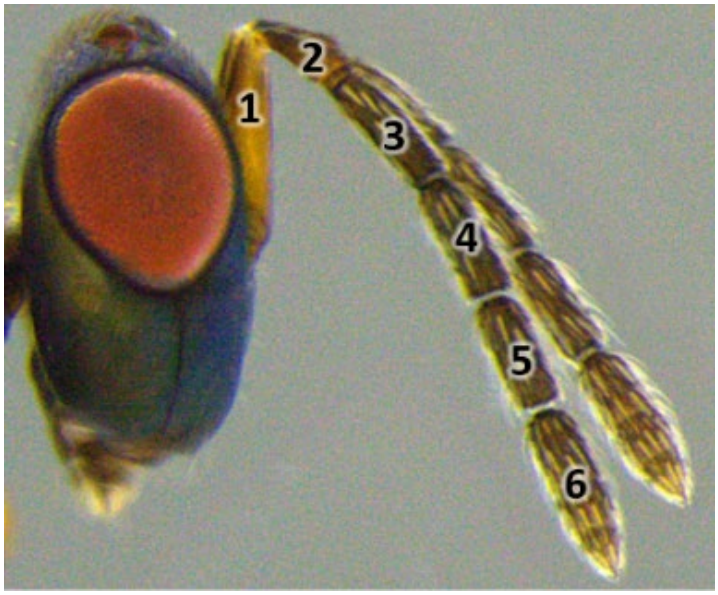
Tetrastichus planipennisi female checklist

Tetrastichus planipennisi females can be identified by five characteristics: 1) dark femurs, 2) 6-segmented antennae, 3) abdomen that is longer than the thorax, 4) last segment of abdomen that is 4x as long as it is wide at the base, 5) smooth thorax with two lateral grooves on the middle thoracic segment. Depending on the condition of your specimen, some characteristics may be hard to see.

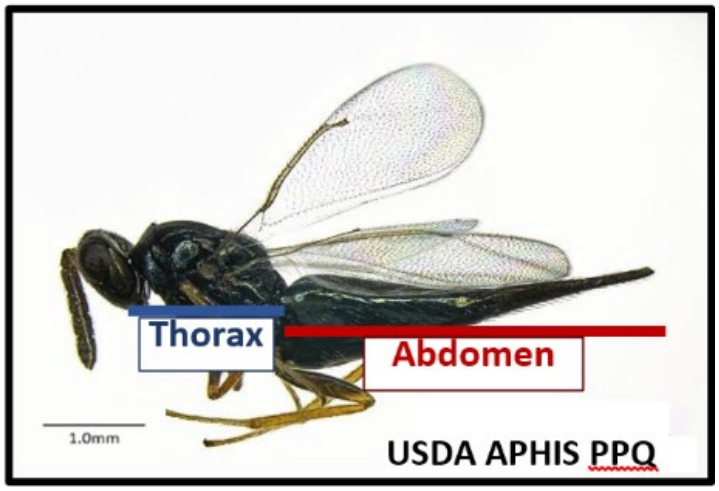
1) **Dark femurs (see arrows).**



2) **6-segment antennae: scape (1), pedicel (2), three funicles (3-5) and clava (6).**

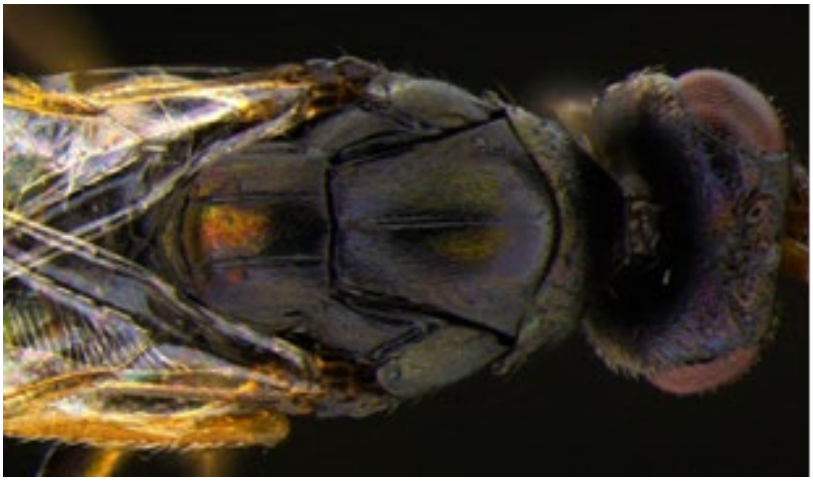


3) Abdomen longer than thorax.



