

**A MODIFIED SIEVE DISH FOR EASIER PREPARATION OF
MICROSCOPE SLIDE MOUNTS FOR TAXONOMIC
STUDIES OF ARMORED SCALE INSECTS
(HEMIPTERA: DIASPIDIDAE)**

NORMANDY M. BARBECHO^{1,2} and IRENEO L. LIT, JR.³

¹Entomology Section, Museum of Natural History, University of the Philippines
Los Baños, College, Laguna 4031, Philippines;

²Zoology Division, National Museum of the Philippines, Padre Burgos St., Ermita,
Manila 1000, Philippines

E-mail: umbarbecho@gmail.com

³Environmental Biology Division, Institute of Biological Sciences,
College of Arts and Sciences and Entomology Section, Museum of Natural History,
University of the Philippines Los Baños, College, Laguna 4031, Philippines.

E-mail: illit@up.edu.ph

ABSTRACT

A modified dish that serves as a sieve for specimens that undergo series of chemical treatments during the preparation of specimens for microscope slide mounts is described and illustrated. The dish serves as a filter for draining fluids. It is assembled using simple materials, namely: the cap of a 15 ml centrifuge tube with the closed end perforated, a glass cloning cylinder, and a piece of fine-mesh fabric (i.e., synthetic satin ribbon) that is slightly larger than the diameter of the cloning cylinder. The use of the modified dish facilitates slide-mounting of more specimens, including crawlers or their exuviae, per batch within a shorter period of time.

KEYWORDS: armored scales, Coccoidea, Cocomorpha, Diaspididae, microtechnique, scale insect taxonomy

INTRODUCTION

Taxonomic studies of armored scale insects require examination of characters that can only be observed in slide-mounted specimens under a compound microscope. However, preparing microscope slide mounts is a time-consuming and tedious process (Williams and Watson, 1988). Several methods developed for this purpose are found in the literature (see review by Sirisena et al. 2013), but we currently follow the method specified by Lit (2002) for preparing slide mounts of lac scale insects (Kerriidae) with slight modifications. The technique was taught to him by Dr Penny J. Gullan (ANU, Canberra, Australia) and can be used for all scale insects, with some adjustments for groups possessing more fat bodies or globules. Slide-mounting of scale insect specimens allows for long-term storage of scale insects in countries with warm, humid climates, such as the Philippines.

The method requires the following steps: (1.a) cold (i.e. ordinary room temperature) maceration using 5-10% aqueous solution of potassium hydroxide (KOH) for 12–24 h — the body contents of alcohol-preserved specimens can be gently teased out preferably using a pair of featherweight forceps before or after maceration; (1.b) if necessary, removal of stubborn fat globules using a solution of 1:1 dishwashing detergent and distilled water for 3 minutes as suggested by Miller (2002) [instead of 1:1 carbol-xylene mixture according to Williams and Watson (1988)]; (2) staining with acid fuchsin for not more than 24 h; (3) gradual dehydration and destaining through a graded ethanol series (i.e. the series ends in treatment with absolute ethanol, followed by two to three changes of absolute isopropanol) before 2 or 3 changes of xylene; and (4) finally mounting on a microscope slide using Canada balsam thinned with xylene.

For each step, individual specimens have to be “transferred” from one treatment to the next using Wilkey’s microtools, or a makeshift microspatula fashioned from coconut midrib, or an insect pin (No. 1) with a bent tip attached to a wooden handle. If there are only a few specimens to prepare then time will not be a constraint. However, normally there are several individuals to process and thus a researcher, especially a novice on slide-mounting or a non-specialist on armored scale insects, may have to spend several hours or even the whole day working on a single batch of specimens. Given that armored scale insects are delicate, especially after treatment through the graded ethanol series after which they become brittle, the researcher must have steady hands because even the slightest shaking or tremor can damage the specimens while trying to pick them up. Moreover, picking them up must be done under a dissection microscope because armored scale insects are minute. This is complicated by the fact that they become glassy transparent after maceration and are thus difficult to see. The process is already very difficult to perform for preparing adult females, but more so for the nymphal stages which are several times smaller than the former (Figure 1). Consequently, the vast majority of taxonomic studies on armored scale insects are based largely on the morphology of the more readily collected and relatively easier-to-slide-mount adult females.

