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A technique to dry mount Hymenoptera (Hexapoda) from alcohol in a few seconds, and its application to other insect orders

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Abstract

A new technique is presented that allows mounting insects directly from alcohol storage, while still wet or soaked, without any chemical treatment. It works particularly well for many groups of Hymenoptera, but not with delicate or soft specimens. Preliminary results are also commented for some Coleoptera, Diptera, Hemiptera, Mantodea, Neuroptera, and Orthoptera. External drying is performed with the aid of a small electric air pump. A 600 L/min air current quickly and efficiently spreads the specimen's wings and pilosity, while helping to position the antennae, legs, and abdomen. Specimens need to be pinned, even if small and pinned with minutens; this generally means the new technique will work for any insect greater than 3 mm long. This is precisely the size range for which an efficient mounting aid for alcohol preserved specimens was lacking.

Key words: Biodiversity, Collections, Diptera, Malaise, mounting, pinning, YPT

Introduction

With the advent of mass-collecting techniques for insects, such as the year-round and simultaneous usage of dozens of Malaise traps (*e.g.*, Hanson & Gauld 1995), collection trips employing thousands of Moericke traps (= yellow pan traps) distributed along several kilometers (*e.g.*, Aguiar & Santos 2010), or canopy fogging (Adis *et al*. 1998), sampling the entomofauna became extremely productive. But the need to mount all the resulting material before it can be studied—that is, processing all field samples into tens of thousands of adequately pinned and labeled specimens—generated a problem of equally impressive proportions. Mounting insects, even the easiest ones, requires some degree of skill and training, as well as time. Because of this, mass-mounting insects is an expensive and time-consuming activity. Mounting some insects becomes considerably more difficult if the specimens have membranous wings (about 30% of all living insect taxa) or well-developed pilosity (*e.g.*, bees and many flies), and were collected or are stored in alcohol. This is because adequately spreading and drying the wings and pilosity of alcohol-wet specimens is not easily performed, and might require expensive and/or dangerous substances and equipment, as is the case with Critical Point Drying (Gordh & Hall 1979), HMDS (Brown 1993; Orozco & Gaimari 2012), and several other techniques (Fisher & Jursic 1958; Vockeroth 1966; Sabrosky 1966; Truman 1968; Martin 1977:156; Noyes 1982; Taylor 1993; Noort 1995). Furthermore, these techniques are mostly indicated for small or tiny specimens (under 2–3 mm), and some of them will seriously damage the DNA of the specimens (Dillon *et al*. 1996).

Notwithstanding, biodiversity research about insects increasingly depends on field trips where copious numbers of specimens are collected, preserved and transported in alcohol, and then need to be dry mounted. And yet, since mounting even a reasonable fraction of such material is quite time-consuming, the vast majority of the collected specimens are usually stored in alcohol, often remaining there for many years.

The present work aims to proposes the use of a new, instantly mastered technique, which reduces considerably the time needed for processing and dry-mounting some large groups of insects preserved in alcohol, while still generating mounted specimens which are perfectly suitable for investigations ranging from taxonomic research to molecular studies.

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Proposed technique

The key idea is to use an air pump to externally dry and reposition the appendages of specimens pinned while still soaked in alcohol. Tests were performed using an INTEX™ air pump (Figs 1–2), with a fixed air flow of 600 L/min. This equipment is cheap and widely available commercially. Specimens must be pinned immediately once removed from alcohol (Fig. 6). They should next be submitted to a frontal air blow of moderate intensity (Figs 7–8), which is achieved at a distance of about 4–8 cm from the air pump. This will quickly (1–4 seconds) remove the excess of liquid (Fig. 7) and a few seconds later will also efficiently dry the specimen externally (Fig. 8), while spreading and pre-adjusting the position of antennae, legs, wings, pilosity, and often also the metasoma (Figs 9–10). For the numerous specimens which are already in a convenient mounting position while still in the alcohol, even a single, quick air blow will dry/mount at once, leaving specimens ready to be set aside to fully dry (or dried in a laboratory oven). In many cases, however, sessions of 10–40 seconds yield better results. Large specimens (e.g., Fig. 28) may of course require longer sessions. The use of a hair dryer is not recommended, but see also the topic *Heated air*, under Discussion.

