Radular Morphology among Chiton in Family Mopillidae

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Abstract

The Mollusca radula is an intricate organ composed of several rows of teeth running along a chitinous ribbon. Several studies have looked at radula of gastropods but fewer have looked at the radula of Polyplacophora. An observational study comparing morphological differences between species of Mopalian radula, including *Mopalia muscosa, Mopalia lignosa* and *Mopalia ciliata*, was conducted. Observations of light microscopy photographs, Scanning Electron Microscope (SEM) photographs, tooth impressions and video were performed in order to look at differences among species. The results show some major morphological differences among species, such that *M. muscosa* had more stout teeth whereas *M. lignosa* had short and narrow teeth and *M. ciliata* had long, slender teeth. Until now, the differences among the three species of *Mopalia* have remained unseen.

Introduction

A common feature among the Mollusca (with the exception of bivalves) is the presence of a radula. The radula is a long, ribbon-like structure that contains several rows of teeth used to scrape food off of rocks or drill holes in shells (Brusca & Brusca 2003). The radula can vary in function and appearance between different classes, even species, of mollusks (Yang & Zhang 2011). These differences can be useful for many things like classifying species, as shown in a study done to show species divisions in the genus/species. The radula of 22

species of Nassariidae were compared to reach a conclusion that the genus *Nassaruis* could not be further divided into subgenera, but they could use certain morphological features to differentiate between species (Yang & Zhang 2011).

Different morphological features can be explained in part by the variation of diet among different species of chiton. Latyshev et al. (2004) looked at the differences in diet composition and food sources between five species of chiton by performing a gut analysis along with fatty acid analysis in the digestive tract tissue. All species were found to co-occur in the same rocky-boulder reef biotope. Significant interspecific selection in food intake was found regardless of having the same food supply available (Latyshev 2004). The diets among the chiton ranged from diatoms and red algae to amphipod crustaceans. Though it was not certain, the differences in diet were suggested to be due in part to shifts in feeding mechanisms (Latyshev 2004). Furthermore, a study by Piercy (1987) looked at differences in diet among six species of chiton, including *M. ciliata* and *M. muscosa.* His results showed that *M. muscosa* appeared to be more herbivorous than *M. ciliata* (Piercy 1987). This finding agrees with those of Latyshev et al. (2004) that even when living in the same biotope, different species will maintain different diets. Even more interesting was a finding that, depending on its environment, *M. muscosa* will eat either only algae or a mixture of algae along with other 'animal food' (Connor 1975). There have also been studies that looked at variability of diet among chiton in relation to seasons. Langer (1983) found that *Tonicella rubra* was omnivorous only in the autumn and was herbivorous all

other seasons as opposed to *Tonicella mermorea* which was herbivorous year round (Langer 1983). The fact that chiton living in the same environment maintain various diets poses the question of what differences appear in the morphology of the radula.

Though some studies have looked at different aspects of chiton radula, little is known about the form and function of radular teeth (Padilla 2004). As suggested, more work needs to be done on the Mollusca radula since it is a defining feature of the phyla (2004). It is still unclear whether species in the same genus, *Mopalia*, have more similarities or differences in their radula. It would seem that species within the same genus, as opposed to those of a different genus, that share a similar diet and lifestyle would have more similarities in their radula than differences. To gain a better understanding of chiton radula, several observational studies were performed to look at the morphological similarities and differences of three species of *Mopalia*, *Mopalia muscosa* Gould 1846, *Mopalia lignosa* Gould 1846 and *Mopalia ciliata* Sowerby 1840.

Study System

All specimens in this study belong to the phyla Mollusca, class (**Figure 1**). All three species are commonly found in high tide zones of rocky intertidal areas stretching along the west coast from Alaska to California. The chiton used in this experiment were collected on San Juan Island from Argyle Lagoon and False

Bay. All three species are also known to eat red algae along with other things such as ulva, bryozoans and sponges (Encyclopedia of Life).

