Bocas ARTS

Video Transcript

How To Dissect A Solitary Ascidian

Rosana M. Rocha

- In this video I am going to show you how to dissect a solitary ascidian.
- Since tunicates are usually fixed in formaldehyde, is a good idea to plan in advance and take them out of the storage jar the day before.
- You are going to rinse it and leave it overnight in water.
- This is an animal that has been overnight in water. I am going to get it with my gloves on because there is still formaldehyde in here and also have glasses or safety glasses for your safety.
- The first thing you do is to pay attention on the tunic of the animal, if there is anything attached to it, any encrustations, its color. Some tunics fade in formaldehyde or even change colors. Then we are going to see this side. Here there is a small bivalve living inside the tunic. Then you can also see where the animal is attached from.
- Here are the siphons, so I am starting to cut the opposite side of the siphons with a scissor with pointed ends.
- If it is difficult to start with the scissors, you can also use a scalpel just to start the first cut, and then you can continue with the scissors.
- The scissors should be very horizontal, not to damage the body wall.
- You are just cutting the tunic, and go all the way towards the siphons because there is where the animal is mostly attached to the tunic.
- You can already see that the animal is not that attached to the tunic. And you can continue all the way towards the siphon.
- The siphon is mostly attached to the tunic around its boarders, so if you cannot detach it, cut the tunic around.
- Now it is liberated and I am going to start cutting towards the next siphon.
- Now you can see the thickness of the tunic and also pay attention to any colors or structures like blood vessels inside the tunic.
- So now we have our animal removed from the tunic, and in the next step we are going to orient the animal.
- Here we can see that in this side, we do not see that many structures, we are just seeing all these through, because this body wall is very transparent.
- But if I turn it on the other side, now you see the guts much better, some of the gonads and also the neural ganglion here.
- Now we have all the structures we need to orient the animal.
- If you follow the intestines, this is the stomach and then you follow the intestine, it makes a first loop, second loop and the anus is right there, pointing to the excurrent siphon or atrial siphon.

- Also gonads are here and the duct is there, the yellow tube is the gonad tube, also pointing to the excurrent, so we can use both the guts and the gonads to understand which is the atrial siphon.
- The other one will be the oral siphon or the incurrent siphon.
- The neural ganglion is between the siphons and marks the dorsal margin of the animal.
- The opposite will be the ventral margin of the animal.
- And since this animal is very transparent, we can see a white line which is the endostyle inside the pharynx.
- The oral siphon marks the anterior region of the animal, and the opposite is the posterior region of the animal.
- To understand which is the left side and the right side, you have to think that the dorsal margin should be up. Now the animal does not have the tunic. So more or less, this will be up, and the oral siphon ahead of the atrial siphon. Then you have the animal in the same direction as you are; so this will be your left side, and the other one the right side.
- We have most of the structures bent to the left side of the pharynx, and on the right side of the pharynx we cannot see much.
- The orientation is important because we are going to dissect our animal in a certain way.
- We are going to start more or less close to the oral siphon and cut along the ventral margin.
- Always do that because all the descriptions and drawings in the publications are this way, so it will be easy to compare your animal to the literature.
- So I am going to start cutting the animal through the oral siphon and following that white band that I showed you before which is the endostyle.
- If the animal is not that transparent and you cannot see, that is not a problem, you just keep going through the ventral margin.
- Now we are cutting both the body wall and the pharynx, so we are completely opening the animal, and be sure you do not cut the intestine.
- We can open the animal and cut all the way to the posterior side until you can really make it flat on the petri dish. You can see that the structures are very transparent, we can almost not see much.
- This is why I am going to transfer this animal to a petri dish with wax, pin it and then stain it.
- And to stain it, we are going to use Hematoxylin.
- Most of the structures are in the anterior region, so this is the important one to stain.
- You leave the stain there for one or two minutes without moving the animal, and then you are going to wash your preparation.
- So after washing it a few times with clean water, this is how our preparation is stained.
- Now we can see here is the oral siphon that we cut and we can see the oral tentacles, in this animal they are simple threads. We can see the dorsal lamina in the middle, it is a membrane that goes all the way towards the esophagus aperture. And this is the

pharynx, and there is a cross of threads here, these are the blood vessels that we are going to see afterwards in more detail.

- And here is the intestine.
- Everything is covered by the pharynx. Because we cut both, body wall and the pharynx, we are going to study first the regions internal to the pharynx. And then we are going to remove his region of the pharynx to be able to study the intestines and the gonads.
- Here is the endostyle; the structure that we cut along.
- Now we are going to remove part of the pharynx to see better the intestine and the gonads.
- To do that, you need very small scissors to cut the pharynx; this one bent this way is easier to work with, because you can work in vertical and have these scissors horizontal, but you can also use small scissors like these.
- The idea is to cut along the ventral margin before the endostyle, and then go around and then cut horizontally above the intestinal loop. And down here underneath the stomach and go up, leaving the aperture of the esophagus in the preparation; just take the pharynx off.
- Then you are going to lift the pharynx a little bit, and pass the scissors underneath because there are lots of small blood vessels linking the pharynx to the body wall or to the intestine.
- Here is our preparation with this region of the pharynx removed, and here you can see the stomach and then the intestine, first loop, secondary loop, rectum and anus.
- Inside the intestinal loop we can see the gonads, the yellow part is the ovary and the white part are the follicles of the testicle.
- And let me show, this is the slide I made out of the removed part of the pharynx. This is important because the pharynx has all the blood vessels and the stigmata and we are going to count how many stigmata per mesh and if there are any papillae or any other structures in the pharynx.
- To do this, you need to put the slide under the optical microscope.
- I am going to show you another animal that has been dissected just as I did before, but this has a more complicated pharynx because these are folded pharynx, but the procedure to remove the pharynx is exactly the same.
- In the case of this species, it has structures on both sides of the body, so it has gonads here and there. So it is necessary to remove the whole pharynx, not just a window.
- And you will also keep the pharynx for a detailed study under the optical microscope.
- Here we have all the steps of our solitary ascidian dissection, the tunic, then the open animal, removing the pharynx, and then studying the internal structures, and the pharynx is there with all the steps, you are going to be able to follow an identification key.