FIGURES 1–2. Air pump of 600 L/min, used in this work. Frontal air exit measures 32 mm in diameter. **1**, Air pump. **2**, Nozzles; from top to bottom: #1 (20 mm dia.), #2 (12 mm dia.), #3 (5 mm dia.).

Bulk drying. To process several specimens at once (Fig. 15) they must all first be pinned while wet, and then returned to alcohol, e.g., in a Petri dish (Fig. 14), to avoid any drying before the blowing procedure. A set of wet pinned specimens are then fixed on a firm surface, such as foam or cork, all facing the same direction, and then blown frontally for a few seconds (Fig. 15).

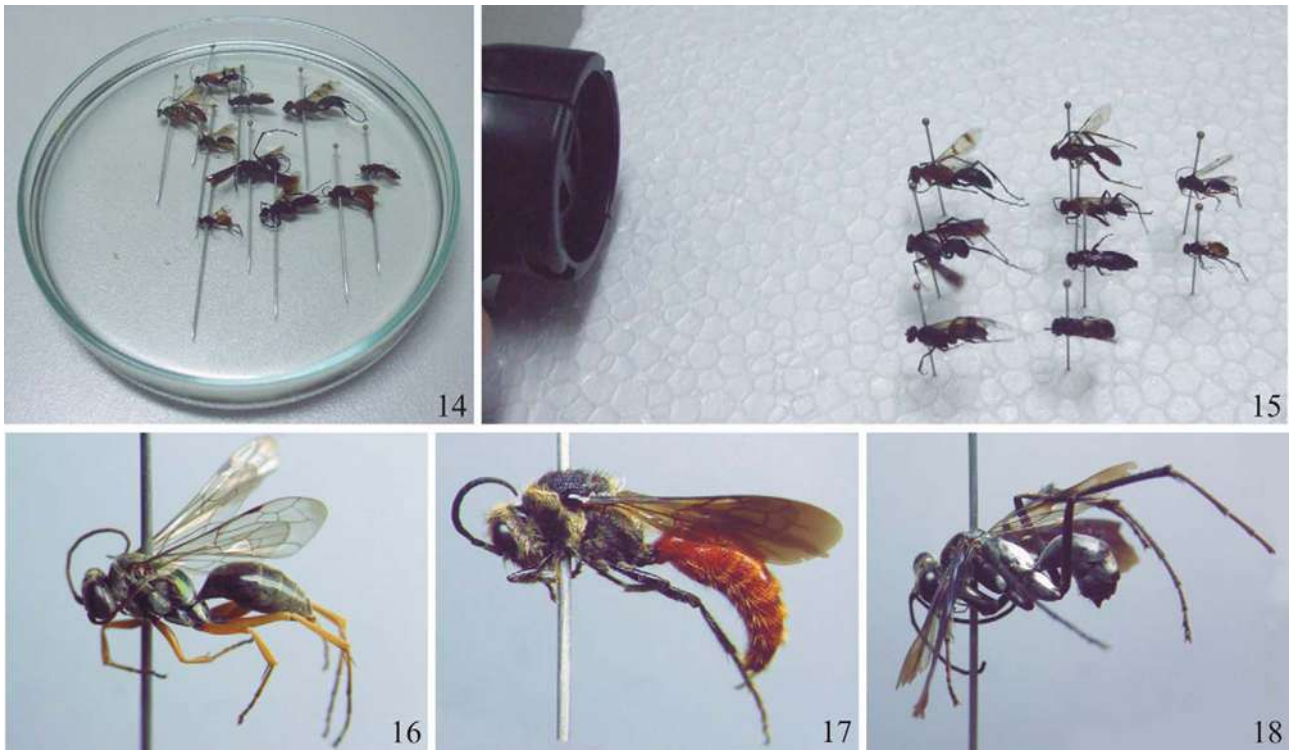
Results

Individual manipulation (Figs 6–8) and bulk drying (Figs 14–15) produced similar final results for the tested specimens (Figs 9–10, 20–21 *vs*. Figs 16–18). Processing specimens one at time, however, will of course allow more accurate manipulation, useful for specimens in twisted or convoluted positions. Excellent results were also obtained with small Hymenoptera, mounted in minutens (Figs 11–13).

