# 2014 Mollusk Survey Reference



#### **Cover image:**

Giant African snail, *Lissachatina fulica* (David G. Robinson, USDA APHIS PPQ, Bugwood.org)

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#### Draft Log

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\*Portions of this document were taken directly from the Tropical and Temperate Terrestrial Gastropods New Pest Response Guidelines (USDA-APHIS, 2010a; 2010b).

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# How to Use This Manual

#### I: Introduction

The first section of this manual describes the purpose of the Mollusk survey. This section provides background information about mollusks and lists the pest species targeted in this survey and their current distribution within the continental United States and Caribbean and Pacific states and territories.

#### **Pest Datasheets**

Pest datasheets have been developed for each target pest genus or species. Datasheets contain specific information on the distribution, survey methods, and identification resources for each pest target. Pest datasheets are linked on the CAPS Resource and Collaboration site manuals page under Mollusk manual.

#### Known food sources (in all Pest Datasheets)

Mollusks do not have true hosts, but rather food sources. Population growth is not limited by the amount of food; however, growth is limited by the lack of high quality food. When high quality food is rare, mollusks will use a lower quality food (Speiser, 2001).

Food sources for mollusks are made up of a variety of plant and non-plant material. Mollusks have been recorded feeding on almost every kind of organic material: plants of all developmental stages (with a preference for senescent plant parts), leaf litter, wood, and dead animals in different stages of decay (including earthworms, mites, and insects). The largest fraction of a mollusk's diet is usually made up of plant material, followed by fungi, animals, and then soil (Speiser, 2001). Many mollusks find food through smell.

Mollusks can occur in a range of different habitats which may have different preferred food sources available. This can result in the same species eating different food sources in different areas. Food sources can also vary greatly over a season due to changes in nutritional needs and availability of food sources (Speiser, 2001).

An important nutritional need for snails is calcium, which is required for proper shell formation and successful reproduction. Calcium can be obtained from alkaline soils as calcium carbonate and from plants as calcium silicates. Shells from other snails can also be used to obtain necessary calcium (USDA-APHIS, 2005). Slugs are not as dependent on calcium-rich environment as are snails (Capinera et al., 2011).

### II: Planning a Survey

The next section describes how to plan a mollusk survey and includes information on the CAPS-approved survey and identification/diagnostic methods for each of the genera or species. General information is provided on survey sites and survey season. When planning a survey, states should consider a pathway approach when deciding on which mollusk pests to survey for. Information regarding the hosts and climate of each pest should be considered as well.

When looking for survey locations, try to choose locations that would have the most hospitable conditions throughout the year, as populations should be highest in these areas if present (Pearce and Örstan, 2006).

# **III: Conducting the Survey**

This section gives specific information on how to conduct a survey for mollusk pests. This section lists symptoms and signs to look for when conducting a visual survey. One of the most important features of a successful survey is picking the right location.

The time of day and time of year are also important. Many mollusks leave their shelters around sunset (Speiser, 2001). Damp weather in autumn is a good time to survey for slugs (Kerney and Cameron, 1979).

When searching during daytime, it may be necessary to search on hands and knees as they are likely to be hidden during this time. When searching under material, make sure to replace anything turned over (Kerney and Cameron, 1979).

# **IV: Sample Submission**

This section gives specific information on how to submit samples for identification.

#### Important!

Consumption of snails and slugs, or of vegetables and fruits contaminated by snails and slugs, may lead to infection by pathogens that are easily transmitted by these pests. Wear rubber or latex gloves when handling mollusks, associated soil, excrement or other materials that may have come in contact with the snails. Immediately after removing protective gloves, thoroughly wash hands with hot soapy water and rinse well. Consult a physician if, after handling snails and slugs, you experience symptoms resembling forms of meningitis, including headache, stiff neck, tingling or painful feelings in the skin, low-grade fever, nausea, and vomiting. These symptoms could indicate an infection by *Angiostrongylus cantonensis*, a parasite carried by snails and slugs.

# I. Introduction

### **Purpose**

The purpose of the mollusk survey is to detect new infestations of target mollusk pest species at low population levels. This document provides standardized guidelines for conducting a mollusk detection survey in the United States and its territories.

## Background

#### What is a Mollusk?

Mollusks include invertebrate animals in the phylum Mollusca. For the purposes of this manual, mollusk refers specifically to terrestrial slugs and snails belonging to the class Gastropoda. All Gastropoda have a well-developed foot and have, or at one point had, a single coiled shell in which the animal can withdraw (Kerney and Cameron, 1979).

Snails have spirally coiled shells, while slugs have lost, or appear to have lost, their shell. The bodies of both snails and slugs are wet as they are covered in a layer of mucus or slime; this coating is usually thicker and more copious in slugs. Immatures are similar to adults; they do not go through metamorphosis or molting. In snails, growth occurs by adding material to the outer edge of the shell with more whorls being added to the shell as they grow. Maturation usually occurs in about one year although larger slug and snail species can take two to four years (Kerney and Cameron, 1979).

#### Snail Anatomy

The body of a snail has two pairs of tentacles: a short, lower pair that are sensitive to touch and chemical signals; and one long, upper pair with eye spots at the tips (Schotman 1989 and USDA 1960). The body is moist, slimy, and rubbery. Body coloration varies with species and/or population. The foot sole is flat, with coarse tubercles most evident on the sides and upper surface of the extended body.

Shell characters that are important for identification are the columella, whorls, sutures, transverse striae, parietal wall, apex, and lip.

Aperture: The major opening of a shell that the body of the animal may be retracted.

Apex: The tip of the spire of a shell.

<u>Columella</u>: The central axis of the shell; originates at the shell apex and ends at the umbilicus.

<u>Lip:</u> The margin of the aperture, which may be sharp or thickened depending upon the species.

<u>Parietal wall</u>: Referring to that part of the interior surface of the shell between the columella and the suture – in effect, formerly the external surface of earlier formed whorls.

