BIOLOGY OF THE PERFORATE DOME SNAIL, *VENTRIDENS DEMISSUS* (GASTROPODA: ZONITIDAE) FROM SEABROOK, TEXAS

by

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Dedication

I dedicate this thesis to my late grandmother.

Acknowledgements

I would first like to thank my thesis advisor Dr. Russell L. Minton for his continued support, critical feedback, good humor, and everlasting patience. I would also like to thank him for allowing me to pursue my interest when developing this thesis project. It has been a pleasure working with him during my time in the Department of Biological and Environmental Sciences at the University of Houston - Clear Lake.

Secondly, I would like to thank Dr. Cynthia Howard for agreeing to be a member of my thesis committee and for the valuable knowledge and skills I have obtained from taking her courses during my time here.

I would also like to Dr. Phillip Harris from the Department of Biology of the University of Alabama for agreeing to be a member of my thesis committee.

Finally, I would like to thank my family, friends, and loved ones for their support throughout my graduate career.

ABSTRACT

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Adrian A. Medellin
University of Houston-Clear Lake, 2018

Thesis Chair: Dr. Russell L. Minton

The perforate dome snail (*Ventridens demissus*) is a terrestrial gastropod in the Zonitidae family. In this study I observed morphological variance, shell strength, reproductive behavior, egg size, hatchling growth, microbial gut content and species distribution of *V. demissus*. Shell height, width, and distance from aperture to callus were measured using a digital caliper. Whorl count was measured visually under a dissecting microscope. Shell strength was observed by placing shells between two metal plates and applying force in 0.1N increments until shell failure. Reproductive behavior was observed visually. Egg width was measured using digital calipers. Hatchling growth was measured by shell width using digital calipers every week. DNA extraction and NGS were used to examine microbial gut content. GEOLocate and DIVA-GIS software were used to create a point distribution map from museum records. Shell measurements were 5.66 mm ± 0.69 mm height, 8.19 mm ±0.71 mm width, 5.0 ± 0.5 whorls, and 1.94 mm ± 1.27 mm aperture to callus. Shell width was significantly positively correlated with shell

height ($R^2 = 0.73$, $p<0.05$) and whorl count ($R^2 = 0.18$, $p<0.05$). Shell width was not significantly correlated with lamellar callus distance ($p=0.53$). Mean shell crushing strength was 4.6 N ± 2.5 N. Shell strength was not significantly correlated with shell width ($p=0.32$). I observed face-to-face simultaneous mating behavior in one couple. Average egg width (n=321) was 1.53 mm ± 0.12 mm. Hatchling growth rate was $y = 0.0632x + 1.4183$. *Mycoplasma, Peanibacillus,* Simkaniaceae, and *Enterobacteria* were the four most abundant microbes in gut content. Species distribution is assumed to be from the southeastern to northeastern U.S. with a concentration in the Appalachian region. Information gathered from this study will fill data gaps in land snail literature and can be used to better understand evolution and systematics of land snail communities.

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INTRODUCTION

Mollusca is the second largest phylum in Kingdom Animalia, consisting of seven classes of organisms that display great variation in biology and natural history (Pojeta and Runnegar 1976). Body form, specialized organs, open and closed circulatory systems, and loss of major characteristics among groups in Mollusca make this phylum diverse and unique (Parkhaev and Paleontol 2017). For these reasons, this group has become of great interest to evolutionary biologists. Class Gastropoda is arguably the most successful group of mollusks due to their ability to thrive in freshwater, marine, and terrestrial habitats around the globe (Strong et al. 2008).

