

SHORT COMMUNICATION

A PROTOCOL FOR DIGESTING INTERNAL SOFT TISSUES AND MOUNTING SPIDERS FOR SCANNING ELECTRON MICROSCOPY

Fernando Álvarez-Padilla and Gustavo Hormiga: Department of Biological Sciences, The George Washington University, Washington, DC 20052, USA. E-mail: fap@gwu.edu

ABSTRACT. We describe a simple protocol for digesting the internal soft tissues of spiders using an enzyme complex known as pancreatin. This technique is preferred over digestions with caustic agents because it better preserves the cuticle surface, allowing its study by means of scanning electron or transmitted light microscopy. In addition, we describe a technique for mounting spider body parts for scanning electron microscopy using an acryloid polymer.

Keywords: Digestion, pancreatin, dissection, SEM

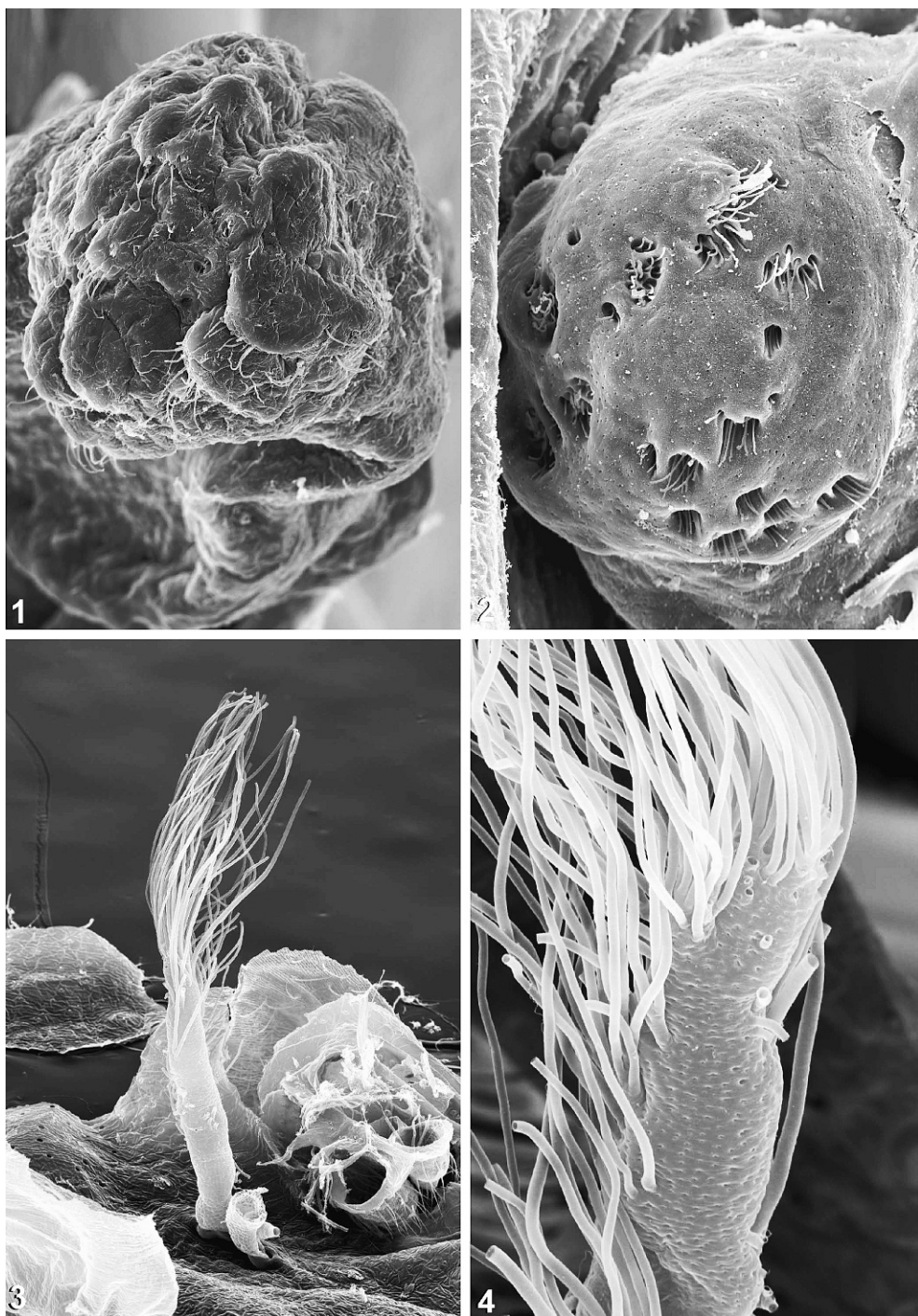
Protocols for digesting internal soft tissues have been widely used in the entomological and arachnological literature either to observe the morphology of internal structures (e.g., Purcell 1909, 1910; Lamy 1902; Machado 1951; Millidge 1984; Wibel et al. 1984; Meyer 1989; Sierwald 1989; Platnick et al. 1999; Ramírez 2000; Townsend et al. 2000; Griswold et al. 2005) or to estimate the proportion of skeletal tissues in insects (Buxton 1932). Potassium hydroxide (KOH), sodium hydroxide (NaOH) and sodium hypochlorite (NaClO, bleach) are some of the most commonly used substances in these studies. The purpose of such digestions is to remove the soft tissues that surround sclerotized organs for study with scanning electron microscopy (SEM) or optical microscopy. Digestions with the above mentioned chemical agents can damage the chitinous cuticle surface of structures such as spermathecae, therefore the use of less aggressive substances, such as trypsin, has been recommended (e.g., Sierwald 1989).