Suture: The junction/seam between the whorls of a mollusk's shell.

<u>Transverse striae</u>: Fine incised groove on shell surface that crosses direction of shell growth, usually parallel with growth lines.

<u>Umbilicus:</u> A navel-like indentation or depression in the center of the shell. It may be described as open (inside of columella visible), partially closed (partly covered by base of aperture) or completely closed (not visible). The width of the umbilicus is a measure of its greatest diameter.

<u>Whorl:</u> A complete spiral turn/growth of the shell of a mollusk. The whorls are counted from the apex outwards.

(Definitions from: http://idtools.org/id/mollusc/glossary.php).



**Figure 1.** Anatomy of a temperate terrestrial land snail (Illustration by Joel Floyd, USDA–APHIS–National Identification Service).

Details about the shell (like shape, size, and color) can sometimes be important when identifying a species, but can also vary greatly within a species (Kerney and Cameron, 1979).

#### Slug Anatomy

Slugs have four main regions: the head, mantle, trunk, and foot.

Slugs have two pairs of tentacles on the head. The anterior (bottom shorter) pair are used for smell and feeling, while the optic (upper longer) pair is used for seeing. The mouth is close to the lower tentacles. The anterior pair of tentacles are similar among slugs, except for Veronicellidae, in which these tentacles are bilobed (Runham and Hunter, 1970). Slugs have a scraping tongue called a radula.

The mantle's main purpose is for protection and breathing. The mantle is made of thicker flesh then the head. It contains an opening called a pneumostome which is used to let air in the respiratory cavity. In snails, the mantle is the fleshy lobe that secretes the shell.

The foot covers the entire bottom surface of the slug. Usually the sole of the foot is the same width of the body, except for Veronicellidae in which the sole is very narrow and surrounded by a deep groove (Runham and Hunter, 1970).

The trunk covers the back dorsal region of the slug.

Unlike many snails, slugs cannot usually be identified to species without dissection. Many species have large variations in size and color, and their bodies usually lack diagnostic features. The main features used for the description and identification of slugs include position and shape of the radular teeth, jaw shape, size and position of various reproductive system parts, and the position of major muscle groups (Runham and Hunter, 1970). For more information about internal anatomy and dissection, see Runham and Hunter (1970) and Kerney and Cameron (1979).

#### **General Biology**

After adults mate, eggs are usually laid in clutches in small holes in soil, under material such as logs and stones, or in rotting material. Egg numbers can range from 20 to 50 in larger species to 100 or more. In general, eggs are laid in either summer or autumn. Mortality is heaviest in the early stages of life due to desiccation, predators, and parasites (Kerney and Cameron, 1979).

Terrestrial mollusks become inactive during dry conditions which help them retain moisture. In dry conditions, snails can withdraw into their shell, which protects them from desiccation. The shell is nearly impermeable to water.

Most species are found near the soil surface; however, some may climb material such as vegetation, rocks, and fences. Due to the risk of desiccation, slugs and snails are

usually most active at night or during wet weather. When conditions are shady and cool, snails can usually rest out of sight – under logs, stones, and vegetation, in leaf litter, or buried just below the soil surface.

When conditions are hot and sunny and soil conditions are too hot, snails can climb plants where the temperature is cooler. When conditions are too dry, snails can survive in a state of suspended animation, called aestivation; when temperatures are below freezing, they can hibernate (Kerney and Cameron, 1979). Snails have a tendency to aggregate before becoming dormant (Pearce and Örstan, 2006). Due to their maneuverability, slugs can burrow into soil during dry weather, sometimes more than one meter. They can also burrow into cracks and crevices in rocks and logs (Kerney and Cameron, 1979). This makes it difficult to survey for slugs and snails when conditions are unfavorable.

There are several types of habitats that are suitable for snail and slug populations including woodlands, wetlands, and grasslands (Kerney and Cameron, 1979).

Although most slugs and snails feed on rotting vegetation, fungi, algae, and lichens, they can also feed on flowers, fruit, seeds, and underground tubers such as potatoes and carrots. Many commercial crops are softer and more nutritious than their wild counterparts and can attract species that normally feed on decaying material (Kerney and Cameron, 1979).

#### Why Are Mollusks Important?

Mollusks can:

- Cause damage by feeding on agricultural and horticultural crops as well as native plants, thereby lowering crop yields and crop quality;
- Transmit pathogens to humans indirectly when humans consume vegetables and fruits contaminated by snails and slugs;
- Transmit pathogens of both plants and livestock in their feces; and
- Displace native species of snails and slugs.

#### **Selection of Target Species**

The target pest genera and species in this survey were selected by the National Committee of the Cooperative Agricultural Pest Survey (CAPS) Program, in cooperation with the USDA-APHIS-PPQ Center for Plant Health Science and Technology (CPHST). All target species included are exotic pests to some area(s) of the United States but not necessarily every state and territory. Specific pests, however, should only be surveyed for in states and territories where that particular pest is not known to occur. Table 1 outlines the targets selected for this survey, their common name, and current level of distribution within the United States (see Table 1. Target Snails for Survey).

Many of the pests listed in Table 1 and their associated datasheets are at the genus level. Although genus level datasheets may have information specific to certain species, any species within the genus can be surveyed for in the mollusk survey.