Materials and Methods

The specimens used in the experiment were identified to be *Mopalia muscosa*, *Mopalia lignosa* and *Mopalia ciliata* (Kozlof) and were collected from San Juan Island, Washington. *Mopalia muscosa* and *M. ciliata* were collected from the rocky intertidal of Argyle Lagoon and M. lignosa was collected from the rocky intertidal area of False Bay. A combination of dissected and live specimens were used to observe variation among species by looking at the radula with light and scanning electron microscopy (SEM), impressions made by teeth when feeding, as well as live video of animals feeding.

Dissection of Radula Each species was anesthetized using a magnesium chloride and sea water mixture before being dropped into boiling water to make the muscles less tough for dissection. After removing the plates and muscles of the dorsal side, the radular sac along with the buccal muscles were carefully retrieved from each specimen by using a pair of fine forceps. Excess tissue was teased away with forceps before the radula went into a diluted bleach solution. Each radula was agitated in a sonicator in a 20/80 mixture of bleach and water to remove the remaining tissue until completely cleaned.

SEM Once each radula was clean light microscopy pictures were taken. Radulae were then put first into vials that contained a 50/50 mix of water and 95%

ethanol. After an hour the solution was switched so the radula were in pure 95% ethanol. After two hours in pure ethanol, the radula were put into hexamethyldisilaxane (HMDS) as its final drying agent. The radula were kept in this solution overnight then put on carbon tabs for SEM and allowed to dry. They were then sputter coated with gold/palladium alloy ten times. The gold coated tabs were then put into a JCM-5000 Benchtop SEM (NeoScope) where images of radula were taken and measured.

Feeding Marks In order to obtain lick marks from each species of chiton, dental wax was melted into shallow dishes and cooled to allow for a smooth, even finish. To enhance the probability of success, a layer of diatoms was added to the top of each wax plate and the plates sat for a day before putting the specimen on the plates. Each species was then put on a separate plate in a sea table and left there for about ten hours.

Video Analysis To observe how the chiton radula moves and functions, videos were taken of them while feeding in tanks. A Nikon D610 camera with a 60 mm MicroNikkor macro lens mounted on a 27 mm extension tube was set up outside of a clear acrylic sea table and each species was placed on a diatom covered surface. Videos were taken at 1280x720 pixels at 30 frames per second and illumination was provided by a Switronix Bolt 220 LED panel.

Results

SEM Analysis of SEM shows variation in teeth among each species. As shown in **Figure 2**, teeth were a height of 0.46 mm in *M. muscosa*, 0.42 mm in *M. lignosa* and 0.53 mm in *M. ciliata*. This figure also shows the distance between rows of teeth to be 0.33 mm, 0.20 mm and 0.35 mm for *M. muscosa, M. lignosa* and *M. ciliata* respectively. The cusp width of the tooth was found to be 0.27 mm, 0.23 mm and 0.22 mm for *M. muscosa, M. lignosa* and *M. ciliata* respectively (**Figure 3**). Also shown in **Figure 3** is the length of the teeth from the curve of the cusp to the point, as well as the distance between the two major teeth in each row of radula. The length of each tooth was 0.40 mm for *M. muscosa*, 0.25 mm for *M. lignosa* and 0.35 mm for *M. ciliata,* and the distance between columns measured 1.4 mm, 1.1 mm and 1.1 mm for *M. muscosa, M. lignosa* and *M. ciliata* respectively.

Feeding Marks Analysis of lick marks left behind by *M. muscosa* and *M. ciliata* are shown in **Figure 4**. These marks show that five teeth contact the substrate for each feeding stroke, with a pattern of a bold mark followed by a less substantial mark. The marks also show that the teeth scrape the surface perpendicular to the body axis. No feeding marks were obtained for *M. lignosa.*

Video Video analysis of *M. muscosa* showed a slow feeding motion, where the teeth came together in a jerky motion. Also seen in the video is the first five teeth coming in contact with the surface per biting motion. Analysis of *M. lignosa* showed a much more rapid and smooth licking motion, where the teeth came

together laterally much quicker than those of *M. muscosa.* Feeding videos for *M. ciliata* were not obtained.