Good or excellent results were obtained with densely pilose bees processed using the new technique (Figs 20–21). Comparatively, specimens of the same species and collecting event ended with lumps of collapsed hairs when mounted through conventional techniques (Fig. 19). Results with all tested Hymenoptera remained stable after specimens were fully dried, with body and appendages in the same condition as observed just after blow drying.

FIGURES 3–10. A specimen of *Liris* sp. (Hymenoptera, Crabronidae) under different stages of mounting. **3**, Stored in 85% alcohol, after one year. **4**, Condition of specimen immediately after removed from alcohol and briefly dried on an absorbent card. **5**, Detail of right wings on glass surface, after receiving a drop of alcohol; note folding of hind wings. **6**, Pinned specimen, still soaked in alcohol; left side. **7**, Same specimen, immediately after it started receiving an air blow – note raised metasoma, and left hind wing still wet, applied over the body. **8**, Same as previous, 15 seconds later; all wings now vibrating; note golden pilosity already drying on gena, mesosoma, and part of metasoma. **9**, Same, a few moments after receiving a 20 seconds session of air blow; specimen not manually repositioned in any way. **10**, Same, dorsal view.

FIGURES 11–13. A 4.5 mm long specimen of *Bracon* sp. (Hymenoptera, Braconidae) under different stages of mounting, after stored in alcohol for 7 years. **11**, Pinned with a minuten, in alcohol. **12**, Double mount, while still soaked in alcohol. **13**, A few moments after a 30 seconds session of air blow.



FIGURES 14–18. Bulk drying. **14**, Petri dish with specimens pinned directly from alcohol, waiting for blow drying processing while once again fully immersed in alcohol. **15**, Same specimens, arranged on polystyrene foam board and under direct air blow. **16–18**, Specimens of *Auplopus* sp. (Pompilidae), *Timulla* sp. (Mutilidae), and *Anoplius* sp. (Pompilidae), respectively, immediately after a 40 seconds session of bulk air blowing. Figs 16–17 show good results. Fig. 18 shows the worst result, but note that position of appendages improved from original situation, in Fig. 14.

FIGURES 19–21. Three specimens of the densely pilose *Melipona rufiventris* Lepeletier (Apidae); all specimens from the same collecting event; stored in alcohol for 9 years. **19**, Specimen mounted manually (technique by Noyes 1982; see also Table 1). **20**, Specimen submitted to air blow for 40 seconds, using air pump without any nozzle. **21**, A third specimen, photographed immediately after a 40 seconds session of air blow using nozzle #2 (Fig. 2).

Tests with a range of Diptera produced reasonable results immediately after the blowing session (Figs 23, 26, 29), but all specimens ultimately collapsed partially (Figs 27, 30) or extensively (Fig. 24) once fully dried. Diptera are in fact difficult to mount from alcohol, requiring more elaborate processing (e.g., Martin 1977: 156–7). A combined approach produced slightly better results: specimens were immersed in absolute alcohol, then in ethyl acetate (for one hour or more in each), and then pinned and submitted to a 20–40 seconds session of air blow. Some specimens processed in this way will remain stable after fully dried, but some degree of collapsing still occurs for most of them.

FIGURES 22–30. Processing of Diptera specimens. All specimens from the same collecting event, stored in alcohol for 3 years. *First column*: specimens as removed from alcohol. *Second column*: immediately after air blow session. *Third column*: two days later, fully dried. **22–24**, A soft, delicate Micropezidae, submitted to a 60 seconds session. **25–27**, Muscidae, 7 mm long, submitted to a 40 seconds session. **28–30**, Sarcophagidae, 15 mm long, submitted to a 120 seconds session.

FIGURES 31–35. Three specimens of *Apis mellifera* L. (Apidae) from the same collecting event, stored in alcohol for 4 years. **31 & 33**, Specimen 1, processed with the air pump, using nozzle #2 (see Fig. 2), for one minute. **32 & 34**, Specimen 2, processed with the hair dryer for one minute. **35**, Specimen 3, immersed in absolute alcohol and then in ethyl acetate, for one hour in each, then bathed in ethyl acetate again after pinned, to loosen the pilosity, then immediately processed with the hair dryer for one minute.

