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A step-by-step protocol and guide for cavity-nesting Hymenoptera sampling and data collection using trap nests V.2

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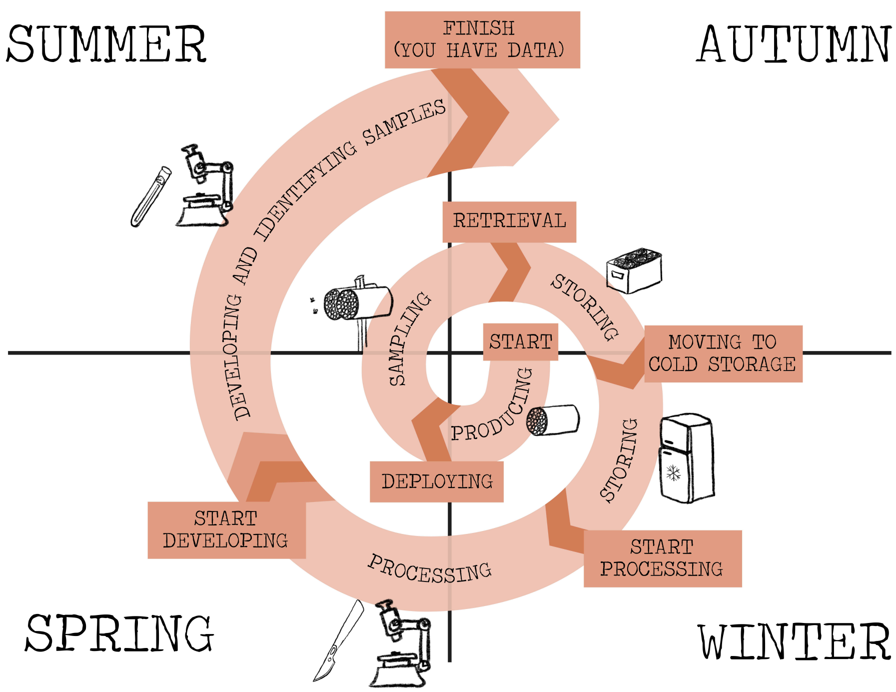
Abstract

This step-by-step protocol details the production, deployment, and processing of trap nests, a sampling tool for cavity-nesting Hymenoptera that enables the collection of detailed ecological, life-history, and abundance data. We start by describing trap nest production and the sampling procedure (production, deployment, retrieval and storing of trap nests; see Section A), followed by processing, which begins with pre-sorting (Section B), and continues with reed processing, and data entry (Section C). Finally, the process of putting away samples (Section D), and the development of immature broods (Section E) is described. The flow diagram below shows the nearly two-year process of obtaining data from trap nests. We have included three supplementary resources to complement the protocol: a **status datasheet** for administrative tracking, a **processing datasheet** which provides help during data entry, and a practical taxa **identification guide**. We mention these resources in the protocol where their use is recommended. Useful information regarding the use of these resources can be found under the Guidelines heading. This publication is a full step-by-step version of the protocol outlined in our short communication in the Journal of Hymenoptera Research.



Attachments

Status datasheet.xls...
123KB

Processing datasheet...
93KB

Identification guide...
3.7MB

Guidelines

The **status datasheet** can help with keeping track of processing status and it is especially useful when multiple people are processing trap nests and/or a large number of trap nests are being processed. Additionally, we recommend recording the following information to make data collection more transparent and retraceable: unique identifier of the trap nest, person carrying out pre-sorting and processing, starting and finishing date of pre-sorting and processing, total number of reeds (step 7), total number of occupied reeds (step 10), number of reeds put away for further development, number of vials containing samples (section D).

The variables we included in the main table of the **processing datasheet** are only recommendations and they should be adjusted for the specific needs of your study. However, we strongly encourage accurate data collection on at least the following properties: number of brood cells and their status (empty, living offspring, etc.), nesting material, provisioned food, and natural enemies. These data will allow you to analyse species interactions and, in cohort with environmental data, allow you to answer ecological questions regarding the habitat preferences and future trajectories of cavity-nesting species.

By examining nest properties, trap nests enable us to approximate the **taxonomic identity of nesting species** at the Family, Subfamily or Genus level without any specialist knowledge of species morphology. Using 1) nest material, 2) diameter of the reed (see Figure 1.), 3) provisioned food type, 4) specialized natural enemies 5), and cocoons of the offspring as clues, one can determine the identity of the nest-building species to the family, subfamily or genus level (see Table 1.) even if imago cannot be inspected due to their immaturity, or if offspring are absent from the nest. **Natural enemy taxons** can also be identified (at higher taxonomic level) based on their cocoons, faeces or the clues of activity. The **identification guide** we have provided and Table 1 allows untrained people to perform this task. During trap nest processing, species identification does not need to be accurate or precise, as samples should later be sent to a professional for more thorough identification, thus identifying the nest-building species at higher taxonomic level is enough.

