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Fixing *Biomphalaria glabrata* snails for dissection

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Introduction

The following procedure describes fixation of the soft snail tissues, which is important for taxonomic purposes. As demonstrated by the innumerable publications of W.L. Paraense and others, many other planorbids can be processed by this general method. It is an especially useful technique for examining the location of development of primary and secondary sporocysts of *S. mansoni*, which are readily apparent in a properly fixed snail.

Equipment

Dissecting microscope

Materials and reagents

Curved forceps
Sodium pentobarbital solution (500 µg/ml)
Fixative: Bouin's solution (https://www.sigmaaldrich.com/US/en/product/sigma/ht10132)

Procedure

- Incubate the snail at 28 °C in the sodium pentobarbital solution for 4-6 hours.
- Remove the snail from the pentobarbital solution and gradually plunge the snail into hot water (70 °C) for a time proportional to the snail's size (approximately 45 seconds for snails ~20 mm in diameter, slightly less time if snails are smaller).
- Plunge the snail into cold tap water.
- Using curved forceps, and holding the snail underwater, grasp the body of the snail posterior to the headfoot and gently pull it with the forceps so that the columellar muscle detaches from the shell. The entire body should come out of its shell as the water fills up the emptying snail shell. If the snail has withdrawn into its shell, the forceps should be used to crack and remove small pieces of the leading edge of the shell until the snail's body can be grasped with the forceps posterior to the headfoot, as described above.
- Place the snail's body in Bouin's solution (fixative) for at least 24 hours. The volume of fixative should be more than 10 times the volume of the snail body. The fixative should be changed once after the first 24 hours.
- The primary sporocysts can be most easily seen as white masses, or swellings, usually in the headfoot and or tentacles of the snail that was exposed to miracidia 10-14 days previously.
- Secondary sporocysts can be observed in various tissues, beginning around 3 weeks post-exposure. By 4 weeks post-exposure, many sporocysts have migrated and embedded in the hepatopancreas and ovotestis of the snail and will occupy a significant part of both organs.

Follow-up comments/recommendations

Although anesthetizing the snail prior to removal is not essential, it does facilitate pulling the snail from its shell. The primary and secondary sporocysts stand in stark contrast to the surrounding snail tissues, although occasionally a primary sporocyst may be difficult to visualize if it is located deep in the headfoot.

Note: An alternative method of removing the snail's body from its shell is to gently crush the shell between glass microscope slides and remove the shell fragments with forceps, exposing the snail's body.

References

1. Paraense, W.L. 1976. A natural population of Helisoma duryi in Brazil. Malacologia 15: 367-376.