Scientific Name	Common Name	Type of Mollusk	Family	Where in United States the Pest is Known to Occur
Belocaulus spp.	caulus spp. No common name, leatherleaf Slug Veronicellidae slugs		<i>Belocaulus angustipes</i> : AL, LA, FL, and TX	
Cernuella spp.	No common name, hygromiid snails	Snail Hygromiidae		Cernuella virgata: Unclear, possibly WA and CA*. Previously reported in NC.
Colosius spp.	No common name, leatherleaf slugs	Slug	Veronicellidae	Not known to occur in the United States
Cochlicella spp.	ella spp. No common name, cochlicellid Snail Cochlicellidae snails		Cochlicella acuta and C. barbara: CA	
Laevicaulis spp.	No common name, leatherleaf slugs	leaf Slug Veronicellidae		<i>Laevicaulis alte</i> : HI, possibly TX
Lissachatina fulica	Giant African snail	snail Snail Achatinidae		FL
Meghimatium pictum Chinese slug S		Slug	Philomycidae	Not known to occur in the United States
Monacha spp. No common name, hygromiid snails		Snail	Hygromiidae	Unknown
Sarasinula spp.	No common name, leatherleaf slugs	Slug	Veronicellidae	<i>Sarasinula plebeia</i> : FL, HI, TX
Semperula spp. No common name, leatherleaf Sl slugs		Slug	Veronicellidae	Not known to occur in the United States
Veronicella spp. No common name, leatherleaf Slug slugs		Veronicellidae	Veronicella cubensis: Santa Barbara, CA. Veronicella sloanii: HI, Questionable record in S. Florida.	

# Table 1. Target Mollusks for Survey

\*California record is likely erroneous (USDA, 2006). See also Burke (2013).

# II. Planning a Survey

# **Choosing Target Species**

Pest targets should be added to your detection survey based on their relevance to a particular state or territory. Determining which target species to survey for should be based on 1) the risk of introduction of the target and pathways of introduction; 2) presence of known or potential hosts in your state/territory; 3) climatic suitability of your state/territory for the target; 4) resources available (financial and staff) for survey and identification of the pest; and 5) the status/importance of a particular pest to your state/territory.

		CAPS-	CAPS-Approved
Scientific Name	Common Name	Survey Method	Method
Belocaulus spp.	No common name, leatherleaf slugs	Visual	Morphological and Molecular*
<i>Cernuella</i> spp.	No common name, hygromiid snails	Visual	Morphological
Cochlicella spp.	No common name, cochlicellid snails	Visual	Morphological
<i>Colosius</i> spp.	No common name, leatherleaf slugs	Visual	Morphological and Molecular*
Laevicaulis spp.	No common name, leatherleaf slugs	Visual	Morphological and Molecular*
Lissachatina fulica	Giant African snail	Visual	Morphological
Meghimatium pictum	Chinese slug	Visual	Morphological
<i>Monacha</i> spp.	No common name, hygromiid snails	Visual	Morphological
Sarasinula spp.	No common name, leatherleaf slugs	Visual	Morphological and Molecular*
Semperula spp.	No common name, leatherleaf slugs	Visual	Morphological and Molecular*
Veronicella spp.	No common name, leatherleaf slugs	Visual	Morphological and Molecular*

# Table 3. Target Pests by Approved Survey Method

\*Differentiation from the native species is only by dissection and only if the specimen is mature enough. All veronicellid samples should be sent to Dr. Robinson for morphological identification. All specimens will then be confirmed through molecular diagnostics performed at the CPHST Mission lab.

### **Pathways**

When planning surveys, states are encouraged to use a pathway approach when deciding on target species and locations to survey. It is understood that risk factors can be examined along a "risk continuum" beginning at offshore sites (points of origin) to points of potential establishment (commodity production areas, natural lands), and numerous risk points in between (wholesale distribution centers, nursery sites, transportation corridors, etc.).

Potential pathways could be identified by searching the EAN database and PestID. Potential pathways may differ from state to state. Surveyors should consider their state's specific risks when planning a mollusk survey.

Some of the targets listed in this manual, specifically *Lissachatina fulica* (giant African snail) could potentially move through intentional movement and smuggling for consumption or the pet trade.

# Outreach

Once a survey site has been identified, the surveyor should contact the property owner to obtain permission to survey on the property. Surveyors should provide the property owner and/or tenant with information about the survey and contact information if they find any suspect specimens. Surveyors should also stress the importance of preventing exotic species from establishing. Mollusks are difficult to detect by a one-time visual inspection and owners or tenants may be more likely to see suspect specimens later when the conditions are more favorable. Owners and tenants can potentially provide surveyors with leads on good sites to survey next, for example where they take their vegetation debris, the shipping companies they use, etc.

### **Survey Sites**

New introductions of mollusks will likely be related to commerce and human-assisted movement. The habitat and land-use type of each survey site may be variable, ranging from agricultural land, to residential or industrial features.

Conduct on-site analysis of the survey area to determine any direct points of introduction, such as ports, rail yards, container yards, and cargo distribution centers.

Other potential survey areas include, but are not limited to:

• Tile dealers,

- Landfills,
- Nurseries,
- Airports, and
- Warehouses.

Also consider habitats with features snails and slugs prefer, such as vegetated areas, and natural and anthropogenic sources of calcium (for snails), including:

- Alkaline soil;
- Limestone quarries and outcroppings;
- Construction sites or dumpsites containing concrete, limestone, or marble;
- Graveyards, headstones, or tombstones; and
- Agricultural fields, orchards, or plantations that use lime to alkalize the soil.

Most snail and slug species will thrive in moist vegetated areas. Some species may prefer slightly drier microhabitats, and others may prefer sandy well-drained soils. Preferences for microhabitats vary with species. Consult the biology of the target pests to identify any habitat or host preference information. If no information is available, or multiple species are targeted, allow surveys to cover a number of diverse habitats.

Remember, slope and orientation towards the sun can influence moisture levels. More exposed topography, like hills and ridge tops will likely be drier than slopes or valleys (Pearce and Örstan, 2006).

### **Sentinel Survey Sites**

A sentinel survey site is a fixed location for doing survey inspections on a repeated basis. This method can be used to detect mollusks. Sentinel survey sites were described in Anonymous (2008). According to the source, if a particular area is considered at high risk for mollusk introduction based on pathway studies, surveyors can use their time efficiently by establishing sentinel survey sites in that area. The best sentinel survey sites within the high risk area are chosen based on the biology and preferences of the target pest. Examples of preferred sites include areas of dense vegetation, garbage piles, or calcium carbonate deposits.