Materials

- Reeds or other tubular plant stems (see Figure 1 for guidance on tube diameter sizes)
- Binding wire
- Wire mesh: roughly 2×2cm mesh size (optional)
- PVC pipe 18-22 cm long and 10-12 cm in diameter
- Wooden or plastic sheet roughly 50*50cm (optional)
- Wooden poles
- Calliper or pre-calibrated diameter measurer
- Test-tubes
- Vials
- Cotton balls
- Alcohol (70%)
- Tape
- Plastic container for reed stems
- Scalpel
- Cold storage system: Trap nests need to be stored at a stable low temperature, around 4°C and constant humidity, around 70% (Richards & Whitfield, 1988; Bosch & Kemp, 2003; O'Neill et al., 2023; Müller et al., 2025). If a **cooling room** with stable humidity and temperature control is available, this is the best place to store the samples. However, during processing trap nests can temporarily be stored in a less sophisticated machine (e.g. refrigerator), but should not be kept at room temperature for an extended time.
- Stereo microscope (see photo in step 14)
- Camera (optional)

Troubleshooting

A. Trap nest production and deployment for field sampling

1 **Produce trap nests**

Note

Some of the materials and dimensions specified below can be adjusted to accommodate different research objectives and target species or different locally available materials. However, we recommend consulting any relevant literature on the effects of such adjustments before proceeding (MacIvor, 2016; Rauf et al., 2022; Eeraerts et al., 2022; Khan et al., 2024).

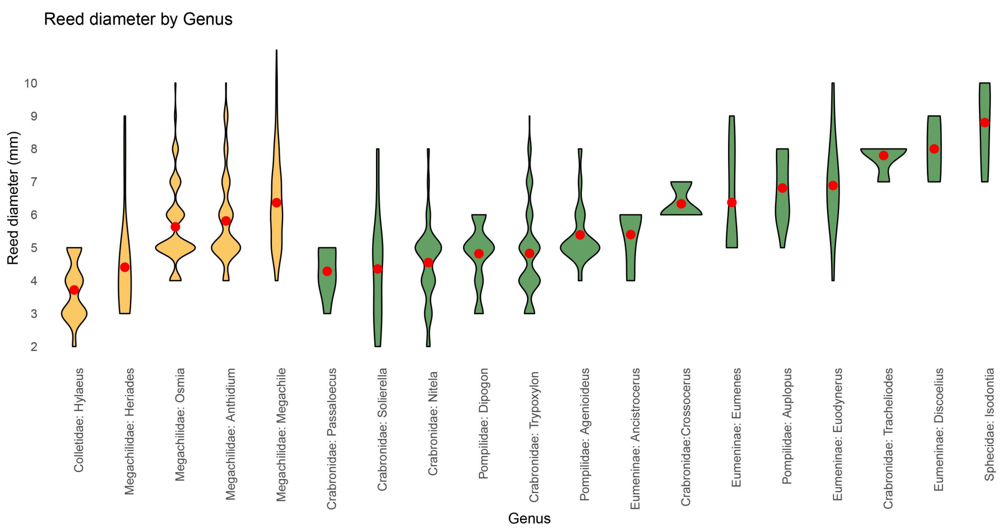
1.1 **Cut reeds** into shorter reed pieces, ensuring that each piece contains one node positioned roughly in the centre (or none at all). Based on our experience, 16-18 cm long sections are of ideal size, as this maximises the chance of obtaining reed pieces with two long internodes and one node.

Note

We recommend using a high-speed electric saw for cutting the reeds to avoid rough or damaged edges, which have been shown to negatively impact cavity occupation (von Königslöw et al., 2019).

1.2 Gather a bundle of reed pieces and **wrap them up** tightly (e.g. with wire or wire mesh), ensuring that reeds cannot fall out.

Note



The **diameter** of the reed stems used for a trap nest should be varied, see Figure 1. The **number** of reeds you use should depend on whether your sampling design allows for regular replacement of occupied reeds, in which case fewer reeds are enough. If replacing reeds is not possible, we recommend using 100-200 pieces, to avoid the limitation of nesting opportunities.

1.3 Place reeds in a protective **PVC tube**. This is optional; however, it can provide important protection against damage from animals and weather.

