Figure 1. Six-metre Malaise trap in author`s yard on the edge of Ottawa, Canada, 28 September 2020. Insert: pinned and labelled tachinids (minus siphonines) caught in trap over nine days, 7–15 July 2020 (specimens turned on angle are interesting taxa or good for images).

How to make your tachinids *STAND OUT* IN A CROWD

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Dedicated to the memory of Monty Wood who taught me the importance of well prepared tachinid specimens.

Introduction

It is a cruel quirk of fate that the most fastidious of insect preparers tend not to be dipterists. They are more likely to be lepidopterists who arrange the wings of moths and butterflies with such precision that one side is a mirror image of the other, or coleopterists preparing specimens of the larger and more showy species. Yet these same collectors tend to treat flies as bycatch and only grudgingly stick pins through some of them for their dipterist colleagues. If a #3 pin is thrust through the centre of a 3mm long *Catharosia* sp. with the legs pinched up tight against the body, is anyone going to care? Sadly, variations of this practise are not uncommon even among some dipterists. How many times have I been brimming with excitement as I opened a box of specimens received in the mail, or pulled out a drawer in a foreign collection, only to gaze in horror at a multitude of ugly and misshapen specimens? It doesn't have to be like this.

Don't get me wrong, I am not advocating specialized spreading boards for flies or elaborate time-consuming methods. Just a little more care: relax your specimens, pin them, push up the wings, pull down the legs, straighten the head, and the basics are done. If your specimens are covered with moth scales then you can make them sparkling clean in a minute or two. If you are so inclined then male terminalia can be exposed for future study, legs that have dropped off can be saved for DNA barcoding, and delicate specimens can be frozen to allow them to dry slowly and not shrivel. These techniques will produce specimens ideal for taxonomic study. Characters that are useful for identification will be maximally exposed and specimens can be compared with less effort.

Early beginnings

In mid-1970s I took an introductory course on entomology taught by coleopterist Henry Howden at Carleton University in Ottawa. Our textbook was the new fourth edition of the beloved *An Introduction to the Study of Insects* (Borror *et al*. 1976). The chapter at the end of the book has 45 pages on collecting, preserving and studying insects. The methods reviewed there are still relevant today, but the same information is now readily available to all on the Internet.

> ***"I believe I can say without hyperbole that Monty's specimens are unrivaled in collections the world over."***

My insect course at Carleton led to a summer job in the Diptera Unit of the Canadian National Collection of Insects (CNC) in 1977 and 1978. I also worked part-time for D. Monty Wood between the two summers, at Monty's personal expense. It was during this time that I learned why properly prepared tachinid specimens are so important. I received some instruction on this topic from Monty but I could also see for myself his meticulously prepared specimens throughout the Diptera collection and especially in the Tachinidae cabinets (e.g., Fig. 11). I believe I can say without hyperbole that Monty's specimens are unrivaled in collections the world over. In the CNC, I can generally spot his specimens immediately, before looking at the labels. It is this gold standard that I try to emulate with my own specimens.

Start fresh

The key to perfect specimens is to start with fresh and relaxed material. Last summer I worked a little harder on this step of the process. Stuck at home due to the COVID-19 pandemic, I ran a 6-metre Malaise trap in my backyard on the edge of Ottawa from April to November to survey local tachinids. This was a continuation of similar efforts in 2016 and 2017 but this time I could empty the catch two or three times a day (e.g., Fig. 2), seven days a week, for the entire period. The trap was run "dry" with a 3cm x 6cm section of Ortho® Home Defense® Max™ No-Pest® Insecticide Strip as the killing agent. The trap head was removed each evening around dusk and replaced early the next morning to cut down on the number of moths mixing with the other insects and coating them with scales.

My former method for holding dead tachinids until pinning time, whether in the field or at home, had been to place them in a container with soft leaves until evening or the next day, often in a cooler or refrigerator. Tachinids tend to stiffen when they die and a little time and moisture will relax them. Although this method is commonly used and produces good results, most of the time the specimens are a little shy of perfectly relaxed and their legs and head often spring back a bit from where they are put.