4) Last segment of abdomen four times as long as it is wide at base.

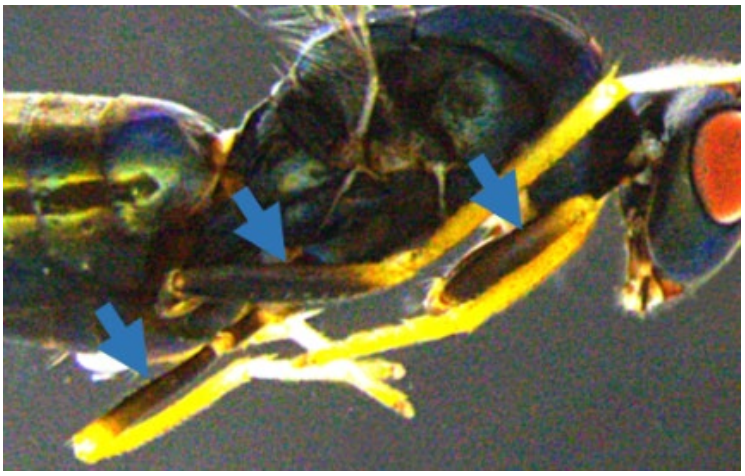
5) Smooth thorax with two lateral grooves on the middle thoracic segment.

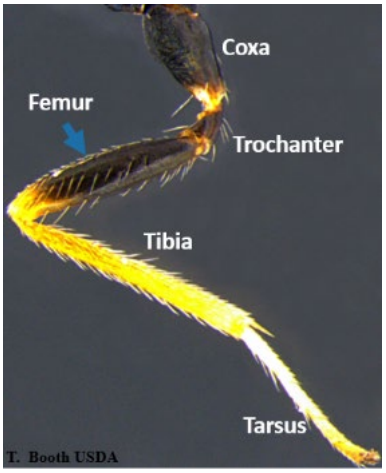


Tetrastichus planipennisi male checklist

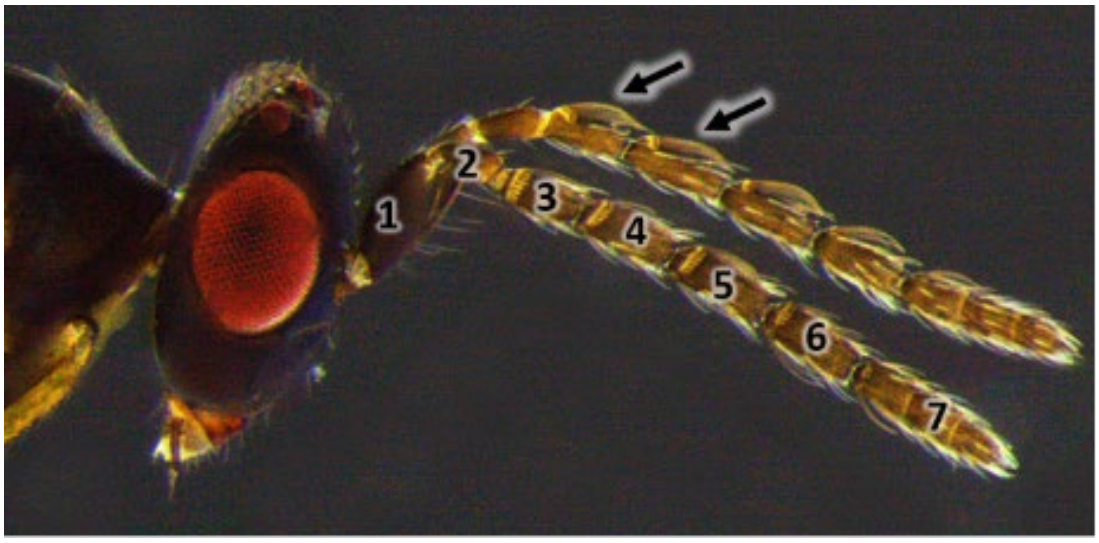
Tetrastichus planipennisi males can be identified by four characteristics: 1) dark femurs, 2) 7-segmented antennae with hairs that look like 'eye lashes', 3) last segment of abdomen not elongated, 4) smooth thorax with two lateral grooves on the middle thoracic segment. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