Note

Using a tube that is a few centimetres longer than the reeds (18-22 cm long, 10–12 cm in diameter), protects the reeds from environmental damage such as direct sun and heavy rain. We recommend using tubes that are 100-110 mm in diameter to ensure space for about 100-200 reed stems – an appropriate quantity if occupied reeds cannot be replaced regularly.

2 **Deploy trap nests**

Note

Different environments will require different deployment procedures, making it hard to generalise. Therefore, the following points are only guidelines. We recommend consulting relevant literature on positioning and orientation before deploying trap nests (e.g., MacIvor, 2016; Yoon et al., 2015; Martins et al., 2012; Khan et al., 2024).

Critical: Deploying your traps at the right time of the year is crucial, as deploying them too late could exclude certain species from your study. Trap nests need to be deployed by the time the first cavity nesting species emerge from their nests. Based on our experience in East-Central Europe, trap nests should be deployed by mid to late February to ensure they are available when the first cavity-nesting species emerge.

2.1 Securely attach trap nests to a tree or pole, to keep them off the ground. Poles should be tall enough so that traps are clearly visible and not hidden by ground vegetation, taking into account vegetation growth that may occur in spring and summer after the traps have been deployed. We recommend deploying more trap nests than are going to be retrieved and processed, to account for potential damage to the traps and to ensure that not all members of the local population are harvested.

2.2 Ensure that the traps are placed in a location where they will not be damaged by vehicles or agricultural equipment.

2.3 Attach a plastic or wooden sheet (roughly 50*50 cm) above the trap nests will provide further protection from heavy rain and direct sun in open habitats.

2.4 Applying wire mesh to the two entrances of the trap nest will keep birds away from the reeds yet still allow access for target species.

3 **Retrieve trap nests from the field.**

Note

We recommend assigning a unique code to each trap upon retrieval. This code will form part of the codes assigned to samples in later steps.

Critical: Retrieve trap nests as soon as the reproductive season is over. For example, in East-Central Europe nests should be retrieved in September. Collection before September may lead to underrepresentation of the full season, while later collection increases the risk of nest degradation by natural enemies and occupancy by non-target overwintering species (e.g. flies, social wasps).

4 **Store trap nests.**

4.1 **Store trap nests at ambient (outdoor) temperature** where further damage can be avoided and broods can finish their natural development.

Note

Collected nests should be stored in a safe place where they are not exposed to any disturbance, such as direct sunlight, extreme warm or cold, excessively low or high humidity, natural enemies, or further occupancy by nesting species or non-nesting species (e.g., flies and ants) looking for overwintering shelter or food resources.

4.2 **Place trap nests in cold storage** (around 4°C and 70% humidity, see Materials for further details).

Note

This step should be carried out once the first frosts occur, as such conditions can pose a risk to the overwintering success of developing offspring. Additionally, holding offspring at a low temperature keeps them from emerging from their cocoons, thus preserving the integrity of the nest and prolonging the optimal processing period.

B. Pre-sorting reeds from retrieved trap nests

5 **Remove a trap nest** from cold storage and free the reeds from their casing and any other binding material.

Note

Enter your name or acronym in the appropriate row of the status datasheet to indicate that the trap nest has been removed from cold storage and processing (pre-sorting) has been started.

6 **Record the number of reeds** in the trap nest (status datasheet).

Note

Reeds that are so damaged that both internodes are uninhabitable for broods should not be included in the count, as they are unsuitable for occupation.

7 **Identify and set aside occupied reeds** for further processing and remove unoccupied reeds.

7.1 Remove reeds that are clearly **unoccupied**. If you can see through the reed lengthwise, the reed is unoccupied.

7.2 Set aside reeds that are clearly **occupied**. If you observe nest material (e.g., mud, leaves, plant hairs) at either end of the reed, it is likely occupied. These occupied reeds will be processed in later steps (see Section C).

7.3 In case of **unclear occupancy** – if you cannot see through the reed, but there are no obvious signs of occupancy (e.g. nest material protruding from either end of the reed) – set it aside.

8 **Cut open reeds** with unclear occupancy (see step 7.3).

8.1 Cut lengthwise into the upper quarter or third of the reed's circumference with a sharp scalpel or blade, and carefully pry it open to expose the contents of the internode.

Note

When **cutting open reeds**, make sure not to run your blade through the centre of the reed, which might house brood. Stop cutting the reed as soon as you find any sign of a nest (e.g. mud or other nest materials) to minimise damage done to the nest. To minimise the chance of injury we recommend cutting into the reed only enough so that the reed can be pried open by twisting the inserted blade.