A far more important concern is the fact that while the later steps are performed, the researcher is constantly exposed to the fumes of toxic chemicals such as xylene. A main impetus for modifying techniques for slide-mounting armored scale insects and other Sternorrhyncha is the growing concern over the toxicity of the chemicals used in these methods. Recently, Sirisena et al. (2013) developed a modified technique that uses less toxic chemicals to address this issue. Along the same objective of safer and easier microtechniques for scale insects, we introduce in this short note, a simple “dish” that we have designed in the course of our research that addresses the issues we have outlined herein.



Figure 1: Illustrating the relative sizes of different stages of the lanzones scale, *Unaspis mabilis* Lit & Barbecho, relative to that of a no. 1 insect pin. The blue arrow points to a crawler (first instar), whereas the yellow arrow indicates the dorsal exuvia of the second instar still attached to the scale cover of the adult female. (Photograph by N.M. Barbecho).

Description of Modified Dish

Our dish is made up of a cloning cylinder and a centrifuge cap with perforated end covered with a fine fabric, which serves as a filter for draining fluids (Figure 2). Simple materials are needed to make the dish: (A) a cap of a 15 ml centrifuge tube with the closed end perforated, (B) a glass cloning cylinder, and (C) a piece of single-faced satin polyester fabric ribbon slightly larger than the diameter of the cloning cylinder (30 mm in width; this ribbon can be bought in craft or art supply stores. Any other kind of fine-mesh fabric can be used as long as it does not readily dissolve in xylene). To construct the dish, first place the fabric over the open end of the centrifuge cap and place the cloning cylinder on top of it. Push the cloning cylinder downward until a fraction of it protrudes from the other end of the cap and the fabric is tightly stretched (D). Cloning cylinders vary in thickness, so simply scrape off the thread of the cap until the cloning cylinder can fit tightly into it. Place the specimens inside and add the chemical to it using a glass dropper. Before

moving specimens to the next treatment, lift the dish containing the specimens and remove the excess chemical from the previous treatment by blotting the dish on a folded piece of tissue paper. Then place the dish in a clean well and add the next chemical.