# **Survey Season**

Most species of mollusks are active during nocturnal hours, when environmental conditions are cool and wet. Some species may also be active during daylight, especially during overcast and rainy days in the spring and fall. During dry, hot periods, mollusk species will seek shelter in cool, moist places shaded from the sun. Refuge preferences can differ between species. Slugs can be found in refuges such as cracks in mud, under rocks, in tree crevices, and under refuse. During extended periods of drought, slugs may move deep into the soil or refuge structure, too deep to be observed during a visual survey. It is not advisable to survey for slugs and snails during extended

hot and dry periods. Many species will be hard to locate as they seek shelter from the hot and dry conditions. If possible, plan surveys during spring and fall, and during early morning and overcast days. Many slugs and snails have diurnal patterns of activity, so early morning and evening hours may be the best time to carry out a survey (Pearce and Örstan, 2006).

In hot and dry environments, some snails will aestivate (remain dormant) to survive. Several snail species have the ability to survive long periods of dry conditions by withdrawing into their shell and sealing the opening with an epiphragm (temporary mucus membrane). Several hygromiid and helicid species will climb rock walls, vegetation, and fences to escape high ground temperatures. These snails can attach themselves to the elevated surfaces and aestivate until more favorable environmental conditions return. Surveys for these species should be directed at plant stems, fence posts, and other elevated surfaces.

Some species of mollusks are difficult to identify in juvenile form. Mature adult specimens possess genitalic features that can help differentiate closely-related species. If possible, plan survey activities during the time when adult life stages are present. If phenology of target species is not known, surveys should be conducted in both spring and fall, when environmental conditions are ideal.

#### **Microhabitats**

Within a larger habitat, populations can vary within microclimates (Pearce and Örstan, 2006). Terrestrial snails and slugs may be found in cool refuges, near vegetation, under rocks, boards, and refuse. Some also climb into sheltered areas, such as tree crevices and plant canopies. In some crops, such as vineyards and orchards, tree canopies serve as a cool refuge site for some snail species.

For survey, examine vegetation and the underside of a variety of structures, refuse, and litter that is in contact with the ground. If the structure is safely movable, the item should be lifted and the underside examined for mollusks. Also consider examining structures, such as logs or wood, that are elevated off the ground up to one meter. Snails and slugs can sometime hide on the undersides of these to avoid predators.

Most snails and slugs require calcium for proper formation of the shell and for successful reproduction in creating new eggshell for their offspring. Snails obtain calcium from numerous sources in the environment. Calcium carbonate (CaCO<sub>3</sub>) can be found in alkaline soils. Plants also provide a store of calcium silicates where free calcium is not readily abundant in the soil. In large populations, snails obtain mineral content from the empty shells of dead snails. When planning the survey route for a particular site, examine the following microhabitats:

- Near heavily vegetated areas, especially gardens and fields where plants have been damaged by feeding;
- Under rocks, asphalt or cement pieces that are in loose contact with the ground surface;

- Discarded wooden boards and planks, fallen trees, logs, and branches;
- Damp leaf litter (not wet or soggy\*), compost piles, rubbish heaps, piles of mulch, bark chips, grass clippings, woodpiles, and potting soil;
- Under flower pots, planters, rubber mats, tires, and other items in contact with the soil; and
- Standing rock walls, cement pilings, broken concrete, and grave markers.

\*It may make sense to survey in wet or soggy areas if it has rained recently or if the target species prefers this type of microhabitat.

Open meadows and pasturelands are usually poor habitats for snails unless many logs are present (Pearce and Örstan, 2006).

While conducting a survey, look for clues that suggest the presence of terrestrial snails and slugs. Evidence may include the following:

- Snails, juveniles and adults;
- Eggs;
- Empty snail shells;
- Mucus and slime trails; and
- Ribbon-like feces, also known as casts.

# **III. Conducting a Survey**

# **Visual Survey**

Visual inspection is the most effective method of survey for snails and slugs. One challenge with using visual sampling techniques for mollusk surveys is the risk of inconsistent sampling effort. Variability in surveyor experience and habitat type may result in different sampling effort between sites and may affect the confidence in the results of a survey. To minimize this variability, a survey program should include surveyor field training in visual sampling techniques. Subjectivity of a visual survey can also be addressed through the use of plot sampling, introduced in the following paragraphs.

Use the following survey criteria to standardize visual inspection techniques and increase the chance that targeted snail and slug species will be detected:

- Conduct visual inspections during the ideal sampling season and in microhabitats attractive to mollusks. Consider taking a flashlight, preferably LED. Flashlights can help illuminate shadows and undersides of structures, even in daytime and especially on a dark day or very sunny day when shadows appear darker. Slime trails will reflect better in the flashlight beam.
- 1. Use a global positioning device if available to determine the coordinates of the survey site or plot. Keep records on all plots surveyed: the number of surveys, the time spent surveying each site or plot, and whether the results are positive or negative.
- Collect and label any suspect snails or slugs and note the exact location of the find (GPS coordinates if known). Note the substrate, microhabitats, and plant hosts.
- 3. Define survey protocols using time-based line survey or plot survey (see below for details).

When conducting visual surveys, it may be necessary to sort through leaf litter and look under rocks and logs, among other places. Most snails and slugs will not be out in the open during times that surveys occur.

There are two main types of visual survey methods, line survey and plot survey. Surveyors can also use a combination of the two.

#### Line Survey

A line survey is a transect across a target property that allows the surveyor the flexibility to choose inspection points likely to shelter mollusks. This flexibility is useful for detection surveys, when several target species and habitat types need to be sampled. Line sampling can be used alone when survey resources are low or can be combined with a plot survey when more quantification is needed, as with delimitation and monitoring surveys.

To conduct a line survey, examine microhabitats that include vegetation, duff, and structures that might serve as diurnal or seasonal refuge sites for mollusks. Estimate the survey area and coordinate line survey routes for each surveyor.