Land Snails

A land snail is any one of the approximately 35,000 species of snails adapted to life away from water (Solem 1984). Most species are members of the subclass Pulmonata (class Gastropoda); the rest are prosobranchs (Wade 2001). In Pulmonata, snails are characterized by having a pallial lung and their ability to breathe air (Ruthensteiner 1997). This separates them from the prosobranch group as members of this group possess their ancestral gills. Land snails exhibit a wide range of habitat preferences, varying with species and region, which generally reflect their main survival requirements: moisture, food, shelter, and a source of calcium for shell building and physiological processes (Burch and Pearce 1990). Most species require a humid environment and seek shelter in microhabitats such as under logs, rocks, leaf litter, in and around bryophytes, coarse woody debris, and moist vegetation (Burch and Pearce 1990). Other places that support

snails include the interface regions of the forest floor, such as the crevices between a log and the ground litter and between exposed tree roots (Burch and Pearce 1990). The high energy cost of movement in land snails makes dispersal limited (Winters 2014). Dispersal behaviors range from sedentary to nomadic (Tomiyama and Nakane, 1993). Land snails are simultaneously hermaphroditic animals which contain both male and female sex organs (Garefalaki 2017). This evolutionary trait has increased assurance in finding a reproductive mate in land snails.

Family Zonitidae

Zonitidae is a family of small air breathing land snails in the gastropod subclass Pulmonata. The land snails in this family are also known as the true glass snails. Tryon (1866) formally described physical characteristics in Zonitidae. The Zonitid snails are animals that are completely retractable within their protective shells. The shells are usually heliciform, transparent, and perforate or umbilicate. Zonitid snail shells are a yellowish or brown color with a glossy or dull appearance. The tail contains a caudal mucus pore which is used for reabsorption of mucus. The Zonitidae family includes several hundred species, usually considered Helices (Figure 1), but differ from the group in the generally thinner, more translucent shells. Tryon (1866:51) noted:

> "There are some pulmoniferous mollusks having Zonitoid shells, whilst their soft parts place them in Helicidae, and vice versa. The animals of most of the species, however, are entirely unknown to science, and we are thus reduced to analogies of the shell in determining their systematic position"

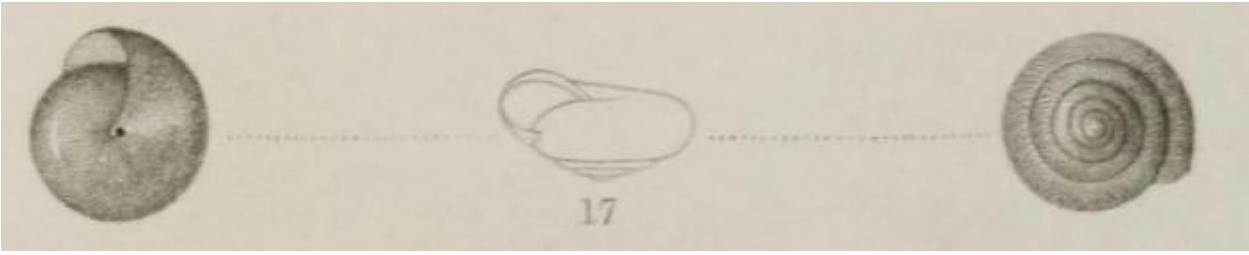

Figure 1. Illustrations accompanying the original description of *Helix* (=*Ventridens*) *demissus* in Binney (1843: plate 16, figure 17).

Genus *Ventridens*

The genus *Ventridens* is described as having a yellow or brown and glossy or dull shell with obliquely striae and 5-8 whorls. A callus is found in young shells when present and continues to lengthen as the shell grows. The sole of the foot is undivided and does not show waves in movement, so locomotion is considered arrhythmic. For reproduction in the genus, the reproductive orifice is located near the right tentacle. In the reproductive system the atrium is long with practically no vagina and the oviduct is free and long. Lower sections of the penis bear the dart sac and is attached to the spermathecal duct by a muscular connective bad. The spermathecal duct is long at its lower third part giving off a branch which opens into a sheath surrounding the basal part of the penis. During mating, the penis transfers the spermatophore to the mate through the reproductive orifice. The different specializations of the shell and distribution indicate old branching of a stock that has diverged little in anatomical structure (Pilsbry 1946). Most of the *Ventridens* shells in collections lack the early stages which are often essential to a thorough understanding of species in the genus. Distribution for the genus has been described as in the eastern United States and Canada and especially the Appalachian mountain system (Pilsbry 1946). *Ventridens* seems to have evolved in the Appalachian system. Southward it barely reaches Texas and is unknown to have reached eastern Mexico (Pilsbry 1946).

