Advanced Imaging Techniques II: Using a Compound Microscope for Photographing Point-Mount Specimens

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Parasitic Hymenoptera offer a particular challenge to scientific photography in this age of digital technology. This group is not only hyper-diverse, but ecologically one of the most important insect lineages for regulating the population densities of other arthropod species. In agriculture, this group is also responsible for the most successful cases of biological control of pestiferous insect species. One particular hallmark of this group, however, is their unusually small size (typically 0.5–5 mm adult size). Couple this with their projected worldwide diversity (225,000 named species, an estimated 15 million species on Earth; Gauld and Bolton 1988, Grissell 1999), and one quickly realizes many species remain to be described.

The small size of parasitic Hymenoptera makes them not only difficult to examine, but quite often impossible to photograph adequately using standard equipment. This has reinforced the taxonomic impediment facing research into the systematics of the group. Hand-drawn illustrations and scanning electron micrographs (SEM) previously were the standard methods for describing characters in the literature. These methods are neither substandard nor outdated, but they can be time-consuming and cost-prohibitive.

We have developed a technique for photographing these minute insects under very high magnification without the use of SEM. Our technique is advantageous over SEM for several reasons: destructive sputter-coating is unnecessary; the specimen need not be exposed to the rigors of low vacuum; color information is retained; and the technique is cost-effective, often using "surplus" equipment.

Electronic dissemination, whether using species pages or e-journals, allows an unprecedented amount of visual information to be coupled with species descriptions, revisions, or morphology-based phylogenetic research. We believe that our work into imaging minute insects such as microhymenoptera will help put a backbone into "spineless taxonomy" (Wheeler 2007).

Materials and Methods

Specimen Preparation. Specimens are ideally selected from cleaned point- or card-mounted series, or sorted from bulk storage in fluids such as ethanol. If taken from ethanol, Heraty and Hawks (1998) offer a solution for dehydrating soft specimens in lieu of using a critical point dryer. Special attention should be paid to finding specimens that have died with appendages oriented in a "planar" fashion (i.e., wing, legs, and antennae all along the same plane).

Specimen Mounting. A key to the success of imaging small insects under high magnification is to exclude as much of the insect mount as possible. The mount can take up an unwanted amount of the field of view, frequently altering the color balance of the exposure (especially if the mount is a white point-mount).

We have developed two alternatives to the traditional museum mount. The first, less labor-intensive solution, is to use clear, archival quality Mylar film; this material is commonly used for protecting valuable documents (we found the easiest source was comic book stores). Simply point-punch this material as you would card stock; it holds onto a pin with as much strength as Bristol board points.

The second technique involves gluing a minuten (size 1/10) pin directly to the insect body. The placement of the pin depends on what angle(s) the specimen is to be shot; we found that mounting on the mesopleuron of a microhymenopteran is particularly useful, allowing for lateral, dorsal, ventral, anterior, and posterior views. With the specimen lying on its side, pick up a minuten with very fine forceps and dip the "blunt" end of the minuten in Super-Glue Gel or similar product (we prefer this material to more traditional glues because of the enhanced drying time, accuracy of placing the glue blob on the pin, and ease of removal). Next, touch the end with glue to the side of the specimen, preferably along the long axis of the mesopleuron, trying to orient the shaft of the pin perpendicular to the midline of the insect (Fig. 1a). For larger insects, the end of the minuten can be bent 90° to produce more surface area.

A key to the success of the second technique is to have at the ready some media in which to insert the minuten-laden pin. We developed a mount that works particularly well in our system. It is composed of gray modeling clay spread along the

underside of a flat metal thumbtack (see below). The gray clay makes for an ideal background color, and the shaft of the pin allows for picking up the mount with minuten-pinned insects with fine forceps when orienting the specimens under the microscope (Fig 1a). If you are using the Mylar point system, simply inserting the pin into tray foam or the like is sufficient.

Optics. Our work on this topic began with the use of Zeiss MZ-16 Apo lens attached to a focusing column and a JVC KY-75C digital camera. At high magnification, we found that for insects around the 0.5–2mm adult size range, the resolving power of the lens was unsuitable. Details such as setae and surface sculpture were often totally out of focus. Buffington et al. (2005) suggested that the use of a compound microscope, with its much shallower depth of field and higher resolving power, might offer an alternative to the M-16. Furthermore, Stephen McJonathan (GT-Vision, Hagerstown, MD) suggested we might try 'metallurgical grade' compound lenses. The advantage of these lenses is that they typically possess a higher depth of field than standard objectives, and they are not color corrected for imaging through coverslips.

The microscope we used was a Leica DMRB compound microscope fitted with Leica HCX PL "Fluotar" 5× and 10× metallurgical grade lenses. Buffington et al. (2005) warned that the use of compound scopes for whole mount insect photography was limited by the need to envelop the specimen in an extremely intense bath of halogen light. Here, the metallurgical lenses we used were rather light sensitive, and we found that two fiberoptic light sources divided into four channels were more than enough to provide sufficient illumination (Fig 1b). Light dispersion of the incident light (Buffington et al. 2005) is essential at this magnification; we found that a cylinder of translucent Mylar film (with a small slit along one side to accommodate an insect pin) fitted to the barrel of the compound lens resulted in an ideal amount of dispersion.