Tests with a hair dryer (ARNO™ Lissima 1000W) did not produce good results. External drying is too fast and intense. In comparative tests with the common bee, the air pump generated good results (Figs 31, 33) but with the hair dryer the quick external drying allowed little time for appendages and pilosity to be repositioned by the air flux

itself, generating particularly bad results for the pilosity – lumped hairs dried at once, remaining impervious to the action of the air blow thereafter (Figs 32, 34). Trials with ethyl acetate did not improve the results with a hair dryer (Fig. 35). Another problem is that it is difficult to ascertain what is the temperature of the specimen, and they seem to overheat easily, damaging the wings and even slightly "baking" the cuticle after about a half minute blow. In tests with large Sarcophagidae and more delicate Stratiomyidae (Diptera), wings became contorted by the heat, specimens begun to collapse during the blow, and once fully dried all did collapse more extensively, as usual with Diptera. Wings of all groups generally were also not efficiently spread or unfolded apparently because the hair dryer used did not produce a potent air blow.

For the Hemiptera, specimens larger than 13 mm, most Membracidae, all Cicadellidae, and some other groups, should all be pointed (Martin 1977), but for those that can be pinned the new technique yielded good results. For specimens with wide and delicate wings, as some Chrysopidae (Neuroptera) and Thespidae (Mantodea), the new technique did not produce good results, even when keeping the specimens far from the air pump or hair dryer, so that the air flow was less intense. In such cases, the wings could not be efficiently unfolded and dried with the air flow alone; faster air blows (e.g., using nozzles) tend to damage the wings, often tearing and even cutting away the tip. Coleoptera and larger Orthoptera withstood well the air blows and the high temperature of the hair dryer, allowing them to be quickly pre-dried, or even fully dried within one to three minutes, thus without the need of a laboratory oven. Note that drying Orthoptera quickly is important for large specimens (see Martin 1977).

Discussion

One difficulty with mounting specimens which are preserved in alcohol is drying the membranous wings into a minimally acceptable position, avoiding circumstances such as dry folded or adhered over one another (as in Fig. 4), or applied to the body (Fig. 7). Strictly, there is only one published technique proposing a solution—the use of an absorbent card followed by manual repositioning of the wings (Noyes 1982). It involves however a somewhat elaborate manipulation and the specimens have to be processed one at a time. The original explanation follows, with superscript numbers added to indicate the requirements or difficulties of the technique:

> "Place the insects [...] in a drop of alcohol on an absorbent piece of card [...] positioned so that the wings, including the hind wings, are completely flat against the card(1). This may be difficult to achieve if the wings are splayed out in front(2), but it can be managed with a little practice. It may be necessary to hold the insect in position whilst the alcohol evaporates(3). [...] The absorbency of the card is very important(4). If it is too absorbent the specimen will dry out too quickly. For average size specimens, very good quality, unglazed library record cards are best(5). For specimens measuring less than 5 mm in length, it may be necessary to use very smooth Bristol board to prevent the marginal setae of the wings becoming matted(6). [...] With smaller specimens it is important to remove them at the instant they dry out or the wings or antennae may stick to the card when the insect is removed(7)."

Wings will indeed often get folded in various positions the moment the specimen is placed wet over a card (e.g., Fig. 4); even after adding a drop of alcohol, wing folding often still occurs, as is particularly true, for example, with the hind wings of Hymenoptera, illustrated in Fig. 5. The situation in which the "wings are splayed out in front," here illustrated by Fig. 3, is also common, and until now could not be quickly corrected—pulling one side will detach the other from the card, thus requiring time, patience, and some skill for a good final result. While a useful technique, the use of cards is notably time-consuming even for a moderate number of specimens, and still does not solve the problem of collapsing pilosity.

