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A TECHNIQUE FOR SETTING AND MOUNTING MICROLEPIDOPTERA

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ABSTRACT. Freshly collected and ammonia-killed microlepidoptera, pinned on minutens, are spread in small, shallow, plastazote-lined boxes with grooves, using either small card points mounted on short pins or translucent setting paper strips to hold the wings. The method produces high-quality specimens, is fast, and uses compact, light-weight, inexpensive equipment. The method is also versatile in that any desired quality of setting, from preliminary, partial setting to the finest setting, can be attained with the same equipment with equal efficiency under any condition, whether at home or on collecting expeditions. The main steps of the method are illustrated. A technique for staging minuten-pinned specimens is also presented.

Additional key words: spreading box, staging, double-mount, ammonia.

During the past 90 years, several papers have presented, with various amounts of detail, techniques for preparing (pinning, setting, and mounting) microlepidoptera (e.g., Kearfott 1904, Calmbach 1921, Lhomme 1926, 1927a, 1927b, Amsel 1935, Holland 1937, Janse 1939, Janmoulle 1943, Charlson 1945, Lindquist 1956, Hodges 1958, Lewis 1965, Tagestad 1974, Zimmerman 1978, Sokoloff 1980, Mikkola 1986). However, our contacts with many lepidopterists indicate that, at least in North America, good and simple techniques for preparing microlepidoptera are not well known. In fact, many North American lepidopterists do not even collect microlepidoptera as routinely as other Lepidoptera, in part because of the perceived inconvenience of preparing them. Microlepidoptera that are collected are often only the larger specimens, in groups such as pyraloids, tortricoids, and large gelechioids.

The paucity of good quality microlepidoptera from North America in many collections is one of the causes for the very slow progress in systematic studies of the Nearctic fauna. Our knowledge of the taxonomy and faunistics of many families of microlepidoptera is shockingly poor. A plea recently has been made for North American lepidopterists to take on the collection and study of microlepidoptera (De Benedictis 1993). Of course the first step in this endeavour is to acquire a *good and efficient* technique for preparing specimens.

There are probably nearly as many ways of preparing microlepidoptera as there are individuals collecting them. The basic method of spreading microlepidoptera is the same as for larger Lepidoptera. How-

ever, some adjustments, both at the time of collecting and of preparation, and in the equipment, are needed because of the small size and fragility of microlepidoptera.

Over the years we have tried every different method and variation of preparation for microlepidoptera that we came to know. While most techniques can yield high-quality specimens, many suffer from being relatively slow or requiring somewhat cumbersome equipment (e.g., spreading boards) ill-suited for prolonged field work under difficult conditions. We sought to develop a technique that offsets these problems, i.e. one that is rapid and usable under any condition with equal efficiency, yet versatile with respect to the quality of preparation desired by the collector. An earlier version of the technique described here was published in French by Landry (1991) but we have modified it slightly, with some additions.

Our method actually combines elements from other methods employed by microlepidopterists, with added refinements. It is based on the concept of setting microlepidoptera on the bottom of a box, which can be traced back at least to Amsel (1935). Modern materials, especially dense polyethylene foam, dramatically enhance the results of Amsel's method. Partial spreading in such boxes is now used by many microlepidopterists on collecting trips (Zimmerman 1978:50–59, Nielsen 1980). The main shortcoming of partial spreading is that special specimens, such as types of new species or those needed for photography, may need subsequent relaxation for final spreading. The technique exposed here offers the possibility of a full range of quality of preparations, from unspread to fully spread with as much care as a perfectionist may wish, all with the same equipment and with hardly any extra time. The technique may be used in the field, in the lab, or at home. The necessary equipment is very compact, light-weight, inexpensive, and easily made. We have tested the method with tens of thousands of microlepidoptera over the past few years, under conditions varying from local day trips to month-long expeditions in the tropics (including camping).

In addition to the actual technique of setting microlepidoptera, we offer some suggestions for handling specimens when they are collected in the field, and for staging (double-mounting) spread specimens. Appropriate handling of collected microlepidoptera is as critical as the actual setting in obtaining high quality specimens, and so is the final staging to insure safe preservation in subsequent handling.