Figures 2–5. 2. Malaise trap sample from 4 July 2020 with Canadian quarter for reference. **3**. Relaxing container with wet sphagnum moss for moisture and tissue-covered tray for specimens (26 July 2020). **4**. Fresh specimen of *Ptilodexia* sp. coated with hairs and scales. **5**. Same specimen after bath and arrangement of wings and legs.

I tried a few variations of the above last summer to increase the moisture content and more fully relax specimens. The method that worked best is shown in Fig. 3 and was used for all the specimens that illustrate this article (except Fig. 11). The bottom of a plastic container (my container having a rim diameter of 12cm and depth of 6cm) was lined with sphagnum moss (depth of 2cm) and generously watered. A small concave dish with a tissue liner was placed on top of the moss and held each day's catch of tachinids. The container was covered with a tight-fitting lid, not refrigerated, and specimens were pinned sometime the next day. The tissue liner was replaced periodically but the same sphagnum moss was used continuously until the end of the season, with just the occasional replenishment of water. Sphagnum moss has anti-fungal properties and, as I learned, is quite resilient because I had no issues with it in a plastic container for six months.

Pinning

Just a quick review here of pinning fundamentals for those getting started. There is a lot of personal choice involved in whether to use stainless steel or black enameled pins (I prefer the latter) and what pin sizes to use (I like #00 to #2 and double-mounted minutens for tiny specimens in the 4mm range or smaller). The standard method for pinning is to hold a tachinid vertically between the thumb and forefinger of one hand and with the other hand push a pin through the thorax to the right of the midline and in front of the wing with an exit hole in front of the mid coxa. Wearing a headband magnifier (e.g., OptiVISOR 2.75X) is a great help for accurate pinning. Position the specimen low enough that it will not be touched when holding the pin and high enough that it will not interfere with a label below. Label height is a personal preference; I like a height of 15–16mm from the pin tip and make my own labelling blocks with this depth (Fig. 6; hole drilled with a 1/32" drill bit).

Figure 6. Labelling block.

Specimens mounted from alcohol or too brittle for pinning can be glued to the side of a pin. This is the method of choice for some dipterists who work with small to medium-sized specimens. There is much debate about what type of glue to use and this topic is nicely reviewed in the first link under Other Resources below. A favourite for decades in the Diptera Unit of CNC is shellac glue but you have to make it yourself and experiment with consistency (Walther 1997). It is also slow to dry and thus best suited for mounting smaller specimens.

Some further techniques and comments pertaining to the mounting of flies are given in:

Mounting tachinids from ethanol (O'Hara 1994)
Minuten mounts for micro-Diptera (Sabrosky & Mathis 1997)
Mounting methods for small Diptera (Wheeler 1997)

It's all in the details

If you are not particular about the look of your specimens, read no further...

For those still reading I will outline what you can do in less than a minute if you are starting with a pinned and well relaxed tachinid. This work is best done under a stereoscopic microscope except for the first step involving the wings.

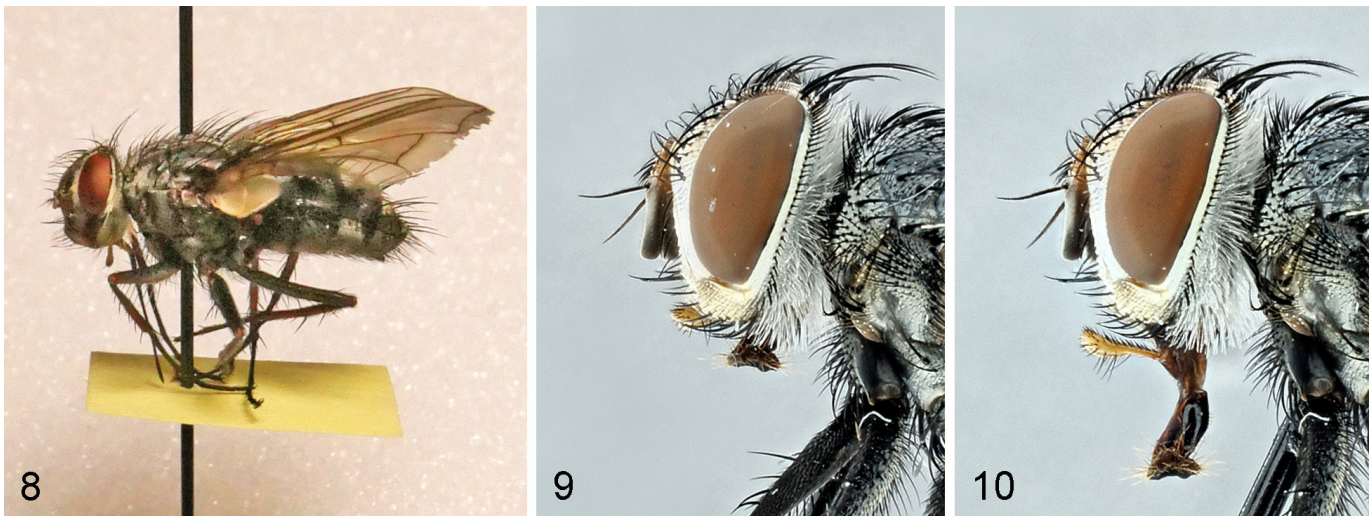
Wings. If these are not already in the upright position, place tips of forceps under them and gently lift upwards, squeezing gently inwards on the thorax to help "lock" them in place when they reach the "up" position. This may have to be repeated a few times until they stay in place. If the wings continue to drop down then they can be braced upright later on a pinning board (Fig. 7).