All other available techniques aim primarily at avoiding the collapsing of small, soft-bodied specimens. These methods involve the need of expensive and sophisticated equipment (Gordh & Hall 1979), or specimen processing through a series of concentrations of various chemical substances, some of them notoriously hazardous (Vockeroth 1966; Sabrosky 1966; Truman 1968; Martin 1977; Brown 1993; Noort 1995; Orozco & Gaimari 2012), or extremely slow drying (Fisher & Jursic 1958; Martin 1977; Taylor 1993) (Table 1). They are in fact rarely used or practical for specimens which can be pinned and do not collapse after drying – a wide range of insect specimens. Specimens larger than 5–8 mm also pose other problems, such as the impossibility to fit more than one or a few specimens at a time in the usually small CPD chambers, or the need to use too much expensive HMDS, etc. These methods are thus much more adequate for small or very small specimens than for medium-sized or large ones

(Table 1). For such specimens, problems with collapsing wings (similar to cases on Figs 4–5), wings in difficult positions (as in Fig. 3), and difficulties with the collapsing pilosity (as in Figs 19, 22, 25–26), all remained without a practical solution until now.

TABLE 1. Comparative efficiency of drying methods for insect specimens from alcohol. *Proc/1* and *Proc/100*, processing time for a single specimen and for one thousand specimens, respectively, in hours (h) or days (d); time required for mounting not considered. *Safety*, lower values indicate higher risks for the user. *Skill*, relative degree of skill required, scaled from 0–10. *US$/10K*, total cost, in US dollars, estimated for processing about 10,000 specimens; expenses with salaries not considered. *Body length*, sizes for which results are unknown (question marks) or impractical appear in gray, sizes for which the respective technique will work best appear in bold. *Safety* and *Skill* were freely rated by the author and reviewers. CPD = Critical Point Drying; HMDS = hexamethyldisilizane.

Method	Proc/1	Proc/1000	Safety	Skill	US$/10K	Body length of specimen (mm)											References
Air pump[1]	0.01 h	0.9–11.1 h	9	4	25	0	1	2	3	4	**6**	**8**	**12**	**24**	**50**	**100+**	This work
Manual	0.07 h	70.0 h	10	8	1	0	1	2	3	4	6	8	**12**	**24**	**50**	**100+**	Noyes 1982
Acetone vapor	0.30 h	1.0 h	6	7	10	**0**	**1**	**2**	**3**	**4**	**6**	?	?	?	?	?	Truman 1968; Noort 1995
Xylene	1.00 h	1.5 h	5	7	20	**0**	**1**	**2**	**3**	**4**	**6**	8	12	24	50	100+	Vockeroth 1966; Sabrosky 1966
CPD[2]	1.00 h	1.0–8.0 h	7	10	10,000	**0**	**1**	**2**	**3**	**4**	**6**	8	12	24	50	100+	Gordh & Hall 1979
HMDS	4.00 h	12.0 h	5	7	500	**0**	**1**	**2**	**3**	**4**	**6**	**8**	**12**	24	?	?	Brown 1993; Orozco & Gaimari 2012
Ethyl acetate	27.00 h	27.0 h	8	6	20	**0**	**1**	**2**	**3**	**4**	**6**	**8**	**12**	24	50	?	Martin 1977:156–157
Freeze drying[3]	3–180 d	3–180 d	9	6–7	1,000	**0**	**1**	**2**	**3**	**4**	**6**	8	12	24	50	100+	Fisher & Jursic 1958; Martin 1977:156; Taylor 1993; Schauff 2005

(1) Processing time range considered for specimens dried one at a time *vs*. bulks of 12 specimens.
(2) Processing time depends on the size of the specimens *vs*. size of the CPD chamber, which will define the number of needed sessions.
(3) Estimated cost considers a regular freezer; specialized freeze-drying equipment is expensive.

The new technique was conceived from trials of applying strong air blows, with the mouth, over specimens just removed from the alcohol, to remove the excess of liquid. This produced fair results, but blowing with the mouth may contaminate the specimen with saliva and, possibly, with mouth cells or microorganisms, perhaps a potential problem for future molecular analyses. Blowing repeatedly is also clearly tiresome, and impossible to perform with numerous specimens. With an air pump, much better and faster results are easily achieved; all excess liquid is instantly removed while cuticle and hairs are quickly dried and cleaned from any eventual fragments, such as scales of Lepidoptera, fibers, etc. At this point, the specimen can still be easily manipulated if any repositioning of the appendages is desired.