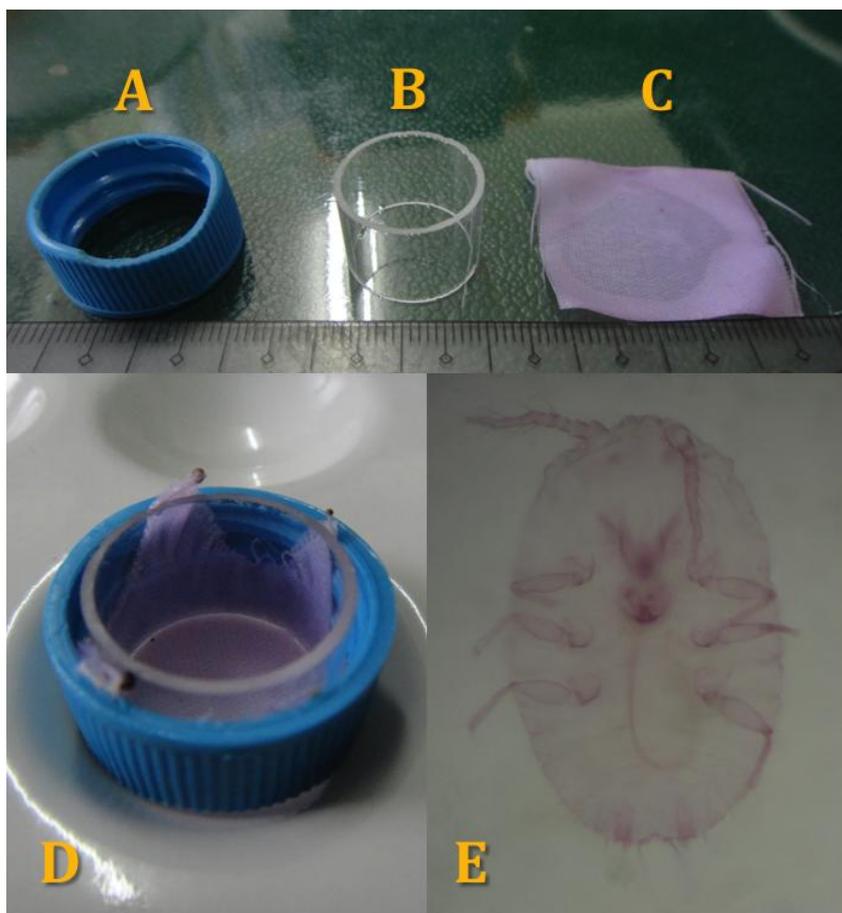


Figure 2 : Materials needed to make our makeshift dish: (A) cap of a centrifuge vial with the closed end perforated, (B) a glass cloning cylinder, and (C) a piece of single-faced satin ribbon about 30 mm in width. (D) The dish is placed on a ceramic spot plate. (E) Slide-mounted specimen of a crawler (first instar nymph) of the lanzones scale, *Unaspis mabilis*, prepared using the dish described herein (not magnified to scale, and image not stacked). (Photographs by N.M. Barbecho.)

DISCUSSION

Our primary aim for modifying the technique enumerated by Lit (2002) is to reduce our exposure to harmful chemicals when preparing slide-mounts of our specimens. Although xylene is an organic solvent, it does not immediately dissolve the polyester fabric of the satin ribbon used in the modified dish. However, carbol-xylene is a stronger organic solvent and will readily dissolve the fabric. In its place we use the detergent-distilled water solution suggested by Miller (2002). Alternatively, preparators may use just two changes of xylene instead of three and prefer to use clove oil for the last treatment before mounting the specimens. While both remove excess alcohol and dissolve trace amounts of wax still left in the specimens, clove oil is less toxic than xylene and the specimens can be left in it for a maximum of two days without significantly fading the stain (Sirisena et al. 2013). However, some slides prepared using clove oil may have the stain fade in a few years as compared to those prepared with pure xylene.

The use of our dish allows us to slide-mount a greater number of specimens per batch within a shorter period of time. It also allows us to slide-mount the minute crawlers (first instars) (Figure 2E) or their exuviae relatively easily. Currently, we use our dish extensively in our research on crawler morphology. Because fewer transfers of individual specimens is required with the use of our dish, the specimens can be handled more easily and thus the risk of accidentally mutilating them can be minimized. Far more importantly, we reduced our exposure to the fumes of harmful chemicals. We are now trying to adopt the modified technique of Sirisena et al. (2013) in tandem with our dish. Ultimately, we wish to come up with a slide-mounting technique that is more efficient and safer. We hope that novice students and non-specialists on armored scale insects, and perhaps even fellow coccidologists, may find our dish useful.

ACKNOWLEDGEMENT

We thank the University of the Philippines Los Baños (UPLB) Museum of Natural History for the use of space and facilities and the UPLB Graduate Mentoring and Apprenticeship Program for the participation of NMB in ILLJ's Museum research activities.

LITERATURE CITED

- Lit, I.L. Jr. 2002. Morphology of the unique structures of adult female lac insects (Hemiptera: Coccoidea: Kerriidae). *Philippine Agricultural Scientist* 85(1): 25–38.

- Miller, D.R. 2002. Coccoidea preparation. *In*: Armored scales of the world: compendium and key to the genera of the Diaspididae (Sternorrhyncha, Hemiptera) by John Dooley and Ramon Dones. Lucid Keys. Accessed from <http://keys.lucidcentral.org> on January 12, 2014.
- Sirisena, U.G.A.I., Watson, G.W., Hemachandra, K.S. and Wijayagunasekara, H.N.P. 2013. Modified technique for the preparation of specimens of Sternorrhyncha for taxonomic studies. *Tropical Agricultural Research* 24(2): 139–149.
- Williams, D.J. and Watson, G.W. 1990. The scale insects of the tropical South Pacific region. Part 1. The armored scales (Diaspididae). CAB International Institute of Entomology, Wellington, UK.