COLLECTING

The facility and rapidity of the technique outlined here rests on working with the freshest specimens possible. Moths are placed indi-

vidually in glass vials upon collecting and kept *alive* until the time of pinning and setting. Upon returning from the field, vials are stored in a cool, dark place if the specimens cannot be prepared immediately. The ideal place is the refrigerator, or a cooler box if one is on a prolonged field trip. We have been able to keep specimens alive for up to five days in this manner, although we recommend delaying as little as possible (some moths will begin to show some wear even after 1–2 days in the refrigerator). Refrigeration is particularly useful if one has had a large catch on one day and there is not enough time to prepare all specimens immediately after they have been collected. We recommend preparing the smallest microlepidoptera as soon as possible, as they will die more quickly from dehydration. Once dead, small moths tend to dry very quickly and become difficult to relax and spread. In the humid tropics, small microlepidoptera will dehydrate quickly inside vials (often in just a few hours) and are best set as soon as possible. Always begin by preparing the smallest specimens first, working up to larger ones. Refrigeration, even if available, should probably not be used for tropical microlepidoptera from those regions that seldom experience temperatures below 10°C, because the relative cold will kill many of them.

Vials. Collecting vials should preferably be made of glass and close with an easily removable stopper that can be opened with a single hand (the other may be busy holding a net). We use glass vials that are 65 mm long and 19 mm in diameter, closed with a rubber stopper. Stoppers should be as little wedge-shaped as possible, otherwise smaller microlepidoptera will crawl in the space between the stopper and the vial neck and damage themselves. We carry about 100 vials for most day-time collecting, at least twice as many for night-time collecting at a light. Experience will dictate the adequate supply. During day-time collecting, care must be taken that the vials are not exposed directly to or heated by sunlight, otherwise the moths will quickly die and dry. If possible, avoid plastic vials (snap-cap type), especially with the smaller specimens, because the static charge that such vials accumulate through handling and friction will damage the squamous cover of the moths and increase the rate of wear.

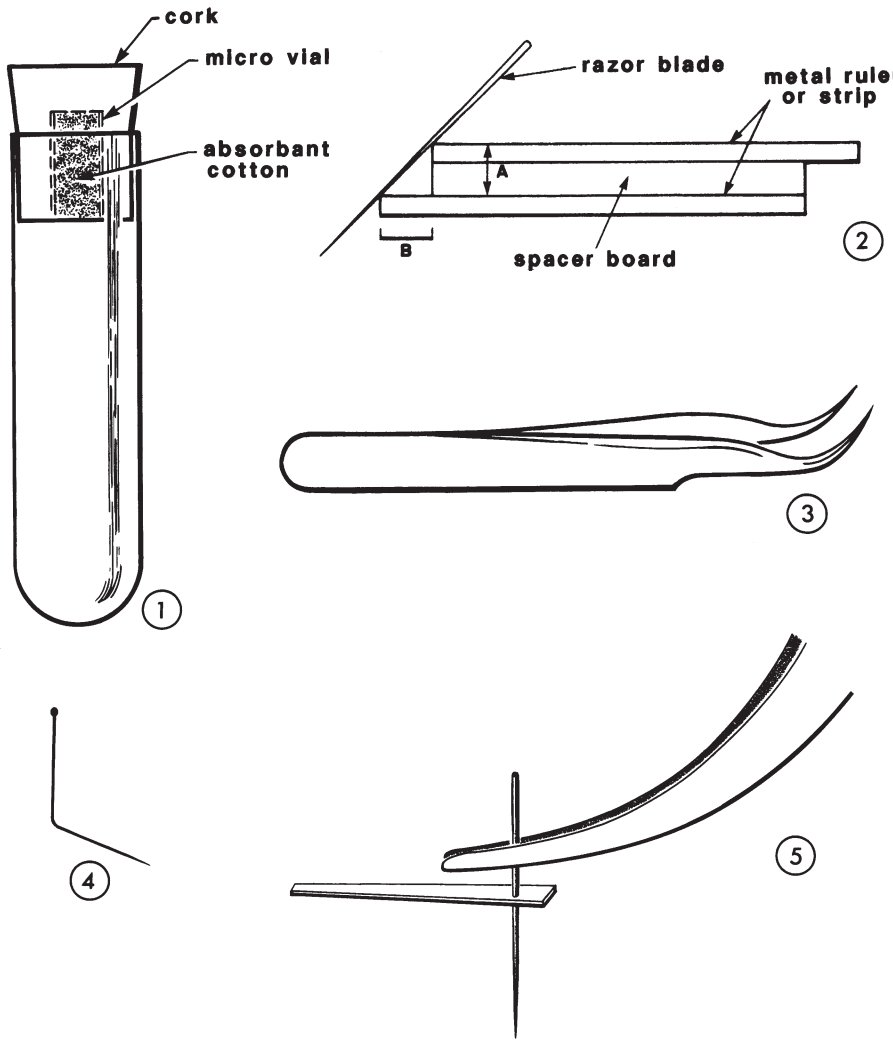
A word of caution is necessary if one is setting reared specimens: *never* set a freshly emerged moth. Allow at least 24 hours (longer if a genitalia dissection may be required) for the moth to harden sufficiently. Without this precaution, wings may curl, crumple, or droop after removal from the setting box, and if the genitalia are later dissected, structures will be insufficiently sclerotized and difficult to prepare adequately.