The Perforate Dome Snail *(Ventridens demissus)*

The perforate dome snail, *Ventridens demissus* was initially described by Binney (1869). The apex of the shell is relatively low with an obtuse spire. The umbilicus is very

small, the area around it only slightly depressed, and the base is generally flat and smooth. The surface of the shell is a yellowish, horn-color and mostly quite smooth, though there are some fine, widely-spaced striae on the outer whorls. The aperture is not especially wide and the lip is thin. Young shells may have an outer basal lamina (Pilsbry 1946-48; Dourson 2010). *Ventridens demissus* is found in a range of habitats including ravines, wooded hillsides with leaf litter, and floodplains;it is even found in urban areas (Dourson 2010). The range of *V. demissus* spreads from southwestern Pennsylvania south to northern Florida and west to east Texas. It is found along the Gulf Coast but generally not the Atlantic Coast nor Piedmont counties. In Virginia, it is mostly in the west (Binney 1869).

Adult shells of the perforate dome are 7.5 to 10 mm in diameter, 5 to 7 mm tall, with a glossy yellow-brown color (Pilsbry 1946). The shell of the perforate dome goes through a transformation as it grows – very young shells may have basal lamina not seen in mature shells (Pilsbry 1946). Biological data for *V. demissus* is poorly described, if any, in the scientific literature. *Ventridens demissus* has been mentioned briefly in land snail diversity studies (Hodges 2018; Stockdale Walden et al., 2017). However, specific studies on *V. demissus* do not exist.

Life History Significance

Life history studies involve the scientific research and observation of organisms, their traits, and reproductive strategies. These studies are significant in gathering evolutionary and systematic information on groups of living organisms. It is crucial to

collect and update life history data over time to allow us to better understand ecological and evolutionary processes (Autumn 2002). Basic data collected form life history studies contribute to other research studies and policies in management and conservation. For terrestrial mollusks ecological studies are rare and the group is poorly studied (Hortal 2009). Snail communities in urban areas are also rarely studied even though they may provide valuable information on ecosystem quality and evolutionary biology (Horsak 2009).

Data collected from morphometric methods is significant in life history studies because it captures variation in body plan of a species that can contribute to systematic studies (Cruz et al. 2012). In snails there is an intimate relation between shell size, thickness, strength and calcium content that may be influenced by environmental factors such as predation and heavy metal pollution (Jordaens 2006). Some snails are more resistant to predation due to their shell strength. Foraging theory suggests that if energetic returns do not offset the sum of searching and processing costs, predators are unlikely to include a given prey in the diet (Pyke, 1984).

The mating behavior of a wide variety of terrestrial land snails has been observed, but the descriptions are often within texts that are not easily accessible, or cannot be searched electronically (Davidson and Mordan 2007). Most land snails are hermaphroditic, meaning each organism possesses both male and female sexual organs (Kiyonori 1996). The spermatophore is transferred by the donor's penis into the receiver's vagina through the atrium. Once inside the receiving animal, the spermatophore releases the sperm, then sperm and eggs meet in the fertilization chamber,

where genetic material is combined (Barker 2001). Many malacologists have also made their own informal observations of mating behavior, but do not publish them for lack of time, or because they are not perceived to be of sufficient worth on their own (Davison and Mordan 2007).

Egg size is commonly related to the survival, growth, and eventual breeding success of offspring, although this is not always the case (Baur 1998). In terrestrial gastropods, provisioning of eggs with energy, nutrients, and calcium carbonate is the most common form of parental investment (Baur 1994a). Growth is an important life-history process, influencing a range of later fitness-related traits such as age and size at maturity and total reproductive output (Stearns 1992; Charnov 2004; English et. al 2014). Proćków et al. (2013) discovered growth rates in other Pulmonate land snails were not continuous but inconsistent. Growth was quicker in the spring and summer seasons. In the winter, snail growth was slowed until spring arrived. With information such as growth, size, and age, species populations can be better described and managed.