Figure 7. Specimens on Styrofoam pinning board showing three with wings braced in upright position and two with legs saved on sticky portions of yellow Post-it® notes.

Legs. These frequently get pinched up against the thorax when the specimen is pinned, obscuring diagnostic features on the side of the thorax. Pull each leg downwards and disentangle legs if necessary. They will partially spring back if not thoroughly relaxed, but this is often enough to see the sides of the thorax. Legs that are particularly long, like those of many Dexiini, can be pushed upwards by a piece of paper and arranged in that position (Fig. 8). The paper is removed when the locality label is attached.

Head. This rotates at 90° to the axis of the body, meaning that in death the vertex is often to the right or left of the centreline. Place one tip of a forceps against the proboscis and rotate the head into alignment with the thorax. As with legs, the head will not stay in its new position unless its flexible connection to the thorax is fully relaxed.

Proboscis. Sometimes the base of the proboscis is retracted into the oral cavity and its full length cannot be seen and the palps are partly obscured (Fig. 9). The palps will generally pop into view with a gentle pull on the proboscis (Fig. 10). I just started doing this last summer.



Figures 8–10. 8. *Mochlosoma* sp. with long legs held in bent position with piece of paper. **9**. *Belvosia unifasciata* (R.-D.) with proboscis retracted and palps partially obscured. **10**. Same specimen with proboscis and palps exposed.

Setae. Some of the larger setae on the head or dorsum of the abdomen (not often on the thorax) are sometimes askew. This is a small matter but they will "click" into position if pushed in the right direction. Do not try this with dried specimens as the setae will break off. Setae on relaxed specimens have a natural position to which they can be returned.

Dirt. The foregoing can be done quickly but removing debris such as pollen, broken setae, lepidopteran scales, etc., takes more time. Forceps are not the right tool for such delicate work. I recommend making a simple "cleaning tool" that bends when it touches setae and body parts. Take a short length of 3mm dowelling (sold at dollar stores in the arts and crafts section) and drill a hole in one end with a 1/32" drill bit, or use an insect pin to create a hole. Insert a 1cm length of hair from a fine paint brush and glue in place (same method as used for terminalia hooks shown Fig. 11).

Pinning boards. Individual preference will dictate where specimens are placed after pinning and before labelling. I like to arrange them on pinning boards made of Styrofoam with a wood backing to add weight. These boards are also handy for labelled specimens if the specimens are not going into unit trays in a collection right away (see insert in Fig. 1). Delicate specimens that are apt to shrivel as they air-dry can be pinned and kept in a freezer for several weeks to slowly "freeze-dry" with less shrivelling.

Bath time

A little-known fact is that most fresh tachinids can be washed. I learned this technique from Bruce Cooper, a long-time technician in the CNC Diptera Unit. This is effective in removing most hairs and lepidopteran scales that are resting on a specimen, as is often the case with fresh material left overnight in a Malaise trap. A *Ptilodexia* sp. pinned from such a moth-filled Malaise sample is shown in Fig. 4. It was held under a gentle stream of water from a tap for about 10 seconds while being rotated to bring all surfaces in contact with the flow of water. The wings were then set in the upright position, some remaining debris cleaned off under the microscope, and legs adjusted (Fig. 5).