Discussion

Looking at both **Figure 2** and **Figure 3**, similarities and differences in the radula can be seen among the different species of *Mopalia*. In *M. muscosa* the width and length of the tooth were larger than those of the other two species (0.4-0.5 mm wider and 0.5-0.15 mm longer). Additionally, the width between the major teeth within a row was slightly larger in *M. muscosa* compared to *M. lignosa* and *M. ciliata,* which were similar. These results suggest the teeth in *M. muscosa* are stouter than the other species' teeth. Both height and distance between rows of teeth in *M. muscosa* fell between the other two species, but the measurements were closer to those of *M. ciliata* than to *M. lignosa.* The most prominent results from looking at *M. lignosa* was the noticeably shorter tooth length and shorter distance between rows of teeth compared to the other two species; the height of the teeth was also smaller. These results may suggest the teeth in *M. lignosa* are smaller and more fragile looking than the other species. The teeth in *M. ciliata* were the tallest and thinnest, and had the largest distance between rows.

Comparisons of the three species' radula suggest *M. muscosa* have sturdier teeth, while those of *M. lignosa* may be more fragile and the teeth of *M. ciliata* long and thin. One suggestion for the differences among radula could be different feeding strategies. *Mopalia muscosa* appear to have teeth that would be good for grabbing a large amount of food at once, *M. lignosa* radula may be able

to pick up finer food materials and *M. ciliata* radula may be well suited for eating off of more porous surfaces. The correlation between structure and function should be further explored to understand the structural differences among different species, as mentioned by Padilla (2004). In addition to the SEM images, light photographs and video images of *M. muscosa* and *M. lignosa* support the idea of differences in feeding habits. Video of *M. muscosa* showed a tendency to move the radula more slowly, which could indicate a more thorough sweep of food. Video of *M. lignosa*, on the other hand, showed a much more rapid feeding stroke, which could be a result of trying to capture smaller particles before the water flow moves them away. Additional studies looking at a more detailed diet and eating environment could help support or disprove these suggestions.

Looking at the tooth impressions in the wax showed a common pattern of five teeth used per feeding stroke and alternating thick marks followed by thinner marks that are perpendicular to the body axis. This pattern would suggest that the tooth first touches the surface with the main cusp followed by the smaller inner cusp as it moves inward. This pattern can also be seen in SEM photos where the tooth is more worn on the main cusp, then the inner cusp and lastly the outer cusp. The pattern is again seen in the videos where the motion of the radula moves in such a way that the center cusp makes contact with the surface first, followed by the inner cusp as the radula rolls inward.

Although the reason is still not apparent, the results from this study showed definite differences is in the morphology among the three species of

Mopalia. Further examination of feeding among these species may help explain the differences in morphology along with the differences in radular movement.