Pilosity. Much better results were obtained with the air blow technique when compared with manual mounting (compare Figs 19 *vs*. 20–21). With bees, faster blows, achieved with nozzles #1 and #2 (Fig. 2), produced better results (Fig. 21) than unaided blows, without the nozzle (Fig. 20). Here, the new technique is superior to all other because it actively *reverts* the compression of the pilosity which is inevitably produced by the manipulation of the specimen when it is being pinned, particularly on the sides of the mesosoma (as in Figs 19, 23).

External *vs*. total drying. While full external drying is achieved in a few seconds, the definitive (internal) drying requires that the specimens be set aside to air dry, or are dried in a laboratory oven. Further drying can be performed with the use of heated air, but the new technique seems however inadequate, as tested so far, to fully dry insect specimens.

Heated air. The unsatisfactory results obtained with heated air blow are perhaps related to the fact that only a very basic hair dryer was tested. Sophisticated equipment could potentially generate good results. Basic requirements would be the need of a fine control of the temperature, and of the air flow intensity, which would need to reach at least 600 L/min. This might however correspond to a kind of hair dryer which is expensive or difficult to find. There are also other disadvantages: (1) Energy consummation of all hair dryers is notably high, and (2) they might require a 220–240 V power supply outlet. (3) The red-hot coiled nichrome wire of hair dryers can ignite the vaporized alcohol or ethyl acetate which is being produced, an extra concern in relation to an air pump.

Delicate specimens. Fragile specimens do suffer with a potent air blow, but no direct damage was observed with the tested Hymenoptera and Diptera. Even delicate specimens could be kept literally at the mouth of the air exit for over a minute without this producing any kind of damage (Figs 22–23), and small specimens did not suffer either (Figs 11–13). The new technique will however not work, and is therefore not recommended, if the intention is to prevent the collapse of soft-bodied specimens (Figs 29, 32, 35) or soft body parts, such as the abdomen. In

those cases, the CPD, HMDS, and other chemical treatments compiled on Table 1 remain as the best available choices.

Pinning. All specimens must be pinned to withstand the fast air current. Note that this can also include small specimens pinned with minutens (Figs 11–13). Specimens in triangle mounts are obviously impossible to process with the proposed technique, since they need be dried before they can be pointed. Pinning before drying might however also represent an advantage: specimens processed with CPD or HMDS, for example, can hardly be pinned, since they are already dry and fragile.

Bulk *vs*. individual drying. Blowing several specimens is desirable because it is clearly time-saving; this approach does work, but it must be noted that even distributing specimens in only three levels (Fig. 15), the second and third lines will receive a much less intense air blow. A more potent pump could be required in such cases, or manual repositioning of the pump over and around the specimens. Drying specimens one at a time, however, allows more agile manipulation of both the air pump and the specimen, leading to better final results.

Concluding remarks

The new technique is a pre-drying and mounting procedure for firmly sclerotinized insects that can be pinned. It fits into a somewhat particular methodological "niche," and is therefore unlikely to replace established techniques such as the CPD or HMDS, which actually serve to a different aim. Specimens do need be fully dried separately, and repositioning of appendages might be needed or desired, although this can be easily achieved. In spite of this, the new technique is an undoubtedly useful new tool, capable of yielding rapid results: even a one second blow can instantly stripe a deeply wet specimen from all soaking liquid, while also unfolding/spreading its wings, antennae, and pilosity. Excellent results are achieved with a 10–20 seconds blow, and at least for Hymenoptera damage is unlikely with blows up to at least 600 L/min, even for delicate specimens. Good results with Diptera seem possible, but an improved or modified technique remains to be developed for this group.

In fact, there is much room for further developments or improvements, such as the use of movable or simultaneous, multidirectional air blows, use of specialized nozzles, development of custom air-blow drying chambers, adaptation of a hose with a pen-shaped nozzle to allow fine control while drying specimens under the stereomicroscope (similar, albeit with a different purpose, to that of Jenkins 1991), development of a rotating plataform to centrifuge wet pinned specimens, use of heated or cooled air, association with other drying methods, etc, indicating that increasingly better results are fully possible.

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