POISON AND KILLING TUBES

The choice of poison is, of course, a matter of personal preference, availability, etc. We strongly recommend ammonia (ammonium hydroxide): it has a quick knock-down action and leaves freshly killed specimens beautifully relaxed and ready to be spread immediately with the greatest ease. We have tried other killing agents and methods, but ammonia is the one that has given us the best results. The ammonium hydroxide solution should be as concentrated as possible. A laboratory-grade solution containing about 30% ammonia and 70% water is preferable because it has a very fast knock-down action. Household ammonia, generally a murky liquid, is weaker and unsuitable.

For killing tubes, we use glass tubes closed with cork stoppers into which a small microvial is inserted, loosely stuffed with cotton (Fig. 1). Five to ten minutes before using a tube, the cotton is imbibed with a few drops of ammonia solution, and the tube closed to let the ammonia concentration rise. This type of killing tube offers nothing inside against which struggling moths may rub; the disadvantage is that the tubes need to be recharged more frequently, approximately once every 2–3 hours of continuous use (when opened several times periodically). When setting large numbers of microlepidoptera, we use up to 10 tubes at a time to minimize recharging, and place only 2–3 moths per tube at a time. It is essential to check for and wipe traces of moisture or sweating on the walls of the killing tubes. Charged tubes may be laid on their side to prevent any ammonia from possibly running down the sides, although this will not be a problem if a modest quantity is used. When tubes are not in use for more than a day or so, it is preferable to leave them open and remove the cotton swab from the stopper to allow then to dry thoroughly.

Ammonia has a few disadvantages: it tends to sweat in a tube if an excessive quantity is used or if it is too warm (tubes must not be exposed to heat or direct sunlight)—but this is a disadvantage common to most liquid poisons; it loses strength relatively rapidly in a frequently open tube; and fumes are choking, irritating. Weak ammonia must *not* be used for moths with green, red, or orange pigments because the long exposure needed to kill them may cause discoloration. If the ammonia is strong though, this is not a problem providing that the moths are removed as soon as they are dead. In case of doubt about possible discoloration, one should use another poison, preferably ethyl acetate (subsequent relaxation may be necessary). With strong, concentrated ammonia, we have not had discoloration problems. Generally we have found that the advantages of ammonia far outweighed its disadvantages, none of which presented a real problem if it was used with the pre-



FIGS. 1–5. Materials required for preparing microlepidoptera. **1**, Killing tube; **2**, superimposed, offset rulers to cut symmetrical V-shaped grooves, A = B for 45° grooves; **3**, curved forceps used to handle minutens; **4**, bent standard pin used to assist pinning and spreading; **5**, card triangle mounted on shortened pin used to hold set wing in the point method.

cautions outlined above, and that it was no more inconvenient to use than any other poison.

Recently we have experimented with a solid form of ammonia, ammonium carbonate, a salt with the appearance of cyanide crystals. Upon

contact with the ambient humidity, the crystals decompose into gaseous ammonia, carbon dioxide and water vapor (Gilligan and Gilligan 1990). Killing tubes are made simply by packing a 1–2 cm thick layer of crystals in the bottom and covering them with a smooth, porous material [e.g. artificial foam sponge (Gilligan and Gilligan 1990)]. We used plastic caps (from snap-cap vials) punctured with many minute pin holes to cover the crystals. Plaster cannot be used because the water it contains will instantly dissolve all the crystals and produce all the ammonia at once. We obtained satisfactory results with ammonium carbonate if used for small numbers of specimens. Disadvantages are that the rapidity of killing decreases markedly compared to liquid ammonia if one opens the tubes frequently; also if there are too many specimens in a tube and it is warm, the moisture content may rise to the point where, upon cooling, crystals may form on the specimens; such crystals are then very difficult to remove. For these reasons we find ammonium carbonate less satisfactory than ammonium hydroxide.