8.2 If the reed is occupied, reseal the reed with tape and place it in a container together with the other occupied reeds identified in step 7.3.

8.3 If you have found no nests in either internode, discard the reed stem.

9 **Record the number of occupied reeds** found in the trap nest (status datasheet).

10 **Mark the container** of occupied reeds with the unique code of the trap nest whence they came from,
close the container and return the reed stems to cold storage (around 4°C and 70% humidity, see Materials for further details) until further processing (see section **C**).

C. Nest processing and data-recording

11 Remove a container of the **pre-sorted reeds** from cold storage.

12 Record the date of processing of the trap nest you are processing (status datasheet).

13 **Prepare reed for data-recording.**

13.1 Place **one** reed on your workstation.

13.2 Remove any tape from the reed, or cut it open with a scalpel if the reed was not cut open during pre-sorting, exposing one or both internodes.

13.3 If you have found a nest in one of the internodes, assign a **unique identifier** to the nest, consisting of the trap nest id, the reed id and nest id. Enter the **unique identifier** into the appropriate row of the processing datasheet.

> Note
>
> The number of nests in a reed is usually 1 or 2, as there should be only two internodes in a reed; however, in some cases more than one nesting species can occupy and share a single internode, in which case there may be 3 or more.

13.4 Produce a physical copy of the unique identifier and **take a photo** of the nest with its unique identifier label also visible.

> Note
>
> **Photographing** the nest placed on graph paper, with the unique identifier clearly visible and in focus, is recommended to help resolve potential data inconsistencies. This step may slow down processing; therefore, it can be omitted if the person has experience in trap nest handling and hymenopteran taxonomy. It is worth taking photographs before and after the cocoons have been opened to be able to verify the status of each brood cell.

14 **Record nest properties and species traits** (processing datasheet).

Example of a workstation setup at the start of data entry.

Note

Each row should contain data for one nest, thus if one reed contains more than one nest, these are to be processed independently, with their properties entered into separate rows.

14.1 Record basic nest properties: nest id, inner diameter of the reed.

Note

The inner diameter can be measured using a calliper or by pre-calibrated diameter measurer (such as a wooden block with various sized holes drilled into it).

14.2 Record the **material** of the closing plug and partition walls.

Note

A list of several possible categories for nest properties can be found in the supplementary excel sheet (processing datasheet/Data_Dictionary).

14.3 Identify the **nesting species** to the closest possible taxon (see the identification guide and Table 1 for guidance on taxa identification).

	Family	Genus	Food	Nest material	Cocoon	Overwintering stage	Typical diameter of nest (mm)
Bees	Colletidae	*Hylaeus*	pollen	membrane	no	prepupa	2-5(-8)
Bees	Megachilidae	*Anthidium*	pollen	plant hairs (cotton-like)	no	prepupa/ adult (imago)	4-10
Bees	Megachilidae	*Heriades*	pollen	resin	no	prepupa/ adult (imago)	3-5(-9)
Bees	Megachilidae	*Megachile*	pollen	**leaf pieces**, mud or resin	brown egg-like	prepupa/ adult (imago)	3-10
Bees	Megachilidae	*Osmia*	pollen	mud or leaf masticate	brown egg-like	adult (imago)	3-9
Wasps	Crabronidae	*Crossocerus*	flies (Diptera)	membrane	no	prepupa	6-7
Wasps	Crabronidae	*Nitela*	barkflies (Psocoptera)	none	two-colored	prepupa	2-6(-8)
Wasps	Crabronidae	*Passaloecus*	aphids (Aphidoidea)	membrane	no	prepupa	2-5(-8)
Wasps	Crabronidae	*Psenulus*	aphids (Aphidoidea)	membrane	no	prepupa	2-5(-8)
Wasps	Crabronidae	*Solierella*	true bugs (Heteroptera)	varied source of materials	mud	prepupa	2-6(-8)
Wasps	Crabronidae	*Tracheliodes*	ants (Formicidae)	membrane	no	prepupa	7-8
Wasps	Crabronidae	*Trypoxylon*	spiders (Araneae)	mud	black-capped bright biege	prepupa	2-6(-8)
Wasps	Pompilidae	*Agenioideus*	spiders (Araneae)	fluffy plant material	black-capped dark beige	prepupa	4-6 (3-8)
Wasps	Pompilidae	*Auplopus*	spiders (Araneae)	mud	no	prepupa	4-10
Wasps	Pompilidae	*Dipogon*	spiders (Araneae)	fluffy plant material	white	prepupa	4-6 (3-9)
Wasps	Vespidae	Eumeninae*	caterpillars (Lepidoptera)	**mud**, membrane, leaf pieces	membranous	prepupa	2-9
Wasps	Vespidae		*e.g., *Alastor, Allodynerus, Ancistrocerus, Antepipona, Discoelius, Eumenes, Euodynerus, Leptochilus, Microdynerus, Parodontodynerus, Stenodynerus and Symmorphus*				
Wasps	Sphecidae	*Isodontia*	crickets (Oecanthinae)	grass	membranous	prepupa	7-10