Evolutionary adaptations have allowed animals to utilize available resources in their environment. The vast majority of land snails are detritivores and many exhibit a generalist feeding strategy (Mensink and Henry 2011). Invertebrates like roaches and termites have evolved unique microbial gut content that allows the efficient breakdown of tough vegetative material (Rajarapu 2017). Microbiome is defined as the collected genomes of the microbes (composed of bacteria, archaea, bacteriophage, fungi, protozoa, and viruses) that live inside the body (Yang 2012). Microbes confer metabolic capabilities such as protection against pathogen, education of the immune system, and

influence directly or indirectly most of the physiologic functions (Debnath 2012). Gastropod microbiome data is largely unavailable despite their variable diet.

Study Goals

For this study I observed and recorded selected life history parameters of *V. demissus* to add biological information to land snail literature. Shell morphology assessment included measurements of width, height, and distance of aperture form lamellar callus to determine morphological variation. I also observed shell strength of *V. demissus*. In addition, I observed mating behavior, egg laying habits, egg size, and hatching growth to gain an understanding of the perforate domes snail's reproductive traits and behaviors. Microbial gut content was observed to discover microbial diversity in the *V.demissus* gut. Finally, I observed species distribution to gain a description of where *V. demissus* has been found. This thesis will contribute to data gaps in terrestrial gastropod biology by observing and describing selected life history parameters of the perforate dome snail (*Ventridens demissus*).

METHODOLOGY

Collection Method

In the Spring of 2017 I collected live and dead *Ventridens demissus* (*n*=190) in the mornings (0800 to 1100) along the Seabrook trail system. The sampling sites included three urban wildlife parks in Seabrook, Texas (Figure 2): Seabrook Wildlife Park (A; 29.584736° N, -95.0066871° W), Robinson Park (B; 29.5875257° N, -95.0003034° W), and Pine Gully Park (C; 29.5914033° N, -94.9960234° W). The parks are interconnected by hiking and biking trails. Although these are urban parks, sampling areas are often undisturbed. The parks selected are primarily for trailing and do not possess tables, water fountains, or playground equipment (except for Pine Gully). I conducted visual searches that were efficient for use in urban areas. This involved leaf litter foraging, log flipping, and light soil digging with small hand shovels, cups, and sticks. The visual search method has shown to be a very effective sampling method for larger obvious snails (Coppolino 2010) and collecting by hand has a quicker processing time than other sampling methods (Emberton et al. 1996). Vegetation surrounding the collection site consisted of tall trees including post oak (*Quercus stellata*), Texas red oak (*Quercus buckleyi*), willow oak (*Quercus phellos*), loblolly pine (*Pinus taeda*), yaupon holly (*Ilex vomitoria*), American sycamore (*Platanus occidentalis*), chinese tallow (*Triadica sebifera*), cedar elm (*Ulmus crassifolia*) , and winged elm (*Ulmus alata*). Larger fauna seen throughout the area included deer, snakes, rabbits, fish, turtles, feral hog, and a variety of birds. Larger trees shaded the collection site with a visual estimate of 90–100 percent canopy cover allowing very little light to the ground underneath. The average temperature in the Seabrook area

was 22° C at time of collection in the morning

(https://www.wunderground.com/weather/us/tx/seabrook).

Figure 2. Collection sites for *V. demissus* Seabrook Wildlife Park (A), Robinson Park (B), and Pine Gully Park (C) in Seabrook, Texas. Map extracted from Google Earth.

Although snails were collected in three locations, I treated all of them as a single population for analysis. Of the 190 snails I collected, five randomly selected individuals were immediately frozen at -20° C for gut microbe DNA extraction. I randomly selected 150 specimens to be measured for morphological assessment. Twenty randomly selected snail shells from the 190 were used to analyze shell strength. Table 1 below displays sample sizes for each data assessment.

Table 1. Sample sizes for parameters measured in this study.