#### Plot Survey

Use the plot survey method if further standardization and quantification is required. A plot is a small, defined area (for example, one square meter) that is used to conduct detailed, standardized subsampling throughout a target site. This method is effective for detecting immature mollusk species.

Construct a plot template of any size that is easy to use. A template is a precisely measured, reusable tool used to define a plot sample. Use PVC tubing or wood to construct a lightweight template. A square template measuring one meter on each side is easy to use, but the size can be adjusted to fit the goals of the survey program.

To begin surveying, randomly toss the template into the habitat. Stand outside of the plot while examining rocks, boards, litter, vegetation and other structures within the plot. Look under leaves, duff, and at the base of plants. Spend a standardized minimum amount of time surveying each plot. Repeat four times per acre of target property. Do *not* overlap plots in a survey site.

Surveyors should get close to the surface (one meter or less) to ensure that they get a good view of the plot. Surveyors may have to get on hands and knees or lay down on their side as needed.

#### **Combined Line and Plot Sampling**

#### **Detection Survey**

If the target property is small (less than one acre), conduct one plot sample and then line survey the entire property.

By using both methods, the surveyor has the benefits of each, including the flexibility and swiftness of the line survey and the standardization and repeatability of the plot survey.

#### Equipment/Supplies to take into the field

Surveyors will require the following equipment:

- Forceps (soft),
- Large permanent marker,
- Collecting vials with water-tight caps,
- Water (1 gallon),
- Blank collection labels,
- Survey forms,
- GPS unit,
- Disposable gloves,

- Plot sampler (PVC for m<sup>2</sup> samplers),
- Re-sealable bags,
- Platform traps,
- Boots,
- Magnifying lens (2 to 3x) or headset (5x+) as needed,
- Re-sealable freezer bags,
- Pocket notebook,
- Flashlight, preferably LED,
- Scoop utensil, and
- Rain clothing.

The equipment needed may vary depending on the environment and target species. Most surveys can be completed with minimal equipment.

#### Safety

Many survey locations may require certain safety precautions be taken before entering the property. Bright safety vests should always be worn when surveying roadways or parking lots. Some sites, like container yards may require hard hats as well. Surveyors should always check with the owner and/or management of the property to see what safety requirements are needed.

<u>Important:</u> Wear rubber or latex gloves when handling mollusks, associated soil, excrement or other materials that may have come in contact with the snails. Immediately after removing protective gloves, thoroughly wash hands with hot soapy water and rinse well.

#### When to Survey

Good times to survey include after rain events and early in the morning as moisture levels will usually be higher. Make sure to carry out surveys during times when visual inspections will be the most productive. Damp weather in autumn is a good time to survey for slugs (Kerney and Cameron, 1979).

### Trapping

Trapping *cannot* be used alone but can be used to supplement visual surveying. Trapping for mollusks is not species-specific and will attract non-target species, including non-mollusks. Platform or baiting traps can be used to supplement visual inspection.

<u>Note:</u> Make sure that your trap is placed in an area that cannot be easily disturbed by other animals or people.

#### **Platform Traps**

Use platform traps as artificial diurnal refuges for mollusks. Platform traps are square cardboard or wood sheets, placed directly on the ground. If target species include large snails or slugs, the platform can be elevated one inch off the ground. Platform traps may be used for repeat monitoring at high-risk sites, or where existing refuges are lacking (for example, open fields) and or difficult to survey (brambles, dense grass). This method may not be effective for some target species.

#### **Baited Traps**

Make a baited trap for mollusks by placing a food attractant inside a cup or bowl set into the ground. Use one of the following dry or wet feeding attractants:

- Bran,
- Molluscicide,
- Beer, or
- Other preferred food source.

Bran-based baits are generally attractive to mollusks but also attract mammals and arthropods. Dry molluscicide baits are also commonly used to attract and kill snails and slugs in back yard environments. Unfortunately, mollusks that ingest the pesticide are not killed immediately, nor are they contained in the trap after feeding. Beer is generally effective at attracting and trapping many slug species; unfortunately, it is not effective for trapping many target species of exotic snails. Another trapping strategy uses trap crop stations, which involve placing a preferred food source such as lettuce or fruit at the site and examining daily.

#### **Cotton/Burlap Sack**

This method is not as specific as the two previous trapping methods listed above. Take a cotton or burlap sack and fold it several times. Soak the sack in water and put out in the survey area. Make sure to stack the sack with rocks to help prevent the sack from drying out (Sturm et al., 2006) and help provide air circulation. This setup provides a cool, shaded, moist resting area. Check the sack after several days to one week for target species (Pearce and Örstan, 2006).

#### **Trap Placement**

Trap placement can occur in the same areas that visual surveys occur. Remember, trapping for mollusks cannot be used alone but can be used to supplement visual surveying. See <u>Survey Sites</u> and <u>Microhabitats</u> for more information.

#### **Checking Traps**

Traps should be checked on a regular basis, usually every few days. The regularity in which a trap should be checked will depend on several factors, including weather and location. When checking a trap, make sure that it is in working order. The surveyor

may need to service the trap and replace baits. Traps can be left out for as long as the surveyor deems necessary. The trap(s) should be removed once surveying has been completed.

# **IV. Sample Submission**

Consult the most recent version of <u>Procedures for Submitting Survey Samples to</u> <u>Domestic and Other Identifiers</u> for information on how to process and submit survey samples.

### **Specimen Handling**

When collecting live samples, specimens should be placed directly into water making sure that no air bubble remains inside. Seal until drowned, then transfer to 70 percent ethyl alcohol. Label the container with the appropriate information. After handling slug samples, hands should be washed in hot soapy water and rinsed in alcohol or a standard disinfectant.