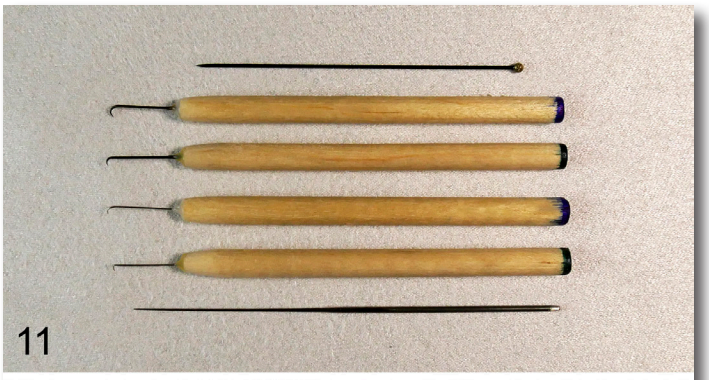
WATER REPELLENCE

A curious fact about tachinids is that many are water repellent. After a wash under a stream of water they are not wet and what water remains is in the form of droplets that can be wicked from the body and setae with a tiny piece of tissue. I have seen some wetness around the mouthparts and a few taxa do allow water to wet their surfaces: the phasiine genera *Gymnoclytia* B. & B. and *Gymnosoma* Meigen, for instance. There could be evolutionary advantages to being water repellent, as well as bristly. Sømme (1989) reviewed literature on adaptations of insects to alpine environments, including increased hairiness in the context of thermoregulation.

Male terminalia

The terminalia of male tachinids represent a wealth of valuable characters for both identification and phylogenetic inferences (e.g., Cerretti *et al*. 2014). A method for removing male terminalia from dried specimens is given further below. Here I will describe quick methods for removing or exposing the terminalia of male tachinids.

Removing male terminalia from a fresh specimen is easy and can take less than 30 seconds. First, make a terminalia hook from a minuten or small-diameter pin (#00, #0). To do this, grab the tip of the minuten or pin with strong forceps and twist with a semi-circular motion to produce a nicely curved hook. Make several using different pin sizes for working with small to large tachinids. I like to embed these hooks in 3mm dowelling (more about the dowelling above under Dirt) (Fig. 11).



Figures 11–12. 11. Four terminalia hooks, a tungsten steel needle, and a #2 insect pin for scale. **12**. A male of *Eribella exilis* (Coquillett) with terminalia exposed (TachImage-00576). This specimen was collected by Monty Wood and its preparation is typical of Monty's style. It was caught at Duncan Lake, Masham Township, Quebec, on 17 July 1997 (Monty's cottage).

Under a microscope, hold the pinned tachinid in one hand and terminalia hook in the other. Turn the fly over so the underside of the abdomen is visible. Insert the hook between the 4th and 5th abdominal sternites, hook the edge of sternite 5, pull out the genital capsule, and wrap the removed parts around the pin below the specimen. Some of the digestive tract will usually come out with the genital capsule and it can be wrapped around the pin to adhere the terminalia to the pin when it dries. If you want to be tidy then shove any remnants of the digestive trait that are dangling from the abdomen back into the cavity of tergite 5. The terminalia can be removed from the pin using very warm water or an insect relaxing fluid. I made a low-quality movie years ago showing the procedure for removing the male terminalia of a tachinid that is now available here:

http://www.nadsdiptera.org/Tach/WorldTachs/TTimes/tachinid.mov

Gluing terminalia below specimens will facilitate their examination later on if they are needed for closer study or a taxonomic revision. The downside is that parts of the terminalia are usually hard to see when stuck to a pin and must be removed and cleared for study, then stored in glycerin. (The steps are the same as given in the next section except the terminalia are already separated from the abdomen.) Some species can be distinguished by differences in the male surstylus and cerci and these parts can be exposed on fresh specimens without the need to remove the terminalia. Insert the tip of a terminalia hook into the gap posterior to sternite 5 and twist to catch on to or near the base of a surstylus. Gentle twists and pulls will usually free the surstyli and cerci enough that they can be viewed *in situ* (Fig. 12). There can be greater difficulty in doing this with some taxa than others and there is some risk of damage when the genital parts do not cooperate.