Parameter	*Sample Size (n)*
Morphological Variation	150
Shell Strength	20
Microbial Gut Analysis	5
Egg Size	321
Mating Behavior	1
Egg Production	1
Hatchling Growth	2-20
Species Distribution	924

Shell Morphology

I measured *V. demissus* specimens (*n*=150) and recorded shell height and shell width to the nearest 0.01 mm using digital calipers. I then measured the straight-line distance from the edge of the aperture to the laminal callus located in the shell to the nearest 0.01 mm. These methods of measurement are shown in Figure 3. I also determined whorl count under a dissection microscope, counting whorls to the nearest quarter-whorl starting from the aperture. I used linear regression to correlate shell height and width, shell width and whorl count, and shell width and distance to the callus. To determine shell strength, I sent 20 empty *V. demissus* shells to Georgia Gwinnett College (GGC) for crushing. My colleagues at GGC placed each snail umbilicus down on a metal plate, and a top plate was pressed on the shell apex. between two steel plates. They employed a Mark-10 series 5 force gauge mounted on an ESM303 motorized test stand to determine the force needed to cause first shell failure to the nearest 0.1 N. Force data were recorded every 0.1 s until first failure of the shell. I then used linear regression to determine the relationship between shell width and strength.

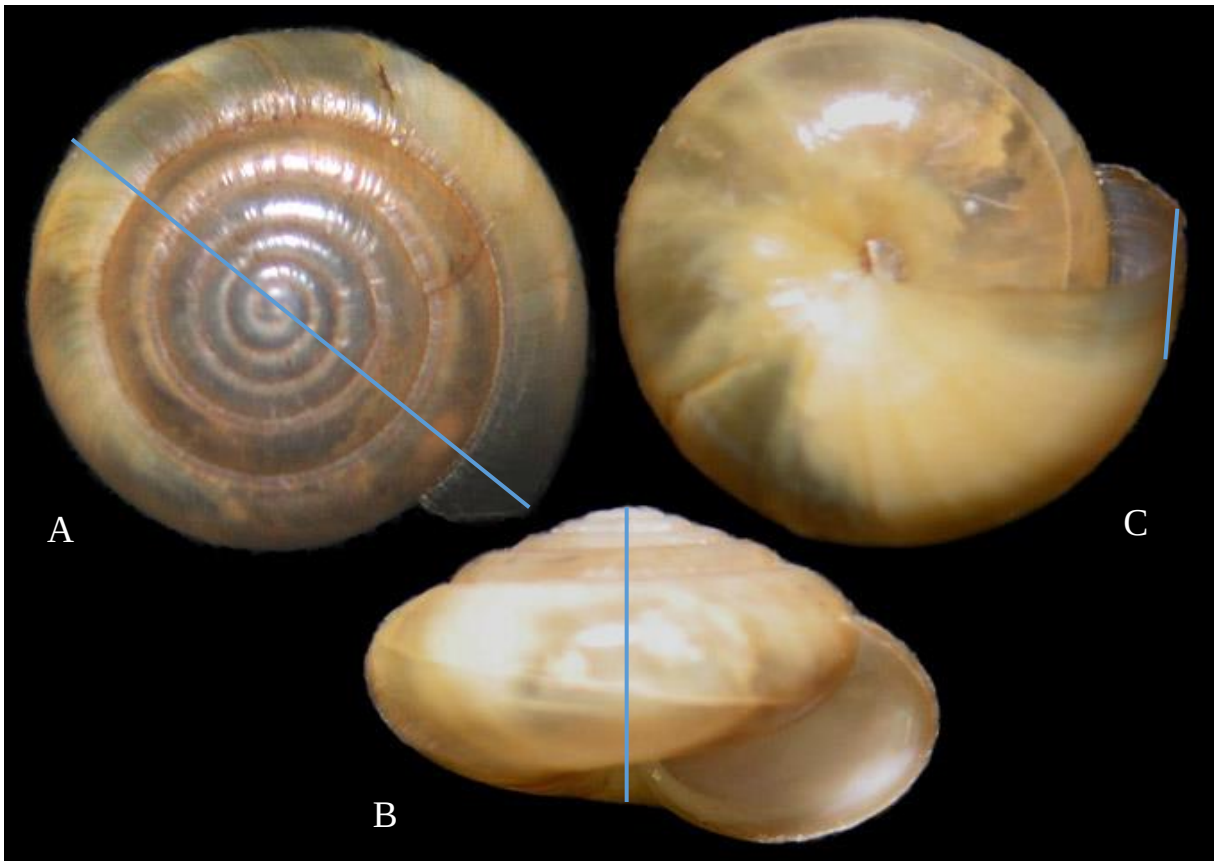

Figure 3. Image of *V. demissus* shells (Coppolino 2009) edited to show how measurements of width (A), height (B) and distance of aperture to the laminal callus (C) were measured.