We describe a protocol for digesting spider internal soft tissues with a mixture of digestive enzymes usually obtained from pig pancreas known as pancreatin. Pancreatin contains trypsin, amylases, lipases, ribonucleases, and proteases. Furthermore, this enzyme complex is active at room temperature and does not require buffers. These enzymes effectively digest the soft tissues without apparent damage to the cuticle surface of sclerotized organs (compare Figs. 1 and 2). We have successfully used pancreatin to study the female internal genitalic structures as well as the tracheal system anatomy of a diversity of spider species (e.g., Figs. 3–8; Álvarez-Padilla & Hormiga unpublished ms.). Furthermore, digestion of soft tissues with pancreatin greatly facilitates the microscopic observation of sclerotized structures in clearing media (e.g., methyl salicylate,

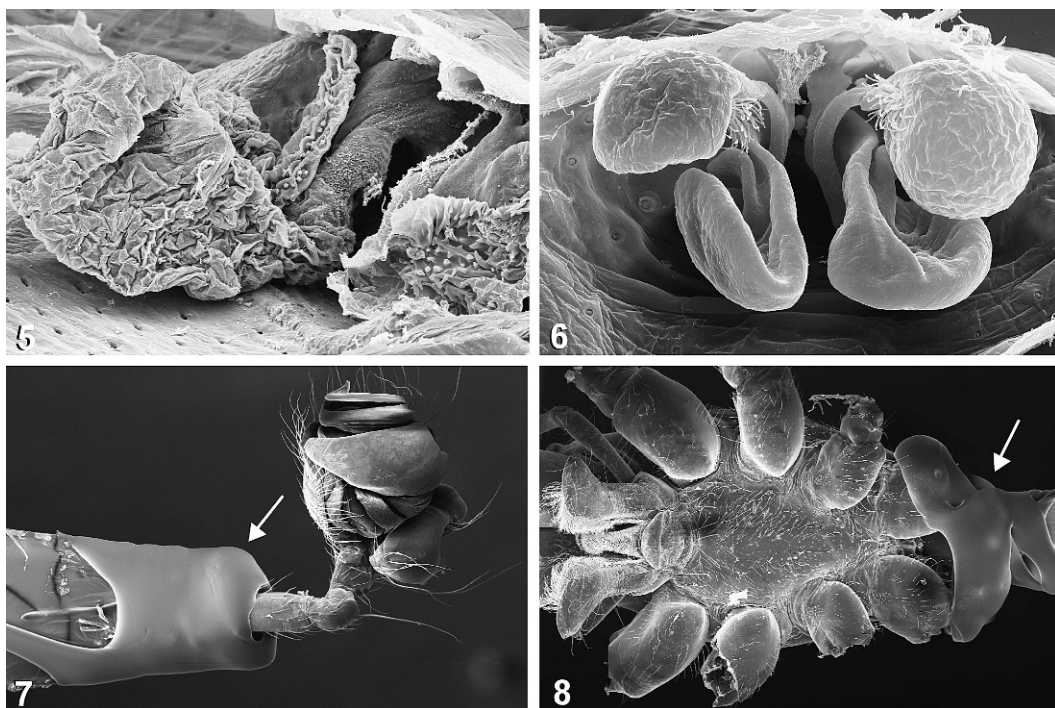
see Holm (1979)), such as spermathecae and accessory ducts.

Examination of entomological or arachnological specimens for scanning electron microscope (SEM) is commonly done by attaching the specimen (or body part) directly onto the mounting stub or by attaching it to the free end of a piece of wire, usually made of gold or copper. Conductive and non-conductive adhesives are available to glue the specimen to the mount. We have used non-conductive acryloid glue, which has the desired characteristic of producing thin threads. These threads are used to wrap the specimen at the free end of the copper tape. This modification allows us to securely glue the specimen to the copper tape by a small area maximizing the observable surface of the specimen (Figs. 2C, D, arrows). In principle, any glue that forms thin elastic threads and dries out relatively fast can be used for this purpose, but an acryloid B-72 solution in acetone has the advantage that its viscosity can be easily modified by changing the proportion of acetone to acryloid in the mixture. If the acryloid pellets are diluted with too much acetone the solution will not form threads or the threads will break easily. Conversely, too little acetone will result in an unworkable mix of high viscosity. The acryloid B-72 glue is easier to manipulate than the standard graphite conductive glue used for SEM, and once it is covered with gold during the sputter coating process the glue surface becomes conductive although sometimes it may present charging problems.