1) **Dark femurs (see arrows).**

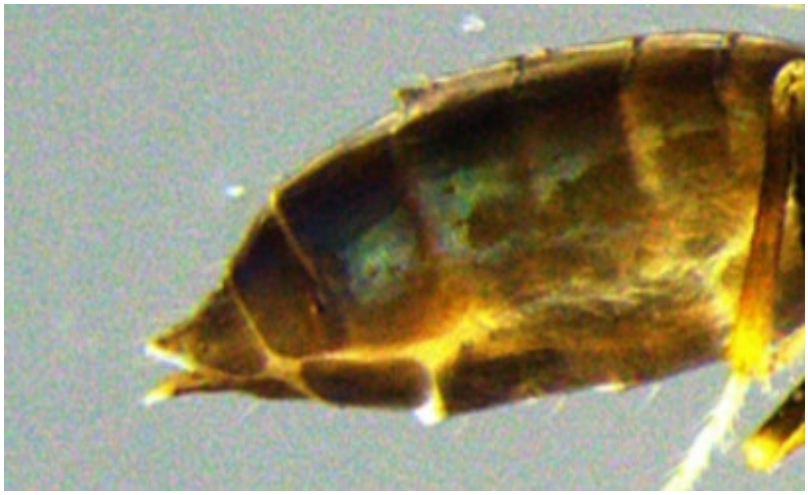




2) **7-segment antennae: scape (1), pedicel (2), four funicles (3-6) and clava (7); clusters of 'eyelash' hairs (see arrows).**



3) **Last segment of abdomen not elongated.** Note that the aedeagus may be visible; not to be confused with an ovipositor.



4) **Smooth thorax with two lateral grooves on the middle thoracic segment.**

Spathius galinae checklist

Spathius galinae can be identified by the following characteristics: 1)forewings tinged brown with clear spots; wings with brown veins, 2) spherical head and eye; back of the head smooth and without wrinkles, 3) base of the tibia of all legs is yellowish, 4) short pedicel, 5) long antennae with >26 segments, and 6) IF FEMALE: long ovipositor. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

1) **Forewings tinged brown with clear spots. Wings with brown veins.**

2) **Spherical head and spherical eye. Back of the head smooth and without wrinkles.**

3) **Base of the tibia of all legs is yellowish (see arrows)**

4) **Pedicel (narrow waist connecting the thorax and abdomen) is short.**

5) **Long antennae with >26 segments. Antennae 1.2x length of body.** Note that antennae of preserved specimens may be broken/missing segments.

6) **IF FEMALE: Long ovipositor.**

Spathius agrili checklist

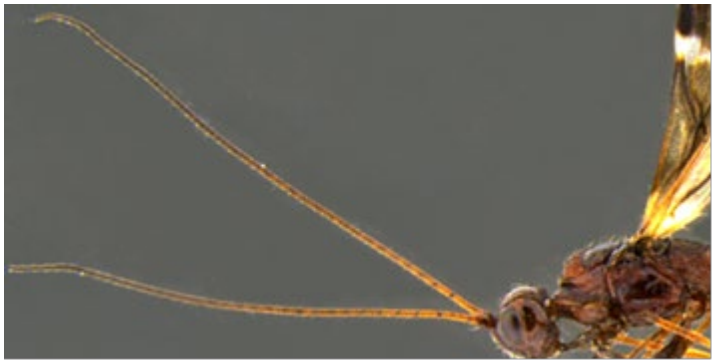
Spathius agrili can be identified by the following characteristics: 1) forewings-tinged brown with clear spots; wings with brown veins, 2) spherical head and spherical eye; back of the head smooth and without wrinkles, 3) short pedicel, 4) long antennae with >26 segments, 1.2 x length of body, and 5) IF FEMALE: Long ovipositor. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

1) **Forewings tinged brown with clear banded areas. Wings with brown veins.**

2) **Spherical head and spherical eye. Back of the head smooth and without wrinkles.**