Dissecting male terminalia from pinned specimens

I gave a description of how to prepare male terminalia for taxonomic study in a revision of the Polideini (O'Hara 2002). That publication is not easy to obtain online so I will quote the directions here (pp. 13–14):

> "The abdomen of a male was carefully removed at its junction with the thorax and placed in 10% hot (not boiling) NaOH[1,2] until slightly flexible (about 15 minutes). During this time, the abdomen was periodically and gently squeezed against the side of the container to remove trapped air bubbles. The abdomen was then placed in a microdish with 50% acetic acid, held against the bottom of the dish with a curved and blunt pin (inserted into the opening at the base of the abdomen) and the terminalia (sternite 5 to cerci) carefully cut out using a fine tungsten needle[3]. The abdomen was taken through 70% and 95% EtOH washes (10–20 minutes each) and a final xylene or toluene wash (30 minutes) before being reattached to the thorax in its original position with shellac glue. If the abdomen was placed in position a few seconds before it was completely dry, then the residual xylene would thin the glue momentarily and a smooth bond would often form. Air-drying an abdomen directly from EtOH is not recommended because it almost invariably results in severe curling of the abdomen. After the male terminalia were removed from an abdomen, additional clearing was necessary so that the structures could be separated further for study. Two methods were used for this purpose. The first method, as described by O'Hara (1983), involved additional clearing of the terminalia in 10% NaOH, neutralization of the base using a 20% acetic acid wash, two EtOH washes (70% and 95%), and examination and storage of the terminalia in glycerine. The concentration of the acetic acid wash in the present study was raised to 50% from the 20% used by O'Hara (1983) because some of my dissections performed years ago for other studies have continued to clear in storage[4].
>
> I began using lactic acid[5] for terminalic preparations late in the present study to alleviate overclearing of terminalia. Lactic acid has two advantages over NaOH for terminalic dissections: less danger of long-term over-clearing, and sclerotized structures are not cleared. The latter is particularly important for the study of the anterior sclerite of the distiphallus, which is easily over-cleared in normal NaOH preparations. Male terminalia, removed from the abdomen as explained above, were placed in ceramic dishes with 100% lactic acid and heated in a microwave oven for 10 seconds[6] at a time until soft tissues had cleared sufficiently (generally 3–5 repetitions at intervals of about 5 minutes). The terminalia were then placed in a microdish of glycerine for further dissection. The terminalia proper were removed from sternite 5 and the ring sclerites. Often the dissection would end at this stage, but if further dissection was necessary for more detailed study or for illustrative purposes then the hypandrium (with pregonite, phallus and postgonite attached) was disarticulated from the epandrium and often the phallus would be separated from the hypandrial complex. Terminalic structures were either placed in special trays (O'Hara & McIntyre 1984) with fresh glycerine for periodic study and temporary storage or in microvials (pinned below adult specimens) with glycerine for permanent storage."

A few notes pertaining to the above (see superscript numbers in text):

1. NaOH (sodium hydroxide) vs. KOH (potassium hydroxide). The latter is more commonly used as a clearing agent. The former is reportedly a little slower acting, which is good if you want to stop the clearing at a precise point, but I have no experience comparing the two.

2. Juan Manuel Perilla López (pers. comm.) has been experimenting with the contact lenses cleaner Ultrazyme™, a digestive enzyme, as an alternative to NaOH/KOH. He does not yet have a protocol for its use. Kanaar (1990) wrote about using Genitase, a proteolytic enzyme, for clearing terminalia.
3. A fine tungsten steel needle is shown in Fig. 11. It can be repeatedly sharpened using super fine emory sandpaper. It is preferable to a minuten or fine insect pin because the steel is stronger and the fine shaft is relatively inflexible. I found several at my place of work early in my career and still use them; I do not know if they can be purchased anymore.
4. Terminalia cleared with NaOH or KOH may continue to clear slowly over time (decades) despite washes of acetic acid that are meant to stop the process. Light-coloured terminalia seem more prone to clearing over the long term. I have a policy of not allowing terminalia of holotypes to be cleared with either chemical unless images or drawings of the terminalia will be published.
5. See Cumming (1992) for more about the use of lactic acid.
6. Microwave ovens are stronger now than when the above was written and heating terminalia in a small ceramic dish for 10 seconds in a modern microwave will likely cause the lactic acid to bubble and/or the terminalia to explode. It is best to experiment with shorter times and expendable terminalia. Another option, albeit slower, is to gently heat a dish of 10% NaOH or lactic acid on a coffee mug warmer. This is my method of choice now.