Note

If the nest does not contain brood, or has been occupied or destroyed by a natural enemy, but shows signs of previous occupancy (e.g. partition walls or empty cocoons), the nest should still be treated as a nest belonging to the original nesting species.

14.4 Identify any **natural enemies** to the closest possible taxon (see the identification guide for guidance on taxa identification).

Note

Here, we use the term natural enemy to refer to any organism that harms the offspring of the nesting species (e.g., nest parasites, kleptoparasites, parasitoids, nest destroyers, predators).

14.5 Record the **total number of brood cells** and allocate each cell into one of the following 6 categories: empty cells, cells with living offspring, cells with dead offspring, cells with natural enemies and living offspring, cells with natural enemies and dead offspring, and cells with only natural enemies.

Note

If there are more than one natural enemies in a brood cell, this should not affect how the cell is categorised, as the number of cells affected are still the same regardless of the number of natural enemies per cell. The number of natural enemies present in the cells should be noted separately as a comment.

14.6 Record the number of cells containing **pollen** and the colour of the pollen.

Note

Pollen can only be present in the nests of bees. If necessary, take a sample of the pollen for identification and further analysis.

14.7 Record the number of cells containing **prey** and identify the prey taxon (e.g. spiders, caterpillars, or other insects).

Note

Prey can only be present in wasp nests (see Identification guide). If necessary, take a sample of the prey (residues) for identification and further analysis.

14.8 Add any relevant comments related to the nest.

D. Put away samples and developing brood

15 Remove **mature offspring** from the nest and store them **as samples**.

15.1 If the **offspring** are present in their imaginal state (imago), remove them from their cocoons and place all offspring into a vial filled with 70% alcohol for further species level identification by a taxonomist. Place a physical copy (label) of the unique identifier of the nest the individuals came from into the vials.

15.2 Place any and all **natural enemies** into a vial separate from the nesting species, together with a physical copy of the unique identifier.

Note

Separate the samples based on taxonomic group, to make it easy to send them to the professional taxonomist for species level identification.

16 Store reeds containing **immature offspring** in test-tubes for further **development**.

16.1 If the offspring or natural enemies are present in their pre-imaginal state (larva or pre-pupa), **reseal the reed** by taping the two parts back together.

Note

Remove any natural enemies that may spread or multiply within the nest(s), causing damage to the sample (e.g., *Trichodes apiarius*).

16.2 Place the reed, together with a physical copy of its unique identifier, in a test tube and seal the end with a breathable material (e.g., cotton).

Note

- Write the **name of the lowest taxonomic group** that you could assign to the nesting species onto the test-tube for easier recognition or grouping.
- If the reed contains **more than one nest**, separate the internodes and put them into different test tubes along with their unique identifiers. If **separation is not possible**, put the reed in a test tube and write the name of all nesting taxa found in the reed onto the test tube.
- **Leave a small gap** between the entrance of the nest and the closing cotton ball, to ensure that the offspring can emerge from the nest once they have fully developed.
- If a nest contains a cocoon but you are unsure whether it contains an occupant in its imaginal or pre-imaginal state, it is best to place the reed in a test tube for further development, as rupturing the cocoon of a premature offspring may induce mortality.

16.3 Store test tubes containing reeds in cold storage to postpone development (see section **E** for further steps)

17 Once you have processed all reeds in the trap nest, record the number of vials and test-tubes containing samples (status datasheet).

E. Brood development

18 Remove test-tubes from cold storage and keep them at room temperature to speed-up development.

Note

We recommend doing this no later than spring, after all trap nests have been processed, and late-emerging bees and wasps have had time to develop.

19 Check the test-tubes every week to two weeks for any offspring that have emerged.

Note

Storing nests of similar taxonomic groups in one container will make processing easier, because these offspring will likely emerge from their nests at similar times.

20 Remove emerged offspring and/or natural enemies from the test-tube and put them in vials with a physical copy of their unique identifiers, according to the instruction of

section D 15.

Note

Before removing any offspring, we recommend returning the closed test-tubes to a cooler for a few minutes, making the offspring slower and easier to handle.

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