3) **Pedicel (narrow waist connecting the thorax and abdomen) is short.**

4) **Long antennae with >26 segments. 1.2 x length of body.**



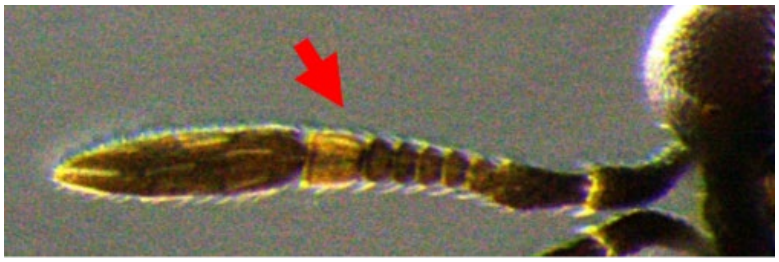
5) **Abdomen very dark in color. IF FEMALE: Long ovipositor**

Oobius agrili checklist

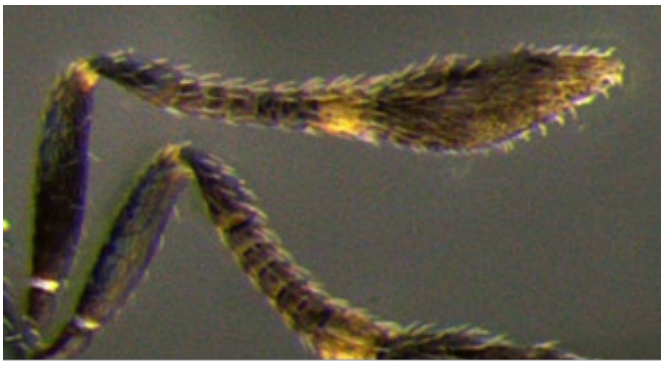
Oobius agrili can be identified by three characteristics: 1) stout body, no waist, 2) second to last antennal segment is light, all other segments are dark, 3) clava (final, club-shaped segment of antenna) is tapered upwards to a blunt point when the insect is viewed in profile. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

1) **Stout body, no waist.**

2) **Second to last antennal segment light (see arrow), all other segments dark.**



3) **Clava tapers upward to a blunt point when insect is viewed in profile.**



Native and Naturalized Parasitoids of EAB

You may come across native or naturalized parasitoids of EAB when you are sorting through your yellow pan traps or peeling trees. You are not required to record or ship these parasitoids, but you may be interested to know they are present at your site.

Figure F1. *Atanycolus* spp. Large native parasitoids with a distinct red or orange abdomen. In contrast, the head and thorax are jet black. The wings are dark grey. Photo by J. Duan.

Figure F2. *Balcha indica*. Naturalized in the eastern U.S. Native to Southeast Asia. Solitary ectoparasitoid of EAB. Pronotum textured. Dark brown with copper, blue, green, and purple lusters under different angles of light. Photo by J. Hansen.

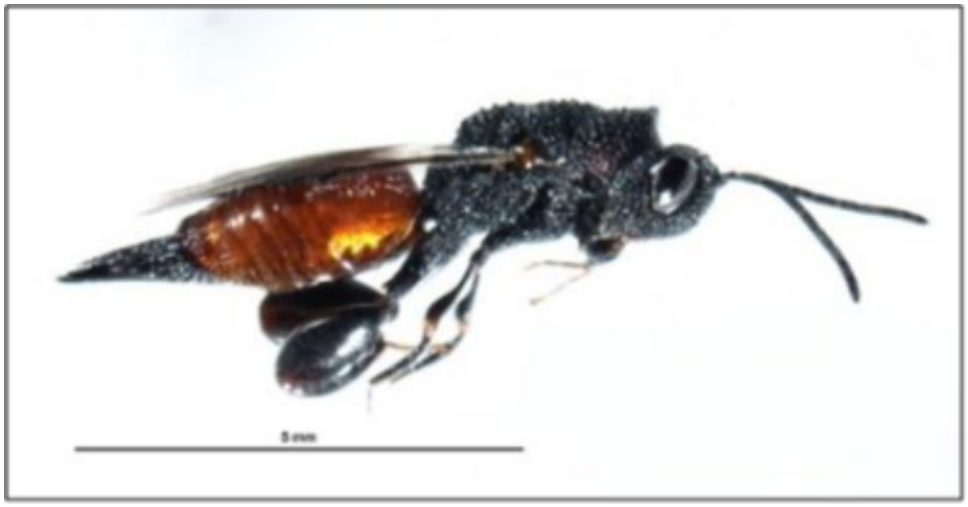


Figure F3. *Phasgonophora sulcata*. A native solitary endoparasitoid. Distinct red/orange section on the abdomen. Thorax is black and pitted. Hind leg femur is swollen with teeth. Photo by J. Duan.