Pancreatin protocol.—Method is modified after Dingerkus & Uhler (1977). Add sodium tetraborate decahydrate (Borax) to one liter of warm distilled water (60° C) until saturation is reached then cool to room temperature (RT ≈ 20° C). (When the solution cools, a layer of borax crystals will form at the



Figures 1–4.—1. Spermatheca of *Chrysometa alajuela* Levi 1986 (Tetragnathidae) digested with KOH; 2. Spermatheca of *C. alajuela* digested with pancreatin; 3. *Glenognatha foxi* (McCook 1894) (Tetragnathidae) tracheal system digested with pancreatin (left tracheal trunk); 4. Detail of the left tracheal trunk and tracheoles of *G. foxi*.



Figures 5–8.—5. Spermathecae of *Leucauge venusta* (Walckenaer 1842) (Tetragnathidae) digested with pancreatin; 6. Spermathecae of *Nanometa* sp. (Tetragnathidae) digested with pancreatin; 7. Left male pedipalp of *Chrysometa alajuela* (Tetragnathidae), mesal view (arrow indicates acryloid glue thread on copper tape); 8. Cephalothorax of *Tetragnatha versicolor* Walckenaer 1842, ventral view (arrow indicates dried acryloid glue thread around coxa IV on tip of copper tape).

bottom.) Dissolve 1 g of pancreatin 4× USP grade enzyme complex in 70 ml of distilled water, add 30 ml of borax saturated aqueous solution and mix well. Filter the solution with cotton and a funnel to remove the coagulated proteins. Distribute the solution into several vials and keep them frozen (-20°C). The solution can be thawed and frozen several times, and it will work for 3 da at RT.

To enable the pancreatin solution to contact internal tissues a wide opening must be made in the abdomen (e.g., Platnick et al. 1999); or a large section of the specimen must be removed to allow the pancreatin solution to enter (e.g., cut a large window on the dorsal cuticle of the abdomen). For specimens preserved in ethanol, transfer the specimens to a vial with distilled water for one to two hours or until they sink; then transfer the samples to the pancreatin solution.

Specimens $\leq 5\text{ mm}^3$ will take overnight to digest completely. Smaller samples, such as epigyna, will take 4–5 h but can be left overnight too. Samples left $> 16\text{ h}$ can be completely digested and destroyed. Digestion times for specimens $\geq 5\text{ mm}^3$ will be $\sim 1\text{--}3\text{ da}$. Incubating the samples at $\sim 37^{\circ}\text{C}$ will reduce digestion times. All samples must be cleaned after

24 h of digestion. The cleaning process must be done carefully to avoid damaging the internal sclerotized structures. Transfer the sample to distilled water and remove the partially digested tissue with streams of water produced by a pipette. Return the sample to the pancreatin solution until digestion is completed. Transfer the specimen to 75% alcohol (= ethanol) and wash it with a pipette until the tissue is completely removed. If some soft tissues remain undigested, repeat the transfer to distilled water and then to the pancreatin solution. Once the soft tissues have been digested, transfer the specimens to clean 75% alcohol.

For inspection with a compound microscope transfer the specimens to clove oil, methyl salicylate or some other clearing substance. Temporary slide-mounts for these preparations are described elsewhere (e.g., Grandjean 1949; Coddington 1983). KOH digestions work fine for study with stereo or transmitted light microscopy. However, the cuticle surface may have been over digested by the caustic process making this type of specimen unsuitable for SEM. For SEM study follow standard protocols for critical point drying and sputter coating of the specimen.

SEM mounting protocol.—Dissolve Acryloid B-72 (Paraloid B-72) pellets in acetone until they form a gluey homogenous mix. The viscosity of the mixture can be adjusted by adding or evaporating acetone. The mix should be such that it will produce a thin thread when a needle touches its surface and is then pulled about one centimeter away. If the preparation of the acryloid solution produces many air bubbles (as a consequence of stirring the pellets in acetone), sonicate the vial until all the bubbles have risen to the surface. Unused acryloid solution can be stored in a closed vial and its viscosity readjusted with acetone when needed. Any glue that forms thin elastic threads and dries out relatively fast could be potentially used, but we only have experience with Acryloid B-72.

Take the critically point dried specimen with a fine brush or soft forceps; conventional forceps will easily damage the specimen. Place the specimen (e.g., cephalothorax, pedipalp, leg, etc.) on the free end of the sticky copper tape (the other end of the tape has been attached to the SEM mount). At this point avoid moving the copper tape because this action may easily catapult the specimen. To permanently attach the specimen to the copper tape, collect enough glue with the tip of a needle mounted on a handle (or a thin and straight piece of metal wire), touch the base of the mount with the glue-loaded needle tip and pull the needle until a thin thread of glue is formed. Wrap the specimen and the copper tape with this glue thread several times by going around the area in a circular motion. Once the glue has dried, examine the mounted specimen with the stereoscope at high magnification. If any dirt is visible on the specimen, try to remove it by gently blowing air with a thin glass pipette connected to surgical latex tubing or with the help of a needle or brush. Carefully bend the tip of copper tape with the mounted specimen to the desired orientation. Proceed to sputter coating the preparation.

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