Cleaning and preparing adult beetles (Coleoptera) for light and scanning electron microscopy

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The coleopterist Arnett (1947) concluded in a techniques paper that 'As with any attempt to outline technique, this is little more than a sketch of some of the points to be considered. Each technician must work out the details for himself.… Do not let the technique become the end, but rather carefully prepared material which will serve the best advantage of the worker in carrying out his research should be the end.' I concur but add that unless entomological techniques are documented, others have to reinvent the wheel, rather than refine it. Here I provide a summary of what is published and document the details that I have worked out for others to use as a starting point.

When one considers the advantages of, and the contribution that scanning electron microscopy (SEM) has made to beetle systematics (Beutel *et al.* 2009), it is surprising that literature lacks a concise techniques paper covering the cleaning, mounting, drying and sample preparation for beetles, or other hard-bodied arthropods for SEM. Those traced are included below: Nelson (1949) in his studies of Elmidae (Coleoptera) covered cleaning insects, where dry specimens are relaxed in Barber's relaxing fluid (Valentine 1934; May 1958) before being cleaned using a custom-made artist's fine paint brush and tri-sodium-phosphate (Na_3PO_4) as a route to removing the naturally occurring layer obscuring their microsculpture. Frank (1978) proposes the use of a 5 % solution of household liquid detergent and water to both kill and clean beetles prior to dry mounting or transferral into ethanol (C_2H_6O). He calls this 'auto-cleaning of captured beetles' and mentions the use of an ultrasonic cleaner, but without specifying how it should be used. Harris (1979) revisited entomological terminology and compiled 'a glossary of surface sculpturing', which remains a good source where invertebrate microsculpture is covered with text and clear SEM illustrations. His materials and methods included the use of soaking dirty specimens (of Hymenoptera) in ethanol or xylene (C_8H_{10}) prior to 10–20 seconds of sonication (note the very short sonication time for Hymenoptera in contrast to what more robust Coleoptera require). Corwin *et al.* (1979) working on ticks (Acari: Ixodida) used a common household glue, like a cosmetic face peal, to adhere to and then remove dirt in order to clean specimens prior to SEM, expanding on an aqueous cleaning technique proposed by Keirans *et al.* (1976). Speirs *et al.* (1986) used an ultrasonic cleaner with ethanol as the surfactant to clean beetle larvae for a SEM study. There are many other papers where cleaning is mentioned, but always so cursorily as to not provide any guidance to a novice trying to prepare dirty specimens. In a novel approach to removing small delicate arthropods collected on yellow sticky traps, Williams & O'Keeffe (1990) used an ultrasonic cleaner with xylene or ethyl acetate ($CH_3COOCH_2CH_3$) to dislodge and clean them. Álvarez-Padilla & Hormiga (2007/2008) provided a pancreatin protocol for digesting the soft internal tissue of spiders, and suggested the use of fine strands of Paraloid B-72 glue (http://en.wikipedia.org/wiki/Paraloid_B-72) in acetone ($(CH_3)_2CO$) as a mountant for small spider parts for SEM examination. More recently, Warner (2010a,b) described cleaning, relaxing and degreasing of beetle specimens.

Consequently, this protocol is written due to the absence of one covering the vitally important cleaning and preparation of especially geotaxic, fossorial beetles prior to light or SEM microscopy. However, much of it is equally applicable to other hard-bodied arthropods.

The following items are required (brand names of those used here are in brackets): specimens for preparation; heat- and vibration-resistant glass-

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