

From:

Avanesyan, A. (2014) Plant DNA detection from grasshopper gut contents: a step-by-step protocol, from tissues preparation to obtaining plant DNA sequences. *Applications in Plant Sciences* 2(2):1300082

## **Appendix S1. Protocol for dissecting grasshoppers and tissues preparation**

Developed by A. Avanesyan

The details for isolating a gut and preparing foregut and [midgut+hindgut] parts are presented in Video 1.

### **Part I. Isolation of grasshoppers' guts (for *M. femurrubrum* grasshoppers\*, Fig. 1A-E, Video 1):**

1. Take a frozen grasshopper from the freezer and rinse it with 70% ethanol.
2. Use forceps and scissors to carefully remove hind legs and wings.
3. Put the grasshopper on its side and use insect pins to anchor it to the dissecting pad.
4. Use scissors to cut the exoskeleton along the side. Start with the last segment of the abdomen and move slowly toward the head.
5. Carefully pull out the digestive system (if dissecting a female, remove bright yellow ovaries and fat bodies which are in the abdomen).
6. Place the whole gut in 1.5 ml *microcentrifuge tube* with 70% ethanol and store it overnight before the DNA extraction. Skip this step if you immediately proceed with DNA extraction (rinse the gut with 70% ethanol for 10s).

### **Part II. Preparing foregut and [midgut+hindgut] parts (for *M. differentialis* grasshoppers, Video 1):**

1. Place an isolated gut on the dissecting pad (Fig. 1E).
2. Review a scheme of the internal structure of the grasshopper to match the main parts of the digestive system (Fig. 2).
3. Find the border between foregut and [midgut+hindgut] parts (Fig. 1E).
4. Use a scalpel to separate foregut and [midgut+hindgut] parts.

5. Place foregut and [midgut+hindgut] parts separately in 1.5 ml *microcentrifuge tubes* with 70% ethanol and store them overnight before the DNA extraction (Fig. 1. F). Skip this step if you immediately proceed with DNA extraction (rinse the gut's parts with 70% ethanol for 10s).

\* Use the whole body of a nymph grasshopper (due to its size) in DNA extraction. Remove hind legs if necessary.

Supplies check list:

Small vinyl dissecting pad (11 3/4 x 8 in, Carolina Biological Supply Company)

Standard dissecting set (fine scissors, straight, 4-1/2"; fine forceps, straight, 4-1/2"; fine forceps, curved, 4-1/2"; scalpel)

Insect pins (black enamel insect pins, size 2, pkg. of 100, BIOQUIP)

70% ethanol

1.5 ml Microcentrifuge tubes (FISHERBRAND, Cat.No. 05-408-129)

Scheme of internal structure of a grasshopper (Fig. 2)

