JHR 22: 133–140 (2011)
doi: 10.3897/JHR.22.2099
www.pensoft.net/journals/jhr

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

# Description of two techniques to increase efficiency in processing and curating minute arthropods, with special reference to parasitic Hymenoptera

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Academic editor: *S. Schmidt* | Received 27 September 2010 | Accepted 18 March 2011 | Published 28 September 2011

**Citation:** Gates MW, Buffington ML (2011) Description of two techniques to increase efficiency in processing and curating minute arthropods, with special reference to parasitic Hymenoptera. Journal of Hymenoptera Research 22: 133–140. doi: 10.3897/JHR.22.2099

## Abstract

We describe and illustrate two techniques for enhancing curatorial and processing efficiency as it pertains to parasitic Hymenoptera (Chalcidoidea, Cynipoidea). These techniques were developed in response not only to the massive number of parasitoids that have been acquired through various biodiversity studies, but also the difficulty in mobilizing the human resources to curate this material. The first technique uses small, crystal polystyrene boxes with tight-fitting lids to store dehydrated specimens prior to mounting. Locality information is affixed to the box and specimens are spread in a layer for ease of examination by researchers. Solutions for managing static electricity within the specimen boxes are discussed. The second involves a vacuum pump connected to a funnel with a filtration membrane and flask apparatus to rapidly dehydrate hard-bodied parasitoids (Figitidae) that are not subject to collapse during air-drying.

## Keywords

*Chalcidoidea, Cynipoidea, crystal polystyrene, vacuum pump, curation, dehydration*

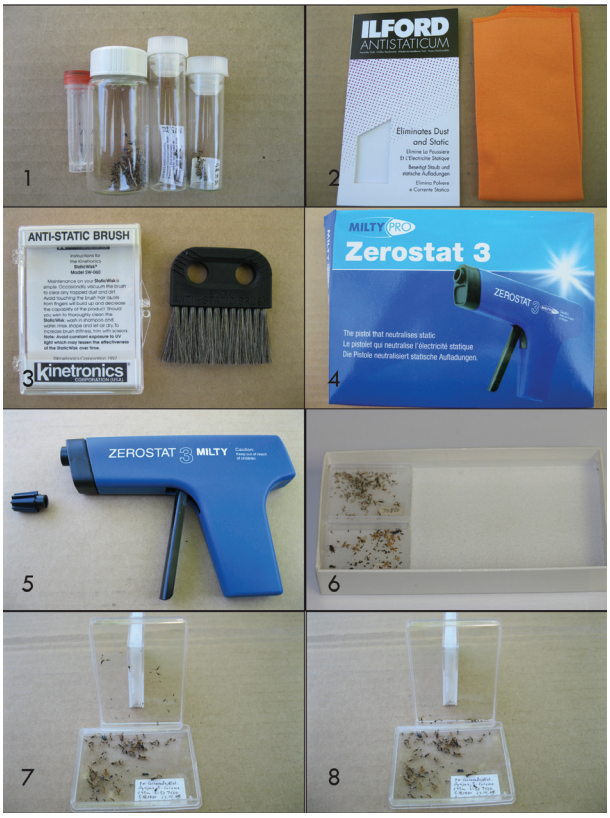
## Introduction

Participation in large-scale biodiversity studies and other ecological research projects (Delabie et al. 2000; Droege et al. 2010; Fisher 2005; LaPolla et al. 2007) involving the collection of arthropods with passive collection techniques (Darling and Packer 1988;

Fraser et al. 2008; Noyes 1989; Townes 1962) generates not only a massive biomass of interest to the conducting researcher, but also a large volume of non-target material, or 'by-catch.' Both of these fractions must be dealt with in an efficacious manner to ensure maximal preservation of the morphological and genomic information of the specimens (Quicke et al. 1999). By using novel, inexpensive separation techniques (Buffington and Gates 2008), and volunteer labor (where possible), samples can be processed and made available for research. Here we illustrate our techniques for processing sorted samples of parasitic Hymenoptera (Chalcidoidea and Cynipoidea). These combined techniques can be considered a model to be applied to organisms of similar size that fit the criteria described herein.