#### Important!

Consumption of snails and slugs, or of vegetables and fruits contaminated by snails and slugs, may lead to infection by pathogens that are easily transmitted by these pests. Wear rubber or latex gloves when handling mollusks, associated soil, excrement or other materials that may have come in contact with the snails. Immediately after removing protective gloves, thoroughly wash hands with hot soapy water and rinse well. Consult a physician if, after handling snails and slugs, you experience symptoms resembling forms of meningitis, including headache, stiff neck, tingling or painful feelings in the skin, low-grade fever, nausea, and vomiting. These symptoms could indicate an infection by *Angiostrongylus cantonensis*, a parasite carried by snails and slugs.

- Always wear disposable gloves when handling live or dead slugs or snails, slime, or other snail products,
- Wash your hands with hot, soapy water immediately after removing gloves.

#### Labeling Samples

Collection information is vital and should be completed immediately after a collection is made. Write directly on the collection container or on a paper label placed inside the vial using a pencil or with alcohol-proof ink. Complete PPQ form 391, Specimens for Determination. Write the date, collector's name, collector's contact information, and location including any transect and plot number. If multiple vial samples are collected from a location, assign individual sample numbers. When transferring the specimens to alcohol, ensure the label accompanies the sample.

### **Screening Specimens**

It is important to sort out the debris and non-target pests. The taxonomic level of sorting will depend on the expertise available on hand and can be confirmed with the identifier.

Use local resources when screening samples. Some states may have taxonomic support, access local training aids or identification guides.

#### Handling and Submission of Suspect Specimens for Identification

Ensure specimens are dead prior to shipping. Use a sturdy cardboard box or heavy styrofoam container so that the samples are not damaged during shipping and handling. When shipping large vials, carefully wrap vials with adequate packing material so that if breakage occurs during transit, the alcohol will not leak outside the shipping box. It is recommended that vials be wrapped in zip-type bags.

When sending to SEL, be sure to include the <u>PPQ 391 Specimens for Determination</u> marked "Prompt" with the sample going forward. Notify and send an electronic copy of the 391 to the PPQ National Identification Services (NIS) Urgent Team at ppq.nis.urgents@aphis.usda.gov, an e-mail group, with the sample number and date forwarded. If you have any questions, contact your regional survey coordinator or the Domestic Diagnostic Coordinator, Joel Floyd, with PPQ National Identification Service in Riverdale, Maryland.

If you have suspect Veronicellidae genera or *Meghimatium pictum*, send the specimen(s) to the following address:

#### Dr. David Robinson

Malacology Identification Specialist USDA–APHIS–PPQ Academy of Natural Sciences 1900 Benjamin Franklin Parkway Philadelphia, PA 19103 Phone: (215) 299-1175

Other suspect specimens should be submitted to Patrick Marquez. He is able to identify (even immature specimens) to the species level.

#### Patrick Marquez

11840 South La Cienega Blvd. Hawthorne, CA, 90250 310-725-1918 Patrick.R.Marquez@aphis.usda.gov

The identifier should be contacted prior to forwarding samples. It is helpful to know how many samples are being forwarded and when the samples will be shipped.

Reporting results are "positive" or "negative." Identifications usually take two to three weeks. However, identification time may take longer based on the identifier's current workload or the volume of samples submitted.

#### **Communication of Results**

If the insect is confirmed by SEL as a CAPS target species or new pest to the United States, the Domestic Diagnostics Coordinator will contact the National Survey

Coordinator of the identification. The notification will then go to PPQ headquarters and regional program managers, and the SPHD and SPRO. One of them will then forward the confirmation to the originator of the sample and other state CAPS personnel. Confirmations of CAPS targets or new species to the United States can then be entered in the NAPIS system.

# Data Entry Guide for Mollusk Pests at the Family, Genus, and Species Level

#### Veronicellidae

In April 2013, the family Veronicellidae, a target on the 2013 and 2014 AHP Prioritized Pest Lists, was broken down into six genera of concern. When conducting a general mollusk survey, if samples are negative for Veronicellidae, then negative data may be reported for each of these six genera: *Belocaulus, Colosius, Laevicaulis, Sarasinula, Semperula*, and *Veronicella*. All positives must be reported at the species level.

#### Cernuella, Cochlicella, and Monacha

Negative data for the genera *Cernuella*, *Cochlicella*, and *Monacha* can be entered at the genus level if no individuals of that genus are found in the sample and if the sampling method used will capture individuals of that genus if they are present in the environment from which that sample was taken. All species of these genera are exotic and not native to the United States. All positives, regardless of genus, must be reported at the species level; no positive entries at the genus level are allowed.

#### Target Species

For states including mollusk species within a commodity or bundled survey, negative data at the species level may be entered as long as the sampling method used will capture individuals of that species and if the species is likely to be present in the environment from which that sample was taken (*i.e.*, the target species would likely be associated with the commodity or environment being surveyed).

For instance, states conducting soybean and/or small grains surveys and targeting *Cernuella virgata* may enter negative data at the species level if no individuals of that species are found in the sample. *Cernuella virgata* is known for crawling onto plant heads and stalks which contaminates crops and clogs machinery. It is also a serious contaminant to wheat in countries where it is established. Therefore, *C. virgata* is more likely to be found in small grains or soybeans due to contaminated machinery; *C. virgata* is a threat to these industries; and it makes sense to survey for this target in these crops.

Please keep in mind that in general, mollusks are not host-specific and they are best surveyed for along a potential pathway of introduction.

All positive data must be identified to species; no positive entries at the family or genus level are allowed.

If these requirements cannot be met, then no data entry should occur.

# Resources

Many of the resources provided here are published by different State Departments of Agriculture and Universities and are available online. Some of the information may be relevant to your state when planning your mollusk survey.

#### **General Information**

Atkinson, J. W., M. Balaban, and K. E. Atkinson. No date. Michigan State University Snail Laboratory. Available online at: <u>https://www.msu.edu/~atkinso9/</u>.