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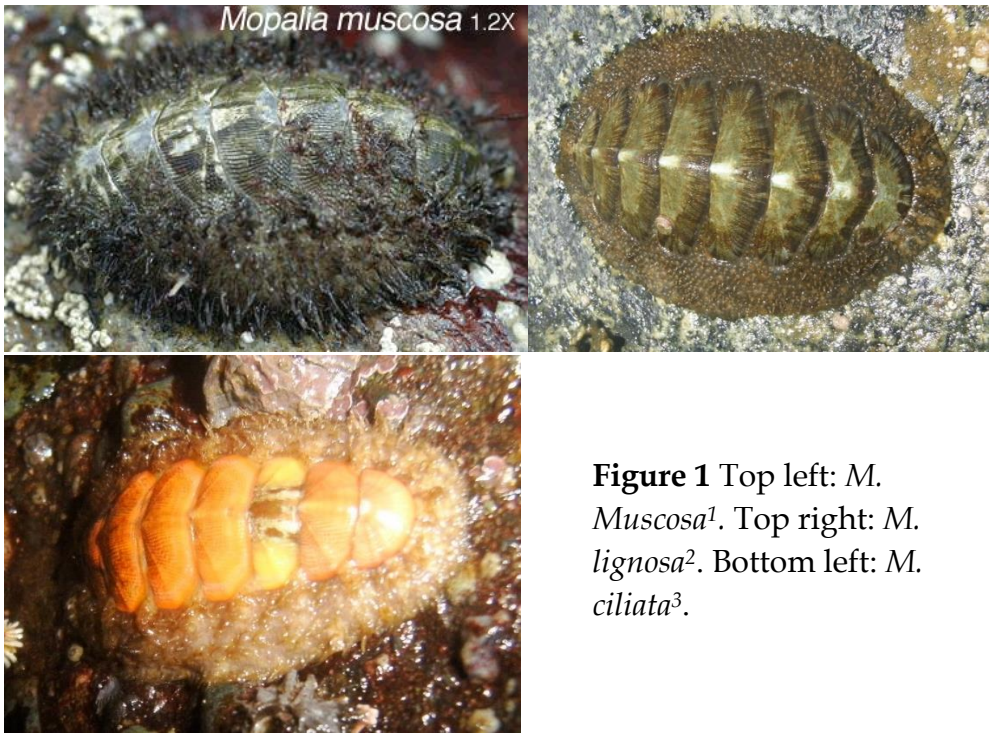

Figure 1 Top left: *M. Muscosa*[1]. Top right: *M. lignosa*[2]. Bottom left: *M. ciliata*[3].

[1]http://www.asnailsodyssey.com/LEARNABOUT/CHITON/chitDesi.php
[2]http://www.dereila.ca/whiffin/
[3]http://www.wallawalla.edu/academics/departments/biology/rosario/inverts/Mollusca/Polyplacophora/Mopalia_ciliata.html