After amassing a large volume of raw insect residues from biodiversity studies from which parasitic Hymenoptera must be separated, we employ an aqueous technique using mesh colanders, plastic tubs, and an orbital shaker to separate each raw residue into a macro and a micro fraction (Buffington and Gates 2008). We then focus on the micro fraction, sorting higher parasitic Hymenoptera taxa into ethanol for distribution to specialists or further processing at the National Museum of Natural History (USNM). In the interim, micro (and subfractions thereof) fractions are stored in –10° explosion-proof freezers (Kelvinator Scientific BT-30C-EXPR) or in our dedicated alcohol sample storage range. Unfortunately, there is insufficient freezer space to properly store our volume of aqueous fractions and the alcohol range is too warm to sufficiently inhibit degradation of aqueous fractions (Vink et al. 2005). An additional constraint to ethanol storage of microhymenoptera is one of handling. Many microhymenoptera are extremely small, making accurate identification difficult while in ethanol; specimens can be difficult to hold in position, and the optical index of ethanol is less than ideal for high magnification examination. Thus, we strive to store many of our micro subfractions in a dehydrated state until resources may be directed for mounting and labeling.

For many taxa (e.g. Chalcidoidea), hexamethyldisilazane (HMDS) is used as part of a chemical dehydration process required to prevent specimen collapse while air drying; we follow the protocols of Heraty and Hawks (1998) for our HMDS processing. For those specimens that can withstand air drying without collapse (e.g., Cynipoidea: Figitidae; Platygastroidea: Platygastridae), we detail a rapid process for specimen dehydration. In either case, the ultimate storage of dried specimens poses problems, and our current method employing a multitude of vials and jars (varying in volume and transparency) is insufficient for the task: examination of specimens ranges from difficult to impossible, storage is non-standard and haphazard, and specimens are periodically at risk of damage during examination (Fig. 1). We summarize here a novel method for storing large numbers of unmounted micro Hymenoptera that maximizes efficient use of space, allows for rapid examination, and protects the specimens from the rigors of handling during examination.

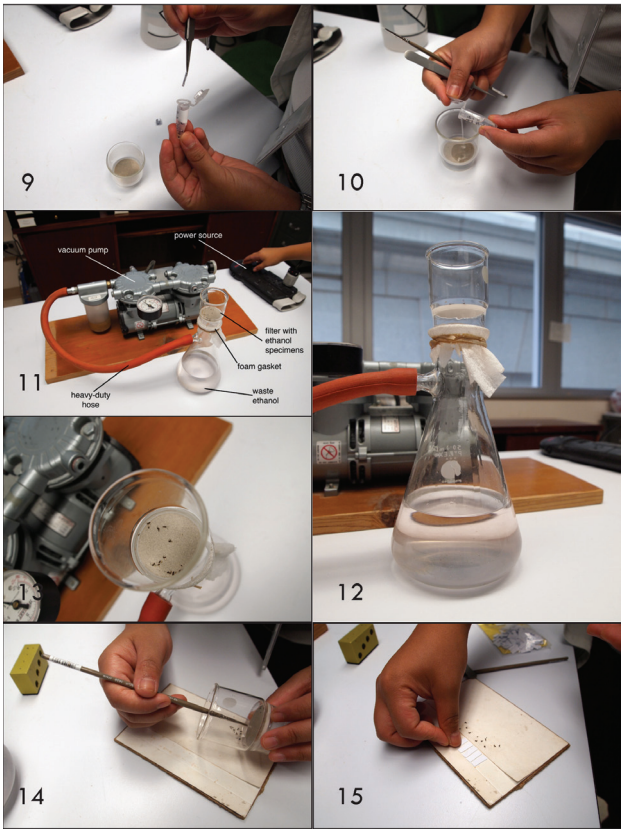



**Figures 1–8. 1** Miscellaneous vials for specimen storage **2** Ilford antistatic cloth. **3** Kinetronics antistatic brush. **4** Zerostat 3 product box. **5** Zerostat 3 gun with cap. **6** USNM ½ unit tray with plastic specimen boxes. **7** Specimens attracted to box lid by static. **8** Specimens at bottom of box lid after static dissipated.

## Materials and methods

Images of supplies, equipment and specimens were taken with either an Olympus PEN EP-1L (Figs 9–15) or a Canon Powershot A3100 digital cameras (Figs 1–8). The crystal polystyrene specimen storage boxes (rectangular cases) are manufactured by Caubère (http://www.caubere.fr/en/produits/carres_rectangulaires/carre_rectangulaire02.htm) and the size we use most frequently is the 56 × 41 × 6 mm. The antistatic cloth (13 × 13" Ilford Antistaticum), antistatic brush (Kinetronics Corp., Model SW-060), and antistatic gun (Zerostat, MiltyPro Zerostat 3) are inexpensive and available online (Table 1).