**Capinera, J. L., J. White, and G. Bernon. 2011.** Terrestrial slugs of Flordia (Mollusca: Stylommatophora: Veronicellidae, Phylomycidae, Agrolimacidae and Limacidae). EENY 494. University of Florida, IFAS Extension. Available online at: http://orange.ifas.ufl.edu/mg/mg\_compendium/pdffiles/in/IN89100.pdf.

**Forsyth, R. No date.** Terrestrial Molluscs of Canada. Available online at: <u>http://www.mollus.ca/</u>.

**McDonnell, R. J., T. D. Paine, and M. J. Gormally. 2009.** Slugs. A Guide to the Invasive and Native Fauna of California. University of California, Division of Agriculture and Natural Resources. Publication 8336. Available online at: <u>http://anrcatalog.ucdavis.edu/pdf/8336.pdf</u>.

**Nordsieck, R. No date.** The Living World of Molluscs. Available online at: <u>http://www.molluscs.at/gastropoda/index.html</u>.

Schmidt, U. 2006. Mollusks of the World. Available online at: <u>http://www.schnecken-der-welt.de/</u>.

**University of Florida. 2013.** Snails and Slugs. Available online at: <u>http://edis.ifas.ufl.edu/topic\_snails\_and\_slugs</u>.

#### <u>Keys</u>

**Baker, H. B. 1925.** North American Veronicellidae. Proceedings of the Academy of Natural Sciences of Philadelphia 77:157-184.

Birch, J. B., 1962. How to know the eastern land snails. Iowa, Wm. C. Brown.

**Pilsbry, H. A. 1948.** Land Mollusca of North America (north of Mexico): Vol II, Pt. II. Acad. Nat. Sci. Philad.

White-McLean, J. A. 2011. Terrestrial Mollusc Tool. USDA/APHIS/PPQ Center for Plant Health Science and Technology and the University of Florida. Available online at: <u>http://idtools.org/id/mollusc</u>.

There is also a Terrestrial Mollusc Key available for download on Android devices. The app is designed to assist in the identification of mollusks of agricultural importance to the United States.

#### Native or Similar Species Information

**Branson, B. A. 1980.** The recent Gastropoda of Oklahoma, part VIII. The slug families Limacidae, Arionidae, Veronicellidae, and Philomycidae. Proc. Okla. Acad. Sci. 60: 29-35. Available online at: <u>http://digital.library.okstate.edu/OAS/oas\_pdf/v60/p29\_35.pdf</u>.

**Burke, T. E. 2013.** Land Snails and Slugs of the Pacific Northwest. Oregon State University Press, Corvallis, OR. 344 pp.

**Hoffmann, H. 1927.** On some North American Vaginulidae. Proceedings of the Academy of Natural Sciences of Philadelphia 79: 209-221.

Hotopp, K.P., T.A. Pearce, J.C. Nekola, J. Slapcinsky, D.C. Dourson, M. Winslow, G. Kimber, and B. Watson. 2013. Land Snails and Slugs of the Mid-Atlantic and Northeastern United States. Carnegie Museum of Natural History, Pittsburgh, PA, USA. Online Resource: <u>http://www.carnegiemnh.org/science/mollusks/index.html</u>.

Pearce, T. A., C. H. Richart, W. P. Leonard, and P. A. Hohenlohe. 2004. Identification Guide to Land Snails and Slugs of Western Washington. Available online at: http://academic.evergreen.edu/projects/ants/TESCBiota/mollusc/key/webkey.htm.

**Thomas, A. K., R. J. McDonnell, T. D. Paine, and J. D. Harwood. 2010.** A Field Guide to the Slugs of Kentucky. University of Kentucky, College of Agriculture. Available online at: <u>http://www2.ca.uky.edu/entomology/entfacts/entfactpdf/sr103.pdf</u>.

#### **Survey Information**

**Kelley, R., S. Dowlan, N. Duncan, and T. Burke. 1999.** Field Guide to Survey and Manage Terrestrial Mollusk Species from the Northwest Forest Plan. Bureau of Land Management, Oregon State Office. Available online at: <a href="http://www.blm.gov/or/plans/surveyandmanage/files/04-terrestrial\_guide.pdf">http://www.blm.gov/or/plans/surveyandmanage/files/04-terrestrial\_guide.pdf</a>

# References

**Anonymous. 2008.** Puerto Rico and the U.S. Virgin Islands: Mollusk Early Detection and Rapid Response Action Plan. 46 pp.

**Burke, T. E. 2013.** Land Snails and Slugs of the Pacific Northwest. Oregon State University Press, Corvallis, OR. 344 pp.

**Capinera, J. L., J. White, and G. Bernon. 2011.** Terrestrial slugs of Flordia (Mollusca: Stylommatophora: Veronicellidae, Phylomycidae, Agrolimacidae and Limacidae). EENY 494. University of Florida, IFAS Extension.

Kerney, M. P. and R. A. D. Cameron. 1979. A Field Guide to the Land Snails of Britain and North-west Europe. Collins, St James's Place, London. 288 pp.

**Pearce, T. A. and A. Örstan, 2006.** Chapter 22. Terrestrial Gastropoda. In Sturm, C. F., T. A. Pierce, and A. Valdés (eds.). The Mollusks: A Guide to Their Study, Collection, and Preservation. American Malacological Society.

Runham, N. W. and P. J. Hunter. 1970. Terrestrial slugs. Hutchinson and Company LTD, London, England.

**Speiser, B. 2001.** Food and Feeding Behavior. In G. M. Barker (ed.). The Biology of Terrestrial Molluscs. CABI International. pp. 259-288.

Sturm, C. F., T. A. Pierce, and A. Valdés (eds.). 2006. The Mollusks: A Guide to Their Study, Collection, and Preservation. American Malacological Society.

**USDA. 2006.** NPAG et Report *Cernuella virgata* de Costa 1778: Vineyard snail. USDA-APHIS-PPQ-CPHST-PERAL, New Pest Advisory Group. 4 pp.