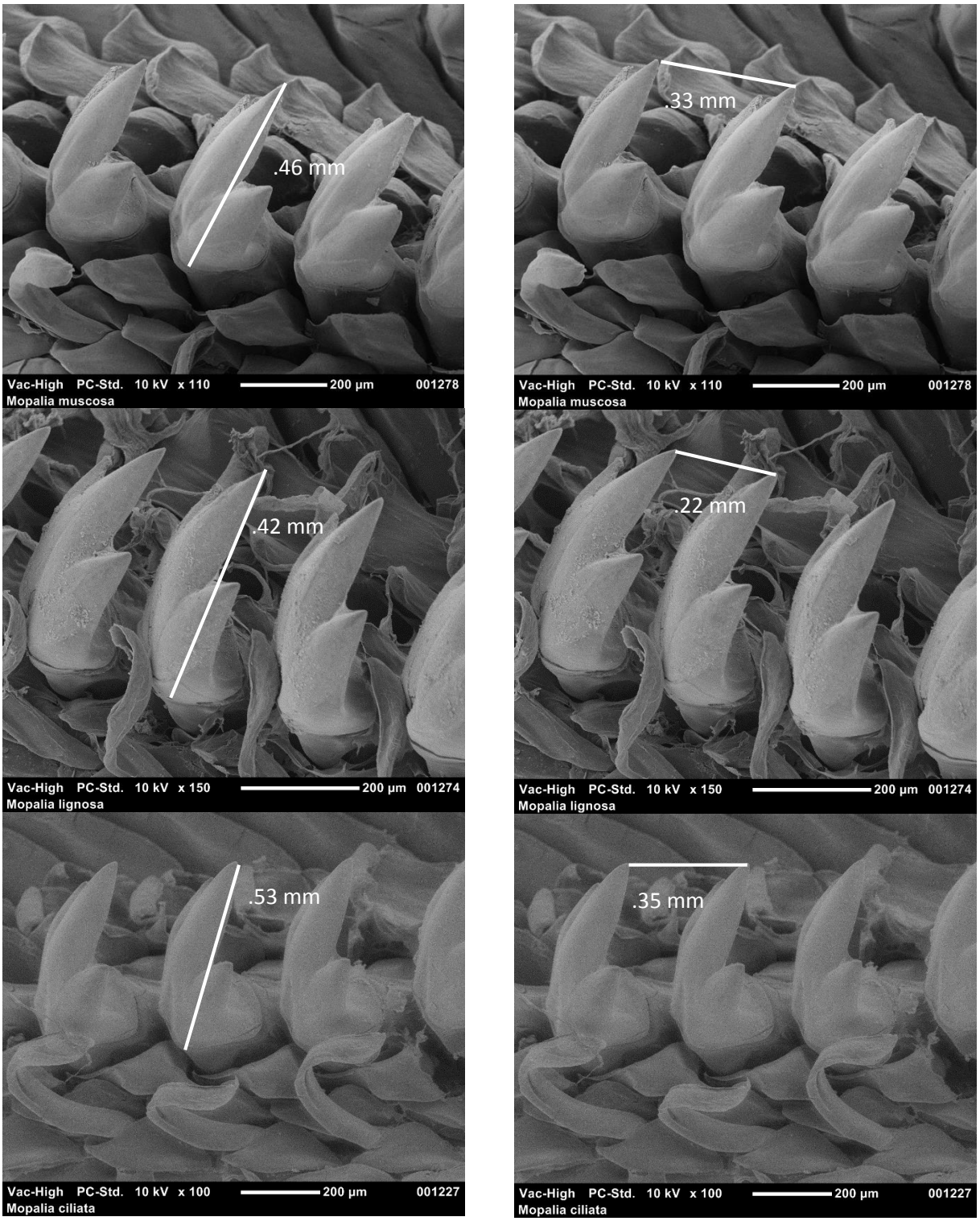

Figure 2 Left column showing height of teeth of *M. muscosa, M. lignosa* and *M. ciliata* respectively. Right column showing distance between rows of teeth of *M. muscosa, M. lignosa* and*M. Ciliata* respectively.