The vacuum pump is Gast LAA-V104-NQ, a model of oilless miniature rocking piston pump/vacuum; glassware associated with the pump are an Erlenmeyer flask, geological filter, styrofoam padding (for a gasket), and high-density hose. Another filter




**Figures 9–15**. **9** Collection labels are removed from the sample. **10** Specimens in ethanol are poured into the filter. **11** Complete vacuum pump system. **12** Filter under vacuum. **13** Specimens dried after running the pump. **14** Specimens removed from the filter onto a mounting board. **15** Glue boards are arranged.

apparatus tested with success was a GE Disposable 'All-in-one Vacuum Filtration Unit' available from Fisher Scientific. The abbreviation for our collection is USNM (National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA).

## Results

**Clear Box Specimen Storage.** 1) Arrange workspace so that it is clean and lay down an antistatic cloth (Fig. 2) on which to work with specimens and boxes. 2) Brush the inner surfaces of the empty boxes (depending on local static electricity conditions, this may be unnecessary) with the antistatic brush (Fig. 3) to eliminate residual static. The blend of natural hairs and conductive synthetic fibers and conductive nature of the brush dissipate static effectively. 3) If static remains an issue, the Zerostat gun may be used (Figs 4, 5). We typically need to use it only on the inner surface of the lid in these

**Table 1.** Cost of supplies.

| Item | Cost | Source |
|---|---|---|
| Milty Zerostat 3 | $100 | http://www.buy.com/ |
| Ilford Antistatic Cloth | $8.00 | http:// www.amazon.com |
| Polystyrene box (#546) | ~$100/500 | http://www.caubere.fr/en/produits/ (must contact for current pricing) |
| Kinetronics 60 Antistatic Brush | $19.95 | http://www.buy.com/ |
| FJC 6909 3.0 CFM Twin Port Vacuum Pump | $115 | http://www.amazon.com |
| Filtration kit w/ 70mm w/ buchner funnel, flask and filter paper | $18.95 | http://www.amazon.com |

conditions, especially if specimens "jump" onto the lid. To test, static was deliberately generated by rubbing the closed box with specimens on carpeting until specimens were attracted to the lid (Fig. 7). The box was opened with specimens on the lid (Fig. 7), the Zerostat was held approximately six inches away from the lid and activated. The specimens from the lid fell off as the static was dissipated. The gun contains two piezoelectric crystals and compression trigger which, by slowly squeezing the trigger, generates a stream of positive ions, and upon slowly releasing the trigger, releases a negative stream of ions. It requires no batteries and is durable (expect 10,000 trigger pulls). 4) The locality label is affixed with clear tape underneath the bottom of the box (Fig. 7), the specimens are deposited into the box, and the lid is placed on the bottom. 5) The specimens are ready for storage in unit trays in drawers (Fig. 6). Based upon the box size used most frequently, 18 boxes easily fit in a standard USNM ½ unit tray for a total of 128 boxes per USNM drawer. Many sizes are available to fit different taxa.

Crystal polystyrene boxes possess several attributes that are favorable for dry storage: 1) transparency; 2) tight-fitting lids; and 3) high stiffness and dimensional stability. These attributes offer several advantages: 1) labels may be affixed to the bottom and can be viewed while simultaneously examining specimens under stereoscope; 2) enables rapid sorting of specimens not yet available as mounted and labeled material; 3) specimens may be removed for use in research projects as needed with minimal disturbance to non-target specimens; 4) specimens may be easily mailed to other researchers, avoiding disadvantages associated with shipping ethanol-preserved specimens (e.g. hazardous materials packaging, package preparation training, restrictions imposed by shipping companies).

**Vacuum Dehydration.** 1) Remove collection labels from the sample (Fig. 9). 2) Pour ethanol-preserved specimens into the filter (Fig. 10). For samples with an abundance of debris in the ethanol (e.g. lepidopteran scales), additional clean ethanol can be added to float off debris from the specimens. 3) The complete vacuum pump system is illustrated in Figure 11. In this example, the pump motor is always 'on', so the power strip also functions as the 'on/off' switch. Note the heavy-duty hose; this is critical because a weaker hose will collapse under vacuum. The foam gasket insures a firm seal between the filter and the flask below. 4) Filter under vacuum; ethanol is pulled through the membrane, specimens remain in filter (Fig. 12). Recycle or discard waste

ethanol. 5) Dry specimens by running the pump for 30 seconds (Fig. 13). 6) Remove specimens from the filter onto a mounting board (Fig. 14). In cases where there are greater than 50 specimens, the majority can be removed by inverting the filter. Some specimens will remain on the filter membrane, and these can be removed using a micro paintbrush. 7) Arrange glue boards in preparation for affixing wasps (Fig. 15).