Life stages of native parasitoids

Atanycolus spp.

A few species of *Atanycolus* exist. All are native solitary parasitoids. The figure below shows the lifecycle of *Atanycolus cappaerti*.

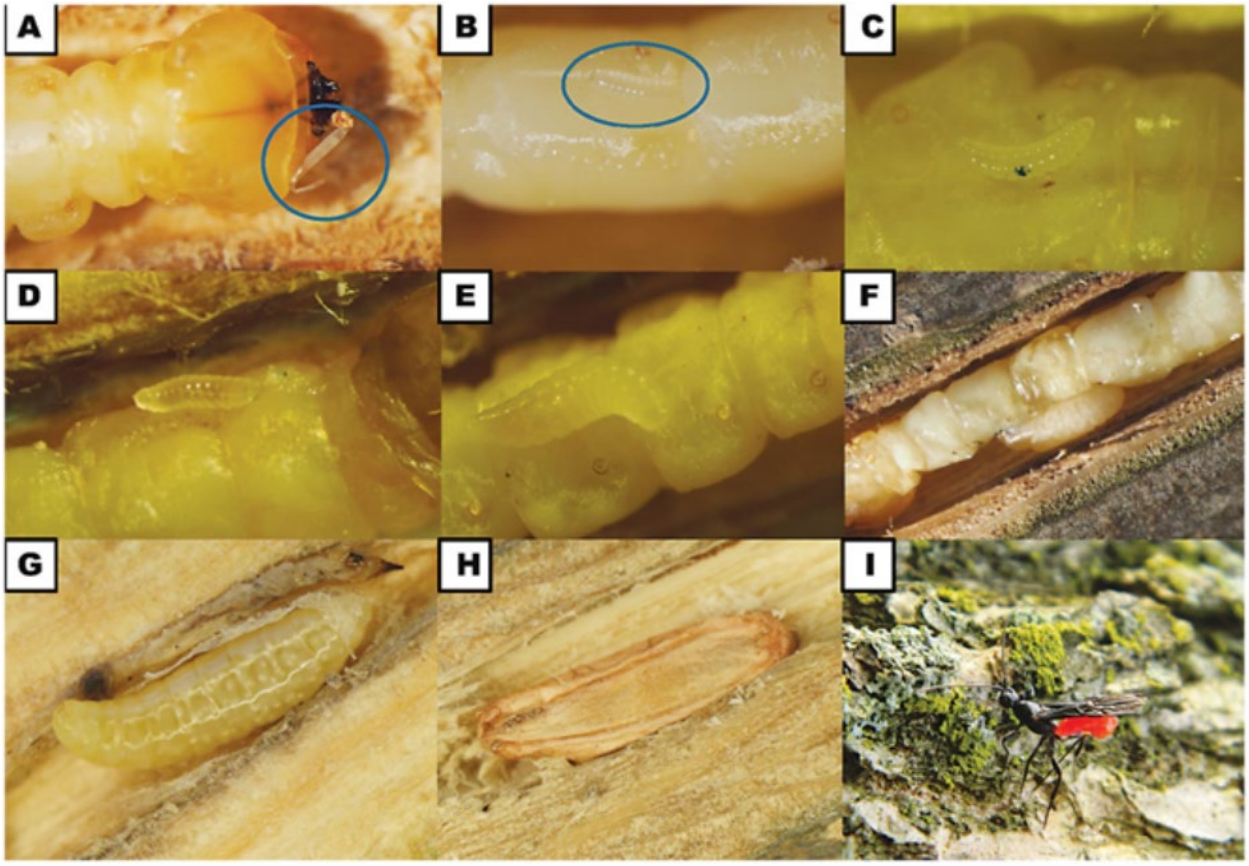


Figure F4. Stages of *Atanycolus cappaerti*. (A) Egg on an EAB larva at 3× magnification; (B) 1st instar at 3× magnification; (C) 2nd instar at 3× magnification; (D) 3rd instar at 3× magnification; (E) 4th instar at 3× magnification; (F) 5th instar at 1.8× magnification; (G) 6th instar at 1.8× magnification; (H) pupal cocoon at 1.8× magnification; (I) Eclosed adult.