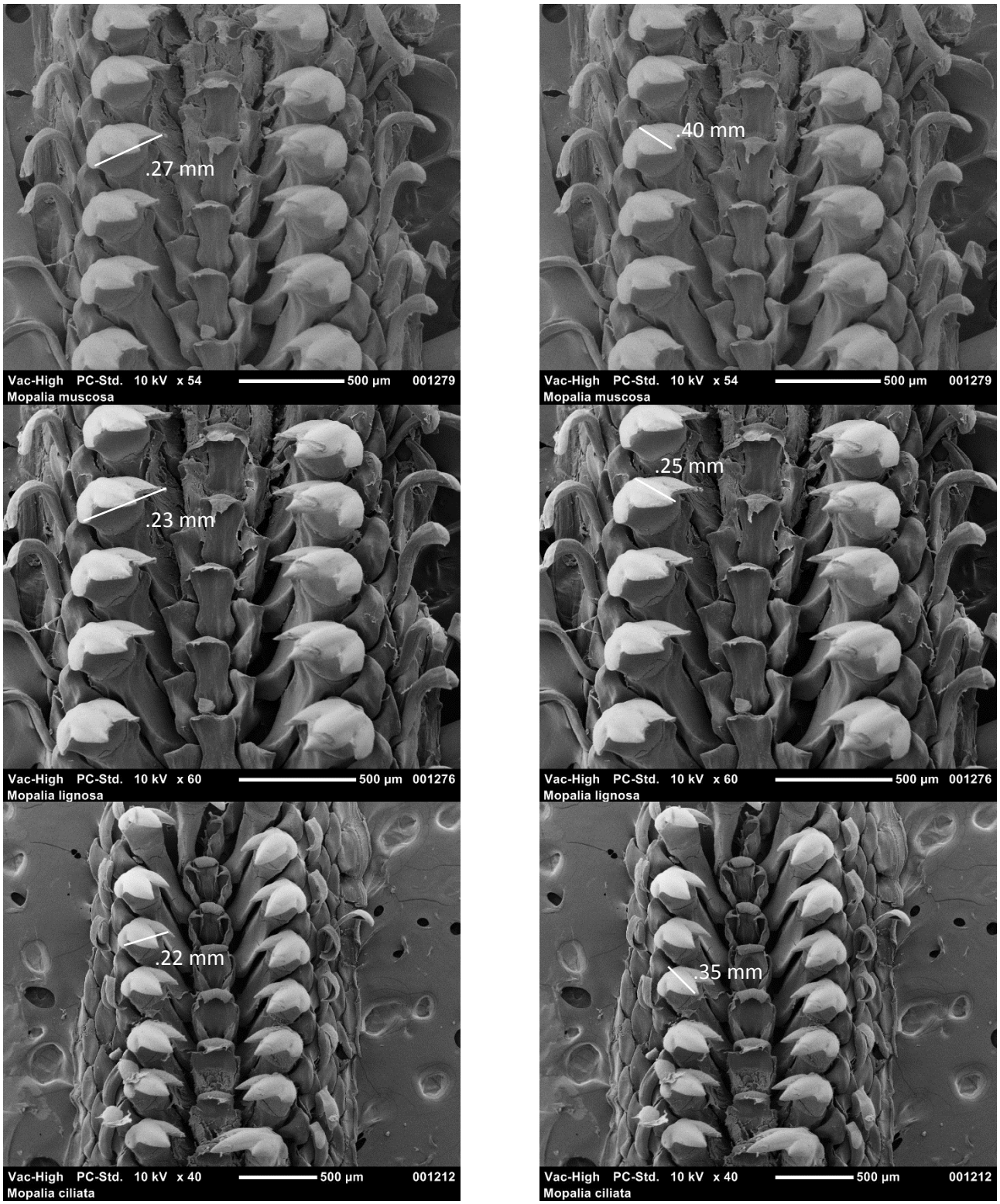

Figure 3 Left column shows width of cusps for *M. muscosa, M. lignosa* and *M. ciliata* respectively. Right column shows length of tooth for *M. muscosa, M. lignosa* and *M. ciliata* respectively.

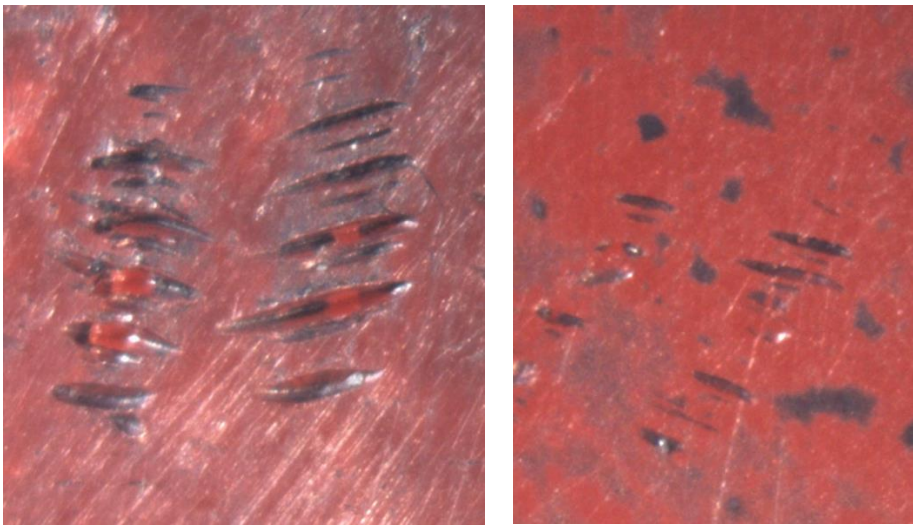

Figure 4 Tooth impressions in dental wax of *M. muscosa* (left) and *M. ciliata* (right)