## Discussion

Researchers attempting to examine dried, unsorted material stored in miscellaneous transparent or translucent vials face the challenge of searching for specimens of interest through the vial wall and within a specimen bolus of various sizes (e.g., Fig. 1). The alternative is the more time-consuming process of emptying the specimens on a tray and sorting through them under the stereoscope, before placing them back into the vial. Clear boxes solve both issues and allow the sorter to open a box, remove the specimens of interest, and mount them immediately for use or put them in a gelatin capsule. Advantages over long-term cold storage include no electrical requirements or storage issues related to flammable liquids. Previously, dried material was stored in a variety of vials of different sizes, making it difficult to efficiently sort specimens. If static is a problem, the Zerostat gun can be used for spot treatments.

Advantages for using the vacuum pump system for drying hard-bodied micro Hymenoptera include: 1) speed of curation dramatically increased (uninterrupted drying and mounting can achieve 500 specimens/day); 2) no need for hazadous chemical handling (including a fume hood, gloves, lab coat, and goggles); 3) low cost; once the vacuum pump system is assembled, there is no further investment required. Further, many laboratories are equipped with 'lab-vac' alongside compressed gas for burners. A lab equipped with such vacuum does not require the acquisition of a vacuum pump. In conjunction with the polystyrene boxes, thousands of specimens per day can be taken from ethanol and stored dried, awaiting examination at a later date.

Success using the vacuum pump drying technique can be influenced by the size of the arthropod being dried. In our experience, larger cynipoids that have thick cuticle (e.g. Liopteridae) tend to take longer to dry. In some instances, a specimen that appeared to look dry upon initial inspection clearly was not done drying when examined under a microscope while mounting. In these cases, ethanol could still be seen evaporating from setae. If this occurs, the specimens are returned to the vacuum apparatus and dried for a longer period of time.

Although the vacuum pump rapidly air-dries specimens, there exists the potentially deleterious side effect of damaging DNA due to the extended contact of specimen tissues with residual water (i.e. enzymatic cleavage in presence of water and oxidation) (Junqueira et al. 2002), particularly in the meso- and metasoma (see comments above). Drying times to ensure tissues are not decomposing any further, yielding a specimen for dissection and/or tissue DNA extraction, are currently unknown. However, the presence of residual water at the drying stage depends in large part on how the speci-

mens were killed (Dillon et al. 1996), subsequently preserved, and stored (Eglinton and Logan 1991). Thus, specimen preservation is more important than other factors (e.g. specimen age, dehydration technique) for DNA recovery (Junqueira et al. 2002). Nevertheless, the speed at which the internal tissues dry can affect the ultimate state of preservation of DNA for subsequent genomic extraction (Quicke et al. 1999; Nagy 2010). The HMDS dehydration method, along with other chemical-based techniques, is demonstrated to yield high quality genomic DNA (Austin and Dillon 1997); some DNA sequence data in Buffington et al. (2007), using the chelex extraction protocol, was generated from cynipoid specimens that were vacuum dried in the manner summarized here, but no quantification of success vs. failure of DNA amplification was documented. Many of the arguments concerning dessication rate are marginalized when one considers recent improvements made to the sensitivity/specificity of DNA extraction and amplification protocols applied to degraded samples (Junqueira et al. 2002). We must underscore that the methods summarized here have been developed in response to biodiversity surveys yielding tens of thousands of specimens, the vast majority of which require mounting to determine morphospecies. We feel the vacuum pump technique helps remove one more barrier in the often difficult process of collection building and generating species inventories.

## Acknowledgments

We gratefully acknowledge Gérard Delvare (CIRAD, Montpelier, France), who initially brought the crystal polystyrene boxes to our attention. We also thank Natalia Vandenberg and Thomas Henry (Systematic Entomology Laboratory [SEL], PSI, ARS, USDA) for their critical review of this paper. Jeffrey Sosa-Calvo (Smithsonian Institution, Department of Entomology) provided useful comments after reviewing this manuscript.

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