Ethyl acetate also works well but we found that it has a tendency to stiffen many microlepidoptera if they are left in the killing tube a few minutes too long; hence, some relaxation is sometimes necessary. Like ammonia, it is volatile, and tubes need frequent recharging and may "sweat" if heated. It is also flammable and will dissolve some plastics. Generally, we have found ethyl acetate to be less satisfactory than ammonia in quickly producing ready-to-spread specimens.

KILLING

Remove the cork, insert one moth, close the cork. Repeat with other tubes. When there is one moth in each tube, start again with the first tube, ensuring that the moth is stunned. Continue until there are 2–3 moths per tube. Stunning takes less than five seconds when the ammonia is strong but may stretch to 10–15 seconds after tubes have been opened several times. Moths should be left in the killing tubes for at least 15 minutes to ensure they are dead. Very small moths (Nepticulidae, small Gracillariidae, for example) can be removed sooner. A time saving strategy in the subsequent setting operations is to segregate specimens by size at the killing stage. This way, at the setting stage, one does not have to switch back and forth among various spreading boxes with different groove widths.

SETTING EQUIPMENT

Spreading boxes (Figs. 6, 17–18). We use shallow, clear polystyrene plastic boxes; currently we have two sizes, 11cm × 11cm × 2cm, and

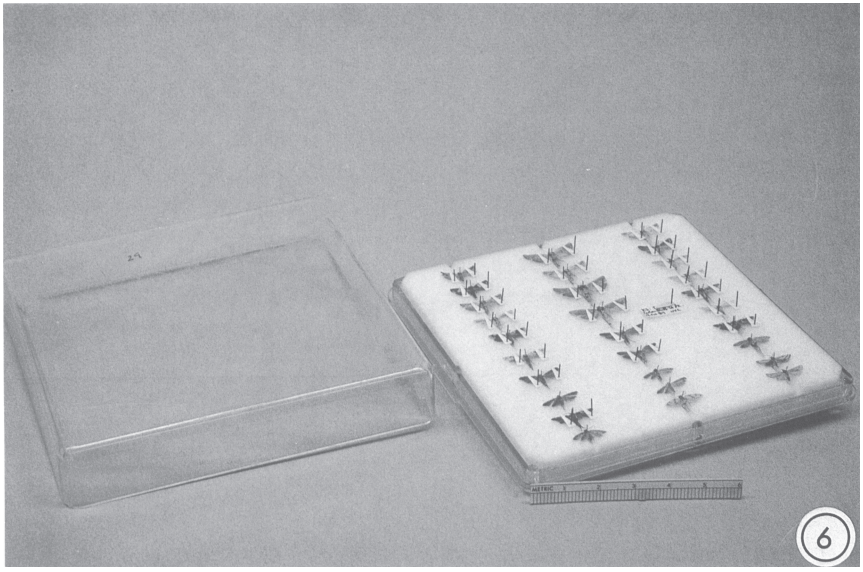


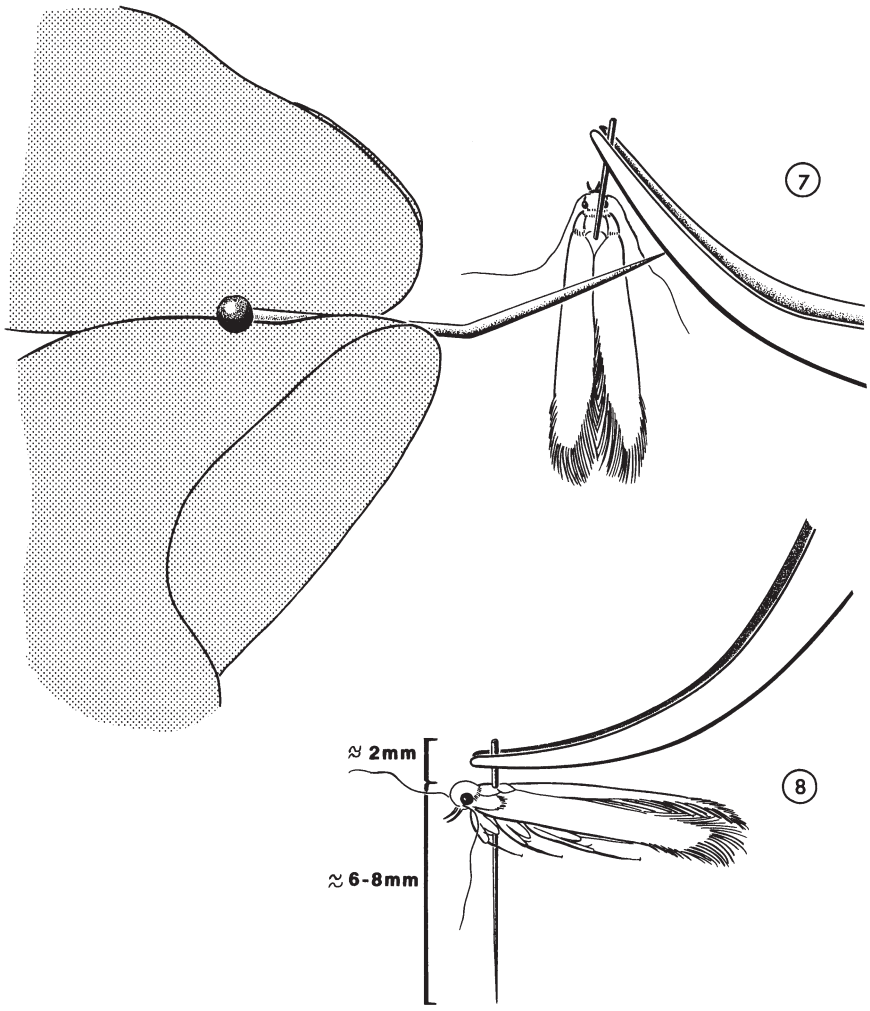
FIG. 6. Spreading box. The actual lid is used as bottom on which the plastazote is glued. Scale in cm.

