GETTING A GRIP ON SIZE, SHAPE, AND EVOLUTION OF OPHICEPHALOUS PEDICELLARIAE IN SEA URCHINS

MEGHAN YAP-CHIONGCO: RICH MOOI
DEPARTMENT OF INVERTEBRATE ZOOLOGY AND GEOLOGY
CALIFORNIA ACADEMY OF SCIENCES
SUMMER SYSTEMATICS INSTITUTE
This summer, I worked with Rich Mooi in the invertebrate zoology and geology department, doing work on the ophicephalous pedicellariae.
Pedicellariae are small, jawed structures that attach to the calcite skeletal structure (also known as the test) of many taxa belonging to the class Echinodea. In this class, 5 different types of pedicellariae can be observed, but little is known about the true functionality, growth, and evolution of these structures. For my project, I only focused on one of the five types, the ophicephalous. This type is believed to be used to grasp onto objects past the point of muscle exhaustion, theoretically aiding the animal in food storage or camouflage. They are micrometers small, and can withstand pull force that is impressive for their tiny size (hold up to 10 grams). The image above shows just how small these things are, and where they can be found on the test.
Ophicephalous are made up of two main parts: the stem and the head. The stem attaches to the test through a small tubercle, and terminates in a cup where the head sits. The head is made up of three valves, containing a hinge, and a loop, called the handle. The three hinges of the valves nest inside one another and are connected here to the stem through connective tissue called the “strap”.

Three valves that make up the head
The image on the left shows the strap that connects the head to the stem. It is speculated that the strap has the ability to stiffen the collagen fibers within the connective tissue. This ability to stiffen collagen is characteristic in other structures, such as the spines, within many echinoid taxa. This would allow the animal to continuously grip to an object for extended periods of time, therefore avoiding exhaustion in a way that is metabolically conservative.
For my project, I focused on members of the class Echinodea. This class is made of the spiny, globular guys you probably associate with the word sea urchin, along with other forms such as sea biscuits and sand dollars (which are flattened urchins with reduced spines). Within this class, I mainly focused on the sub order Clypeasterina, along with sampling from other out groups. The phylogeny above shows the relationships between these taxa and the evolutionary history of the ophicephalous.

There are two forms of ophicephalous found within the sea urchins, what we have deemed the “U-Shape” and the “Space-Man”. The U-shape form is found in all earlier forms of Echinoïds that have this type of pedicellariae. The Space-Man shows up first in the Echinolampadoida, and then again in the Clypeasterina. Clypeasterina’s sister group, the Laganina, however, display the U-Shape form once again. There are two ways to describe the evolutionary history of the ophacephalous, Option 1. in which the Space-Man is first derived in the order Echinolampadoida and then
To better visualize the creatures themselves, here are a few of the specimens I will be talking about. The Clypeasters are sea biscuits, which are, of course, sea urchins.
In order to answer any of the following questions, one key question must be addressed first: can we measure these tiny things and get anything of significance out of the data? Fortunately, the answer is yes. Knowing that we can get relevant data from these micro structures, we can now answer the following questions.

1. What are the growth patterns of the ophicephalous?
2. Are there any differences in growth patterns between species?
3. Are there any absolute differences in size and shape among irregular echinoid clades?
For my project I performed an allometric analysis which determines the relationship between the size of an organism and the size of a particular structure. This analysis was used in order to do determine the size relationships between the ophicephalaous and the specimen, and between the structures that make up the ophicephalous themselves. To determine this relationship, I focused on the genus Clypeaster, doing one series myself on C. ravenelli, and then combing that to allometric data previously done by Rich Mooi on C. rosaceous and C. subdepressus. To do an allometric series, you first have to get a good size range of the species you are focusing on.

Above is a picture of the 15 C. ravenelli specimens that I used in this project, ranging in length from 27mm to 127mm. 5 pedicellariae and one of each type of spine.
I worked under a dissecting microscope to pluck each pedicellariae off of the test and put it on a slide with a drop of bleach on it. The bleach is used to strip the tissue from the calcite, leaving the valves and stem separated from each other. We found that scented bleach works best for stripping tissue, with the added bonus of smelling like lavender. After everything was separated, the slide was then prepped and put under a compound microscope attached with a camera lucida. This allowed for the structures on the slide to be traced onto a piece of paper and measured in millimeters using a caliper.
After the drawings were measured and converted using a standard based on what magnification the image was drawn at, 40x for the stems and spines and 10x for the valves. This standard was determined for each magnification, using a stage micrometer. Measurements of the cup, stem, valve, and hinge were taken from each data set and converted into micrometers. The image above shows how each structure was measured. Data was then entered into excel and transferred to stat view for data interpretation. Here, the averages of the five pedicellariae from each specimen were taken, transformed into logs, and plotted on a scattergram. The same methods were used to measure and record specimens from 15 additional species of Clypeaster, and 10 species from both Laganina and Echinolampoida.
For allometric data, the closest your slope is to one the more equal the allometry is, meaning that the the two structures are growing at equal rates (called isometry). When the slope is less than one, you have a negative allometry. This happens when the organism grows at a faster rate than the structure you are comparing it to. If the slope is greater than one, you have a positive allometry. This is when the structure your comparing grows more rapidly than the organism itself. What this data set tells us is that the structures that make up the ophicephalous have near isometric allometries. This means that these structures have a plan, and that the size of each structure in respect to another aides in a particular function. The valve and hinge, for example, each need to be a particular size with respect to the other. If hinge kept growing while the valve stopped, the function that they serve would be effected.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>VALVE WIDTH VS. VALVE LENGTH</th>
<th>HINGE WIDTH VS. VALVE LENGTH</th>
<th>CUP WIDTH VS. STEM LENGTH</th>
<th>VALVE LENGTH VS. STEM LENGTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAVENELLI</td>
<td>Y = 0.903x + 0.336, R² = 0.862</td>
<td>Y = 0.947x + 0.263, R² = 0.907</td>
<td>Y = 0.702x + 0.586, R² = 0.794</td>
<td>Y = 0.71x + 0.106, R² = 0.833</td>
</tr>
<tr>
<td>ROSACEUS</td>
<td>Y = 0.822x + 0.606, R² = 0.943</td>
<td>Y = 0.861x + 0.723, R² = 0.963</td>
<td>Y = 0.705x + 0.51, R² = 0.889</td>
<td>Y = 0.753x + 0.397, R² = 0.897</td>
</tr>
<tr>
<td>SUBDEPRESSUS</td>
<td>Y = 0.97x - 0.191, R² = 0.944</td>
<td>Y = 0.927x + 0.447, R² = 0.833</td>
<td>Y = 0.776x - 0.194, R² = 0.882</td>
<td>Y = 0.65x + 0.798, R² = 0.927</td>
</tr>
</tbody>
</table>