From: Duan, J. J., & Schmude, J. (2016). Biology and life history of *Atanycolus cappaerti* (Hymenoptera: Braconidae), a North American larval parasitoid attacking the invasive emerald ash borer (Coleoptera: Buprestidae). *Florida Entomologist*, *99*(4), 722-729. https://doi.org/10.1007/s10526-011-9408-0

Balcha indica

This species is native to southeast Asia but has naturalized in the eastern US. It is a solitary ectoparasitoid of EAB.

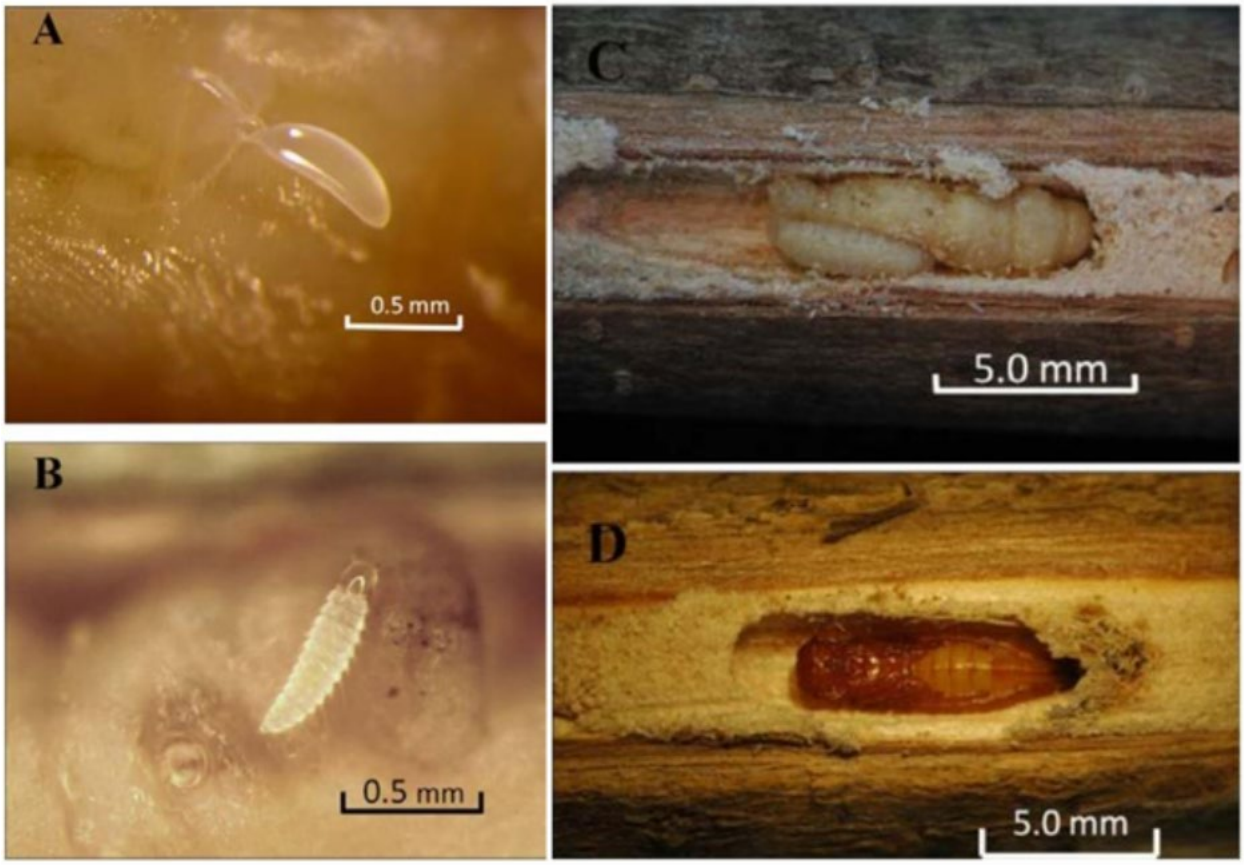


Figure F5. Stages of *Balcha indica*. (A) A single egg laid on the surface of an EAB host; (B) 1st instar larva feeding on the EAB pre-pupa; (C) the "maggot-like" intermediate-stage larva parasitizing EAB pupa; (D) pupa in cell.