12cm × 8cm × 2cm, obtained from different suppliers. Actual dimensions are not important, as long as boxes are relatively small, preferably shallow (for compactness), with a low-edge lid, rigid, and relatively airtight (or pest-proof).

For a spreading surface we use Plastazote®, a dense, smooth polyethylene foam. We found this material best because it affords the following advantages: the surface acquires a small static charge through handling, which helps wings cling slightly and facilitates spreading; it grips the pins firmly and leaves no pin holes; it sustains hardly any wear.

A 1-cm thick piece of plastazote is glued inside the lid of a spreading box (we use all-purpose, non-toxic white glue). Gluing the foam inside the lid (using the bottom as lid) eliminates edges to the spreading surface, greatly facilitates work of the hands, and maximizes use of the spreading surface.

Before gluing the foam into the boxes, we cut three or four V-shaped grooves with a razor blade. To obtain grooves with perfectly symmetrical sides, we use two metal rulers or strips, with one being taped on top of the other and propped up by a thin board; the edge of the top ruler is offset from the edge of the lower one by a distance equal to that of the ruler + board thickness (Fig. 2). To cut, the blade is slanted



FIGS. 7–8. **7,** Inserting the minuten while holding the body with a bent standard pin; **8,** Minuten-pinned specimen, showing approximate height on 1-cm long minuten.

and abutts both edges. Symmetrical grooves facilitate spreading. We use a series of spreading boxes with various groove widths, these varying from 1–5 mm (2mm and 3mm are the most frequently used widths). It is not necessary to have square grooves with vertical sides, as on standard spreading boards. In fact, the sides of V-shaped grooves often provide direct support for the abdomen.