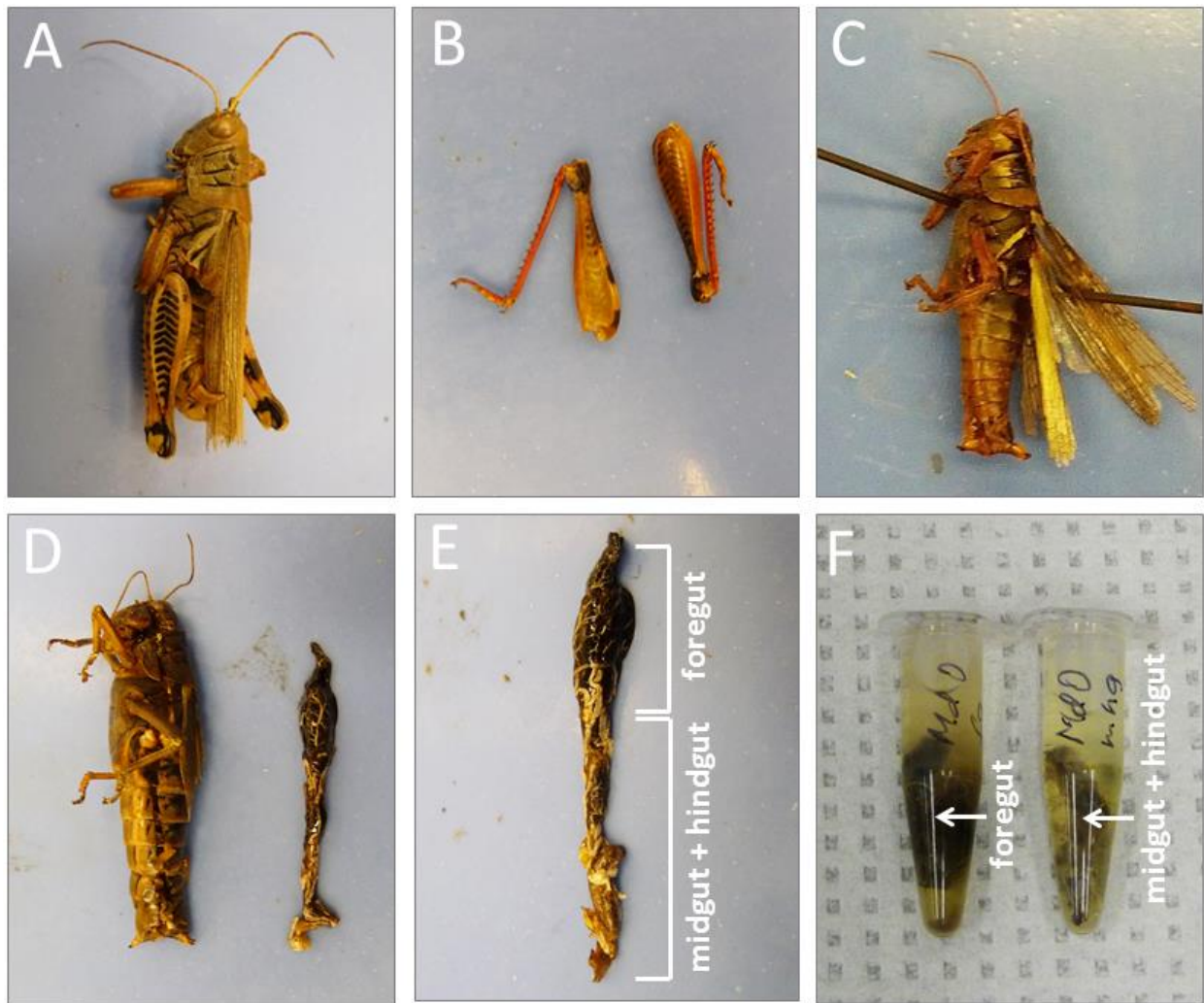


Fig. 1. Basic steps of dissecting grasshoppers and preparing their guts: removing hind legs and wings (A-C); cutting the exoskeleton along the side and pulling out the digestive system (D); separation of foregut and [midgut+hindgut] parts (E); storing different parts of the gut in 70% ethanol. Step E is not needed if the dissection is immediately followed by DNA extraction (Images by A. Avanesyan; Video 1).

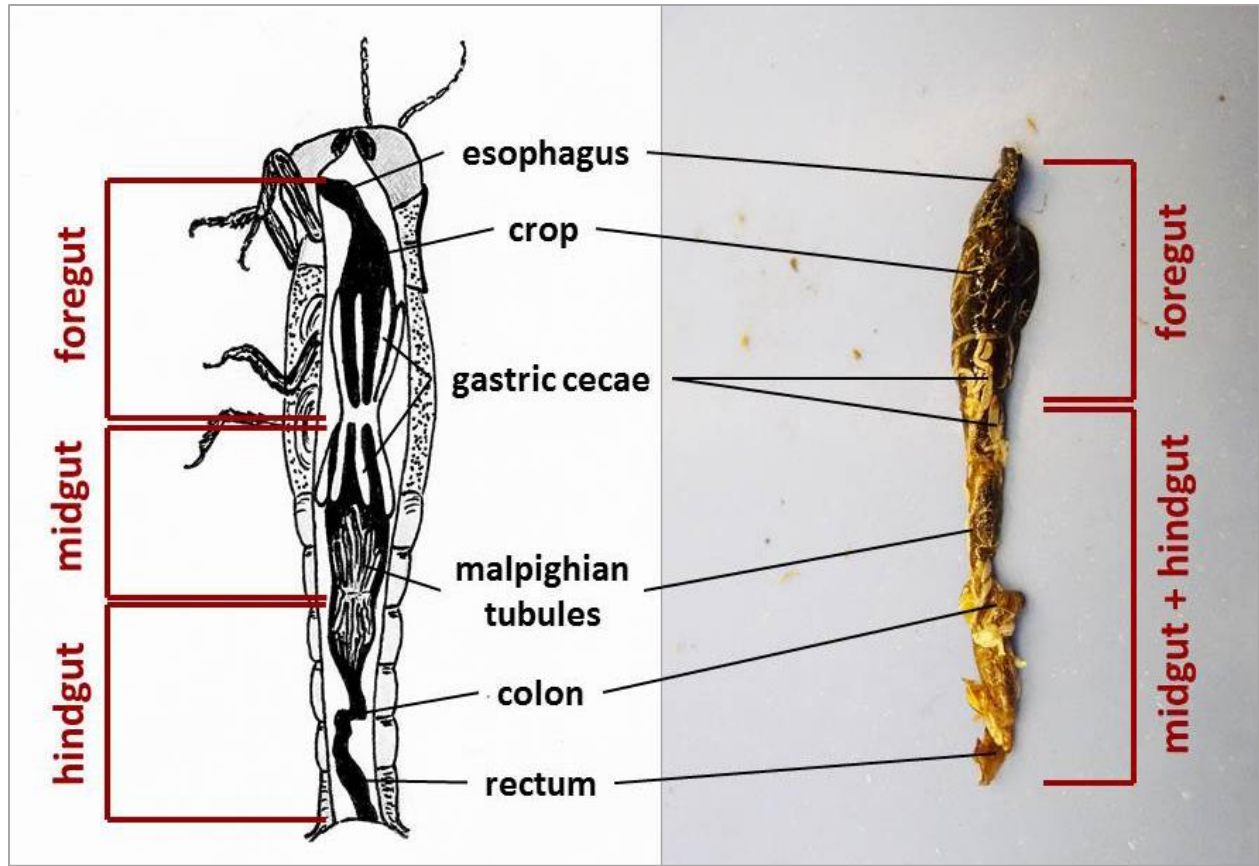


Fig. 2. Scheme of a grasshopper's digestive system (on the left) and corresponding parts in the gut pulled out from the grasshopper (on the right) (Images by A. Avanesyan).