**USDA-APHIS. 2005.** New Pest Response Guidelines. Giant African Snails: Snail Pests in the Family Achatinidae. USDA–APHIS–PPQ–Emergency and Domestic Programs– Emergency Planning, Riverdale, Maryland. http://www.aphis.usda.gov/import\_export/plants/manuals/index.shtml.

**USDA-APHIS. 2010a.** New Pest Response Guidelines. Tropical Terrestrial Gastropods. USDAAPHIS- PPQ-Emergency and Domestic Programs-Emergency Planning, Riverdale, Maryland. <u>http://www.aphis.usda.gov/import\_export/plants/manuals/</u>

**USDA-APHIS. 2010b.** New Pest Response Guidelines. Temperate Terrestrial Gastropods. USDA-APHIS-PPQ-Emergency and Domestic Programs-Emergency Planning, Riverdale, Maryland.

http://www.aphis.usda.gov/import\_export/plants/manuals/

# Appendix A: PPQ 391 Specimens for Determination

	This report is authorized by la your cooperation is needed to	aw (7 U.S.C. 147a). While o make an accurate recor	e you ar not d of plant pe	required t	o respo ons.	nd	S	ee revi	erse for addition	al OMB informa	FOR ation. OMB	M APPROVED NO. 0579-0010
U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE SPECIMENS FOR DETERMINATION			Instructions: Type or print informat when handwritten. Item 1 - assign year, followed by collector's initials John J. Dingfe): 83-JJD-001. <u>Pest Data Section</u> - Complete Item applicable. Complete Items 17 and				nation requested. Press hard and print legibly ign number for each collection beginning with als and collector's number. Example (collector xems 14, 15 and 16 or 19 or 20 and 21 as and 18 if a trap was used.			d print legibly ginning with aple (collector, d 21 as	FOR I	BIII USE
	1. COLLECTION NUMBER		2. DATE			3.	SU	BMITT	ING AGENCY			
			MO	DA	YR		ן היו	State Cooper	ator	PPQ 0	Other	
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ND OR	6. ADDRESS OF SENDER		TION S			7.	7. NAME AND ADDRESS OF PROPERTY OR OWNER					
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Do	B. Damaging Crops/Plants				F.	F. Possible Immigrant (Explain in REMARKS)						
N	C. U Suspected Pest of Regulatory Concern (Explain in REMARKS)				G	G. U Survey (Explain in REMARKS)						
D.         J.         Stored Product Pest         H.         Other (Explain in REMARKS)												
	9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".											
	10. HOST INFORMATION 11. QUANTITY OF HOST NAME OF HOST (Scientific page decision)											
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DA	12 PLANT DISTRIBUTION 13 PLA					LANT P	NT PARTS AFFECTED					
ST		Leaves, Upper Sur	face	Trunk	k/Bark				Bulbs, Tubers	, Corms	Seeds	
Ŧ		Leaves, Lower Sur	Leaves, Lower Surface Branches						Buds			
	SCATTERED	Petiole		Grow	ing Tip	s			Flowers			
	WIDESPREAD	Stem		Roots	5				Fruits or Nuts			
	14. PEST DISTRIBUTION						MA	TODE	s		MOLLUSKS	
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ATA		ALIVE										
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PES	16. SAMPLING METHOD	PLING METHOD 17. TYPE OF TRAP AND LURE 18. TRAP NUMBER										
	19. PLANT PATHOLOGY - PLA	NT SYMPTOMS ("X" one and ENERAL	describe syn	nptoms)								
				DLING		= GETATI	VE		FLOWERING/	FRUITING		
	22. REMARKS											

24. DETERMINATION AND NOTES	(Not for Field Use)	FOR IIBIII USE
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		LABEL
		SORTED
		PREPARED
		DATE ACCEPTED
SIGNATURE	DATE	RR
PPQ FORM 391 Previous ed	itions are obsolete.	
(AUG 02)		<b>Click to Submit Form</b>
This is a 6-Part form. Copies	must be disseminated as follows:	
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 PART 1 – PPQ
 PART 2 – RETURN TO SUBMITTER AFTER IDENTIFICATION

 PART 4 – INTERMEDIATE IDENTIFIER
 PART 5 – INTERMEDIATE IDENTIFIER

23. TENTATIVE DETERMINATION

PART 3 – IIBIII OR FINAL IDENTIFIER

#### **OMB** Information

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0010. The time required to complete this information collection is estimated to average .25 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

#### Instructions

Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

BLOCK	INSTRUCTIONS						
	ber for each collection beginning the year, followed by the s and collector's number						
1	EXAMPLE In of	2001, Brian K. Long collected his first specimen for determination the year. His first collection number is 01-BLK-001					
	2. Enter the colle	ection number					
2	Enter date						
3	Check block to in	ndicate Agency submitting specimens for identification					
4	Enter name of se	ender					
5	Enter type of pro	perty specimen obtained from (farm, nursery, feedmill, etc.)					
6	Enter address						
7	Enter name and	address of property owner					
8A-8L	Check all approp	priate blocks					
9	Leave Blank	Leave Blank					
10	Enter scientific name of host, if possible						
11	Enter quantity of host and plants affected						
12	Check block to indicate distribution of plant						
13	Check appropriate blocks to indicate plant parts affected						
14	Check block to indicate pest distribution						
15	<ul> <li>Check appropriate block to indicate type of specimen</li> <li>Enter number specimens submitted under appropriate column</li> </ul>						
16	Enter sampling method						
17	Enter type of trap and lure						
18	Enter trap number						
19	Enter X in block to indicate isolated or general plant symptoms						
20	Enter X in appro	priate block for weed density					
21	Enter X in appro	priate block for weed growth stage					
22	Provide a brief e	xplanation if Prompt or URGENT identification is requested					
23	Enter a tentative determination if you made one						
24	Leave blank						

#### **Distribution of PPQ Form 391**

Distribute PPQ Form 391 as follows:

Send Original along with the sample to your Area Identifier.

Send Original along with the sample to 2. Retain and file a copy for your records.