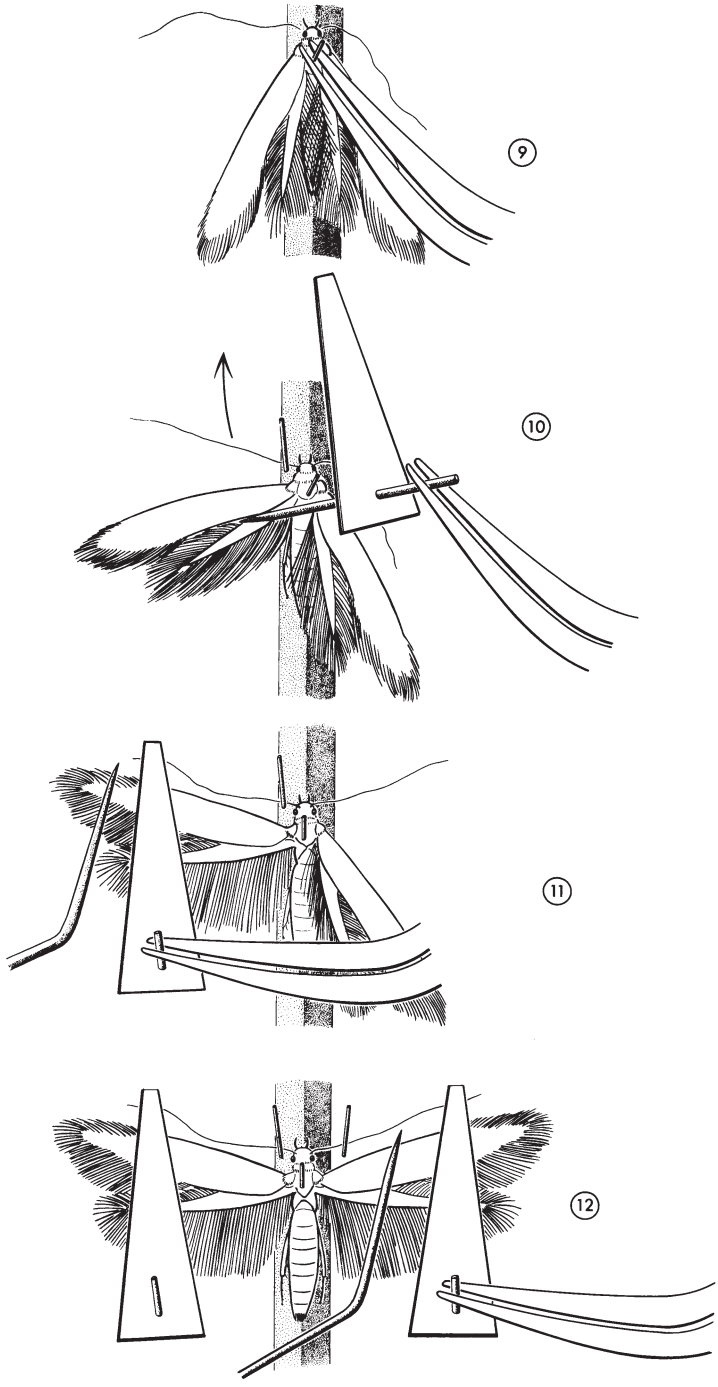
Minuten pins. Use of minuten pins involves subsequent staging or

double-mounting, so this is distasteful to many a lepidopterist. Whatever the perceived difficulty, inconvenience, or time factor involved, we emphasize that this is by far the best and safest way of obtaining fine-quality microlepidoptera. Double-mounted specimens can sustain rougher handling without damage and are far less likely to lose their abdomen, a very frequent problem with microlepidoptera that are mounted on fine standard pins (00 or 000), which are very springy. The genitalia are critical for the specific determination of numerous species of microlepidoptera, hence the abdomen must not be lost.

There are different qualities of minutens available on the market. For the best results, and to avoid frustration, one should use the best quality stainless steel minutens. Avoid black-enameled minutens, which have a tendency to rust (guaranteed if one is in the tropics) and have tips that more easily "hook" (being made of softer metal). The difference in price between stainless steel and black-enameled minutens is small. Diameters of the most useful sizes are 0.20 mm, 0.15 mm, and more rarely 0.10 mm (for nepticulids and other tiny microlepidoptera); some British brands label their minutens A1 (0.14 mm) and B1 (0.19 mm) (1 referring to the shortest length, usually 10–12 mm).

Most minutens are excessively long and must be shortened down to no more than about 1 cm for the larger ones (0.20 mm) or 6–7 mm for the finer ones (0.15 mm and 0.10 mm). If minutens are not shortened, the excess length jutting either above or below the specimens will greatly increase the risk of breakage or damage during handling of the double-mounts (fingers pinching the minuten while grasping the stage-supporting pin will spring the specimen and likely send parts flying, most commonly the weakly-attached, all-precious abdomen). A rapid method of shortening a large number of minutens is to cut narrow strips of plastazote (often the latter's thickness is conveniently 1 cm or 7 mm), to insert minutens all the way through the strips (ensuring that their tips do not extrude), and trimming off the excess length close to the strip surface with good scissors or pin cutters. To maximize efficiency later in the setting process, we prepare large quantities of trimmed minutens in advance. Minuten-loaded plastazote strips can be packed side by side in an insect mounting tray or small shallow cardboard box. A protective layer of plastazote is glued on the bottom of the tray or box. Strips are then laid upright, side by side, and held in place with pins inserted through the sides of the tray or box; any remaining space can be filled with plastazote. Use a box narrow enough for the holding pins to pierce through at least half of the strips from one side.

Tools. We use curved forceps for handling minuten pins (Fig. 3). The inner surface of the grasping end must be smooth (not striate). While fine straight forceps could be used, we found curved forceps to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