The Leica DMRB is far from being an entry-level microscope. We believe that some researchers may not be able to afford such a piece of equipment, so we experimented with alternative microscopes commonly available for reasonable prices on online auctions as well as university surplus. The Leitz Ortholux series of microscopes were made from the late 1940s through the mid-1970s and remain some of the highest quality scopes ever made. We obtained a "black lacquer" model from the early 1950s and a gray "Labolux" model from the early 1970s. Both came with a camera port, and both only needed a minimal amount of cleaning.

Specimen Manipulation. An advantage to using a compound scope for microphotography work is ability to use the standard x-y stage for specimen manipulation. To use this system, however, the thumbtack specimen stage must be adhered to a standard microscope slide that fits into the clips on the microscope stage. We covered a standard glass slide in gray card stock to match the gray modeling clay on the thumbtack. If the user is interested in the Mylar point mount system, simply roll a ball of gray clay and adhere the ball to the microscope slide and insert the insect pin into the clay (note that the clay can be molded into a cone/tower to raise the specimen off the surface of the slide). Alternatively, the point-mounted specimen can be slid off the pin, and the base of the point inserted into the clay.

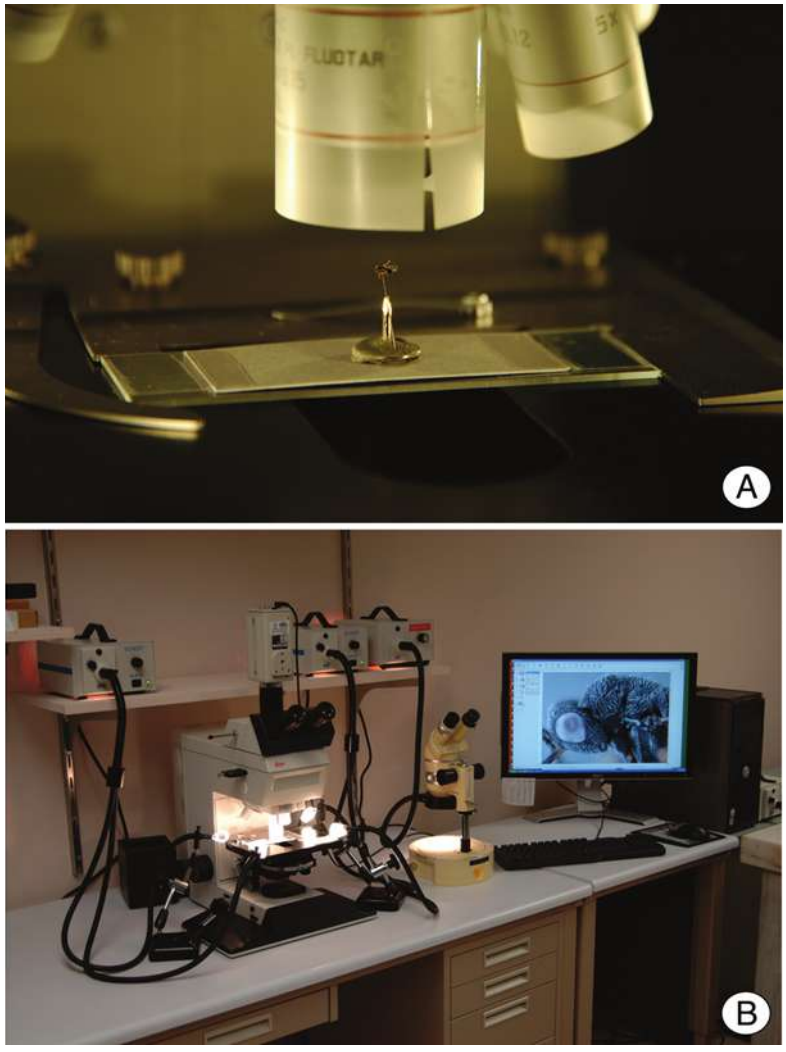

Fig. 1. Compound microscope setup. (a) Eurytomid specimen mounted to minuten pin, held in position with modeling clay on a thumbtack. Thumbtack is adhered to the gray card on the microscope slide via a smear of clay. Mylar wrapped around the lens is lowered around the specimen to reduce glare. (b) Leica DMRB microscope with dual light sources; yellow Wild microscope for staging/cleaning specimens.

Digital Imaging. Images were obtained using an EntoVision Imaging Suite, which included a firewire JVC KY-75 3CCD digital camera mounted to either a Leica M16 zoom lens via a Leica z-step microscope stand, or to a Leica DMRB compound microscope fitted with metallurgical grade lenses and fiber optic light sources. The Z-16 system fed image data to a desktop computer where Cartograph 5.6.0 (Microvision Instruments, France) was used to capture a fixed number of focal planes; focal planes were manually captured using the DMRB via Archimed 5.5.0. The resulting focal planes were merged into a single, in-focus composite image.