- Valve length and width are growing at essentially the same rate.
- Hinge width is growing at the same rate.
- Width of the cup is not keeping up with growth of the stem.
- Stem is growing slightly faster than the length of the valve.
These are the graphs that illustrate what was on the previous table. This regresses the valve width vs valve length and the hinge width vs valve length. These both display good lines, high R-values, and slopes close to one. This meaning these structures are close to isometry.
This shows the relationship between cup width vs stem length and valve length vs stem length.
The slopes for the structures regressed against test length, show slopes less than one in every category. This shows that there is a negative allometry occurring in all of these relationships, meaning that the test is growing much more rapidly in respect to the ophicephalous.
This shows stem length vs test length and cup width vs test length. Each color represents a species, green is C. rosaceus, red is C. ravenelli, and blue is C. subdepressus. Note how even though the data sets were combined, the data naturally divide into individual species, but still display similar slopes.
These graphs show valve length vs test length, and hinge width vs test length. Once again, the data split off into their respective species, while still displaying similar slopes.
This final graph shows valve width vs. test length. This graph is a great representation of the splitting of the data into their respective species. This splitting shows us that there are differences in growth rates between different species. With this knowledge of differences in growth, you should be able to take one of these pedicellariae, measure them, and
Before I did this project, it was believed that in the 3rd valve, the T-zone, as shown above in C. rosaceous, was unattached to the rest of the valve. I first noticed that this varied in C. annandalei, such as in the image above. As I collected diversity data, I realized more and more had this “fusion” of the T-zone. After recording every species that had this fusion, I began noticing a possible phylogenetic trend. This view, however, changed when I was looking at specimens of ravenelli that I went back to in order to get more data. On one ophicephalous, I noticed fusion on one side of the “T” while the other side was free. From that point on, I looked at the third valve of every ophicephalous I measured, and found that whether the T-zone was fused or unfused depended on each individual ophicephalous, not on what species it came from. By doing this, we are able to infer
In addition to the C. ravenelli allometry I performed this summer, I also did species diversity within Clypeaster. For this, I have preliminary data on 15 additional species of Clypeaster, as well as preliminary data on 5 species of Echinolampodiidae and 5 species of Laganids.
These are the species of Echinolampadidae That I completed with average ophicephalous size. Note that the Laganids (Peronella and Personella) are the smallest type of ophicephalous that I sampled.
Here, are some very good comparisons to what these things look like in comparison to one another. Both of the images are scaled to 100 micrometers.
1. We conclude that there is strong negative allometry in the relationship between test size and ophicephalous size. This shows that the test grows in much higher rates than the ophicephalous. This shows that just because you have a large test, does not necessarily mean you have large ophicephalous.

2. There is variance in the growth rates of ophicephalous species to species: this means that you could tell what species each ophicephalous is from based off of its measurements, and never have to look at the specimen from which it came from.

CONCLUSIONS

1. There is a strong negative allometry in the relationship between test size and ophicephalous size.

2. Species vary in growth rates within the structures of the pedicellariae themselves.
There are many future projects that can be done on the growth, morphology, and evolution of ophicephalous. As Rich put it, you could get a masters or a PhD out of it. Here I have some future avenues that I would like to explore.

1. **Micro-CT**: This would allow for a scan of how the connective tissue is arranged while the pedicellariae is whole. The true function of the strap is mere speculation, and having CT data for this would offer some clarification on the matter.

2. **Expansions of existing data sets**: The data sets for *C. rosaceus* and *C. subdepressus* only contain 12 specimens, while the data for *C. ravenelli* contains 15. I would like to fill in some gaps in size with 3 additional specimens from each species. In addition, I would like to go back to my diversity data sets and bring up the
I’d like to thank the following people for their help in my project:

1. Rich Mooi for introducing me to these amazing things, answering my questions, photoshopping all the great images in this project, and being an all around great mentor.
2. Stephanie Castillo for taking time away from her project to help me with the SEM images of C. ravenelli.
3. Isaac Krone and Shaina Villalobos for helping me make the Jaws poster at the beginning of the presentation.
4. NSF for the funding to be able to be here today and present my findings to you all.