From: Duan, J. J., Taylor, P. B., & Fuester, R. W. (2011). Biology and life history of *Balcha indica*, an ectoparasitoid attacking the emerald ash borer, *Agrilus planipennis*, in North America. *Journal of Insect Science*, *11*, 127. https://doi.org/10.1673/031.011.12701

Phasgonophora sulcata

Phasgonophora sulcata is a solitary endoparasitoid, so you will only see a single larva (rarely two, but one is usually dead). The larva is usually found near the tail end of the EAB larva. *Phasgonophora sulcata* has a distinctive head end and tail end, unlike a *Tetrastichus planipennisi* larva.

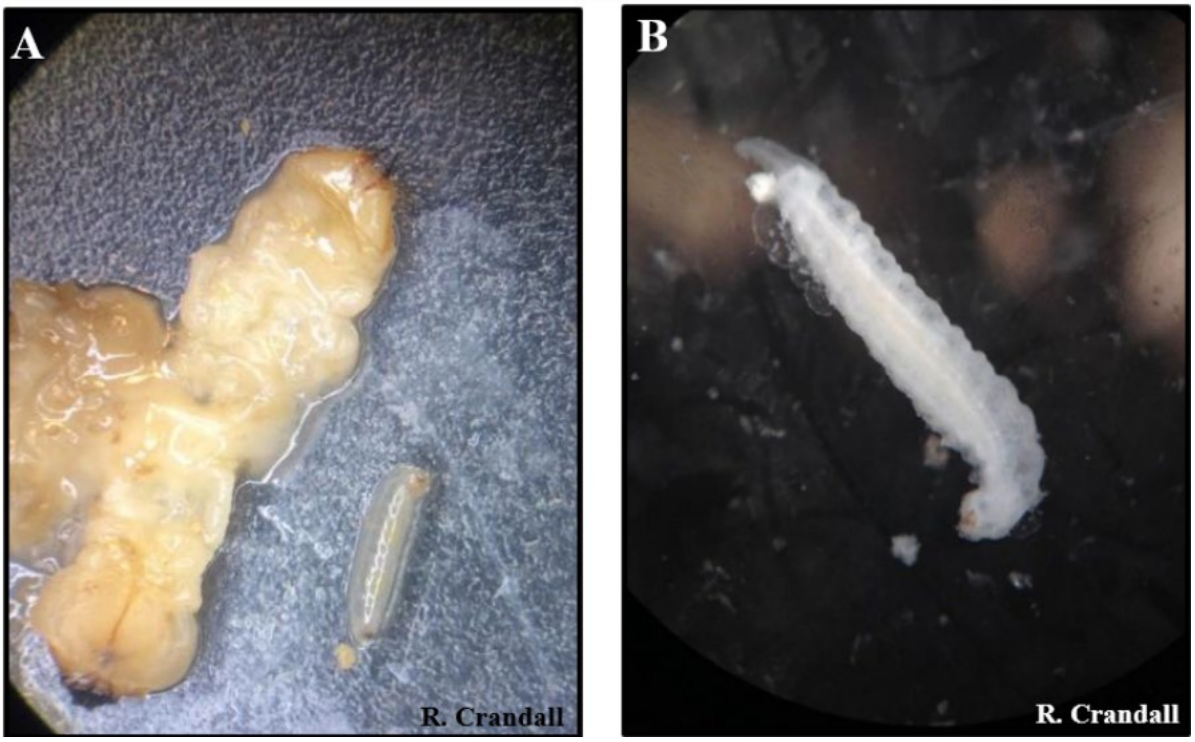


Figure F6. (A) A dissected EAB larva on the left and a *Phasgonophora sulcata* on the right. (B) *Phasgonophora sulcata* larva with the tail at the top and the head at the bottom.