The Final Setup. Bringing the whole system together is initially somewhat time-consuming, but with regular use and "tricks" learned along the

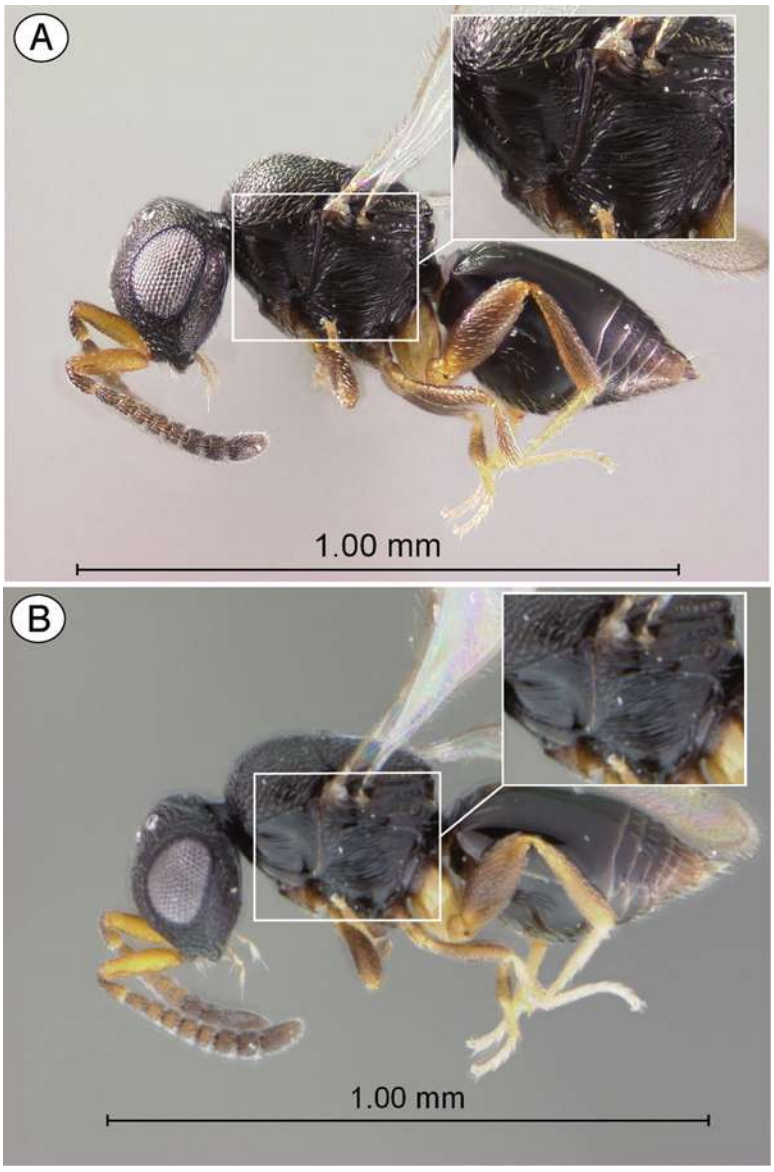

Fig. 2. Comparison of compound microscope versus dissecting microscope using *Aphanogmus* near *goniozi*. (a) Leica DRMB compound microscope using a Leica HCX PL "Fluotar" 5× metallurgical grade lens. (b) Leica MZ-16 dissecting microscope lens.

way, setup can be achieved routinely in minutes. The first task is to position the thumbtack stage on the microscope slide. We found that a thin layer of modeling clay on the slide makes an effective, temporary mount for the thumbtack. Finalize the position of the specimen with a stereomicroscope kept adjacent to the compound scope (Fig. 1b); this allows more freedom of positioning compared with the compound scope.

Before transferring the slide to the compound scope, its imperative that the stage be at its lowest setting. This is necessary to ensure that the lens itself will not disturb the specimen once it is rotated into position. Next, the lights need to be positioned; the optimal system is a "quadrant" setup, with four fiber optic light guides stemming from two individual light sources. Two per side of the scope, positioned at 90° intervals and directly aimed at the specimen, provide even illumination. The sleeve of Mylar, which had been slid up the barrel of the lens, may now be lowered around the specimen. If you are using the Mylar point mount system, you will need to provide a slit in the Mylar to accommodate the insect pin.

Conclusion

The field of systematic entomology continues to be challenged by dwindling financial and environmental resources. At the same time, Web-based resources such as LucID, Encyclodpedia of Life, MorphBank, and BugGuide make information about insect species, their biology, and distribution widely accessible. The ability to capture high-resolution digital images for the dissemination of these data has been recognized in several recent publications (Buffington et al. 2005, Kerr et al. 2009) and symposia. However, efficiently capturing high-quality images that are affordable is a challenge we are only now coming to appreciate.

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