Save that leg

The legs of tachinids are prone to falling off no matter how gently they are touched during pinning. It is rare to pin 25 specimens without some legs left over. This is more common with the smaller and less robust specimens but no species is immune. I could not be bothered in the past to save these cast-off appendages but with the advent of molecular systematics and DNA barcoding there is more reason to keep them. I simply cut a small rectangle from the sticky end of a Post-it® note and press a leg against it, then pin this below the specimen (Fig. 7). There is not a firm attachment and the leg is easily removed for molecular use. So far I have not seen legs fall off on their own during normal handling but I would not recommend this method for permanent storage.

Comparing two specimens at once

At the beginning of this article I wrote: "Dedicated to the memory of Monty Wood who taught me the importance of well prepared tachinid specimens". I want to explain what I mean by this. Flies of some families are fairly recognizable based on external features (e.g., Bombyliidae, Syrphidae) or male terminalic features (e.g., Sarcophagidae, Sphaeroceridae). Other families, like the Tachinidae, fall somewhere in the middle with some taxa exhibiting one type of difference or the other, rarely both types in the same taxon, or sometimes only subtle differences in any characters. *Lespesia* R.-D. and *Winthemia* R.-D., for example, fall into this last category. In closely related species, slight differences in the width of the parafacial, length of postpedicel, shades of silver or gold on the fronto-orbital plate, abdominal setation and colouration, are most easily compared between specimens that have all their parts similarly aligned. Differences can be so slight that looking at two specimens at once is the best way to compare them; one pin held below the label in one hand and the other pin held by the pin head with the other hand so the specimens can be moved back and forth in tandem. Trying to do this with badly pinned specimens with heads rotated at odd angles and legs all over the place is frustratingly difficult. DNA barcoding has been confirming that the slightest of differences can be species specific.

Other resources

Museum Specimen Preparation Guidelines.

There are a number of guides to preparing insects. This one is more detailed and comprehensive than most about labelling, pinning and gluing specimens (by Heraty, J., Yanega, D. & Triapitsyn, S., undated):

https://entmuseum.ucr.edu/specimen_preparation/index.html

Note: I agree with the authors that "anything bigger than about 15x7mm is getting too big" for an insect label but I differ with respect to font type, font size and maximum number of lines. They recommend "Times (or maybe New York)", "4 point lettering" and "5 lines". They note that letters can bleed together using Arial font. This is true, but I like the uniform thickness of Arial letters and I avoid letting them touch by selecting 105% kerning (this moves the letters apart slightly). With this kerning I can print at 3.5 point with letters sharp and not touching provided the printer is up to the task. I reduce line spacing to 95% to bring the lines closer together. With these adjustments I get 6 lines of data in the same 7mm width recommended by the authors (compared to their 5 lines). In recent years my 6th line is often a database number like "CNCxxxxxxx". I prefer to have this number on the locality label rather than print a second label for it.

Entomological supplies.

There are suppliers around the world with specialized equipment for entomologists. In North America I recommend *BioQuip Products* (https://www.bioquip.com). They are knowledgeable, reliable and professional. Among other things I like their insect pins and label paper because both are high quality (the paper is 100% rag and acid-free, the gold standard for permanent labels). If you can locate the original sources you might save a little money but you also risk buying inferior materials.

Collecting methods for tachinids.

My review paper on the Tachinidae of Gila National Forest in New Mexico, USA (O'Hara 2012) was written for a general audience and briefly outlines common methods of collecting tachinids: handnetting, sweeping, sugaring, Malaise trapping, hilltopping and blacklighting.

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