Snail Reproduction and Egg Size

Six plastic aquaria (29.8 cm x 20.3 cm x 19.7 cm) were used to house snails collected from the collection site. Snails (*n*=190) were equally distributed between the 6 aquaria. Snails were provided approximately 5 cm of soil in the aquaria from the collection sites. We also included twigs, leaves, and rocks to provide natural small hiding places. We provided commercially available salad mix and mushrooms to provide nutritional energy. Daily misting was provided to keep moisture in the soil. I observed one occurrence of each snail mating and egg laying while checking the aquaria. Once snails produced eggs, I collected 321 *V. demissus* eggs from the soil in the plastic aquaria and recorded the width of each egg with digital calipers to the nearest 0.01 mm. A skewness and kurtosis test were conducted in excel assess distribution of egg sizes.

Hatchling growth

Eggs that hatched (Figure 4) were placed into a separate aquarium with soil from the collection sites and fed a diet of salad mix and mushrooms. I determined hatching growth by measuring shell width using digital calipers of newly-hatched *V. demissus* over a 21 week period. Snails hatched within the same week were used a starting cohort for average width and were measured every week until death.

Figure 4. *V. demissus* hatchling from our laboratory aquaria.

Gut Microbe Analysis

I assessed gut microbe content using five individual *V. demissus*. I gently cracked each shell and dissected out the digestive tract from each snail. I extracted genomic DNA from the snail tissue using the MoBio PowerSoil kit and sent the samples to MrDNALab (Shallowater, TX) for analysis. The V4 region of the 16S rRNA gene was amplified by PCR with the 515/806 primer pair of Caporaso et al. (2011). Amplicons were sequenced on an Ion Torrent PGM following manufacturer's guidelines. Sequences were depleted of barcodes and primers, then sequences less than 150 bp were removed, as were chimeras and sequences with ambiguous base calls and with homopolymer runs exceeding 6 bp. I took the assembled reads and used the SILVAngs pipeline (http://www.arb-silva.de/ngs; Quast et al. 2013; Yilmaz et al. 2104; Glöckner et al. 2017) to generate operational taxonomic units (OTUs) at 97% similarity and classify the OTUs taxonomically.

Estimating Species Distribution

I created a species distribution map for *V. demissus* by georeferencing museum species collection records. I downloaded collection records from the Carnegie, Delaware, and National Museums of Natural History, and the Museum of Comparative Zoology at Harvard University. I then used GEOLocate (Bart and Rios 2010) georeferencing software to estimate latitude and longitude coordinates from the collection records. Coordinates were then entered into DIVA-GIS software (Hijmans 2001) to create a point distribution map of *V. demissus* in the United States.

RESULTS

Shell morphology

The mean shell measurements of 150 *Ventridens demissus* shells were 5.66 mm ± 0.69 mm height (Figure 5), 8.19 mm ± 0.71 mm width (Figure 6), 5.0 ± 0.5 whorls (Figure 7), and 1.94 mm ± 1.27 mm aperture to callus (Figure 8). Whorl count frequencies appeared bimodal with peaks at 4.5 and 5.5 whorls. Shell width was significantly positively correlated with shell height ($R^2 = 0.73$, $p<0.05$; Figure 9). Shell width was significantly positively correlated with whorl count but showing a weak association ($R^2 = 0.18$, $p<0.05$; Figure 10). Shell width was not significantly correlated with lamellar callus distance ($p=0.53$; Figure 11).

Shell strength

An example of the crushing data is shown in Figure 12. Shell failure was recorded at the first peak and subsequent drop. Mean shell crushing strength was 4.6 N ± 2.5 N. Shell strength was not significantly correlated with shell width ($p=0.32$; Figure 13).

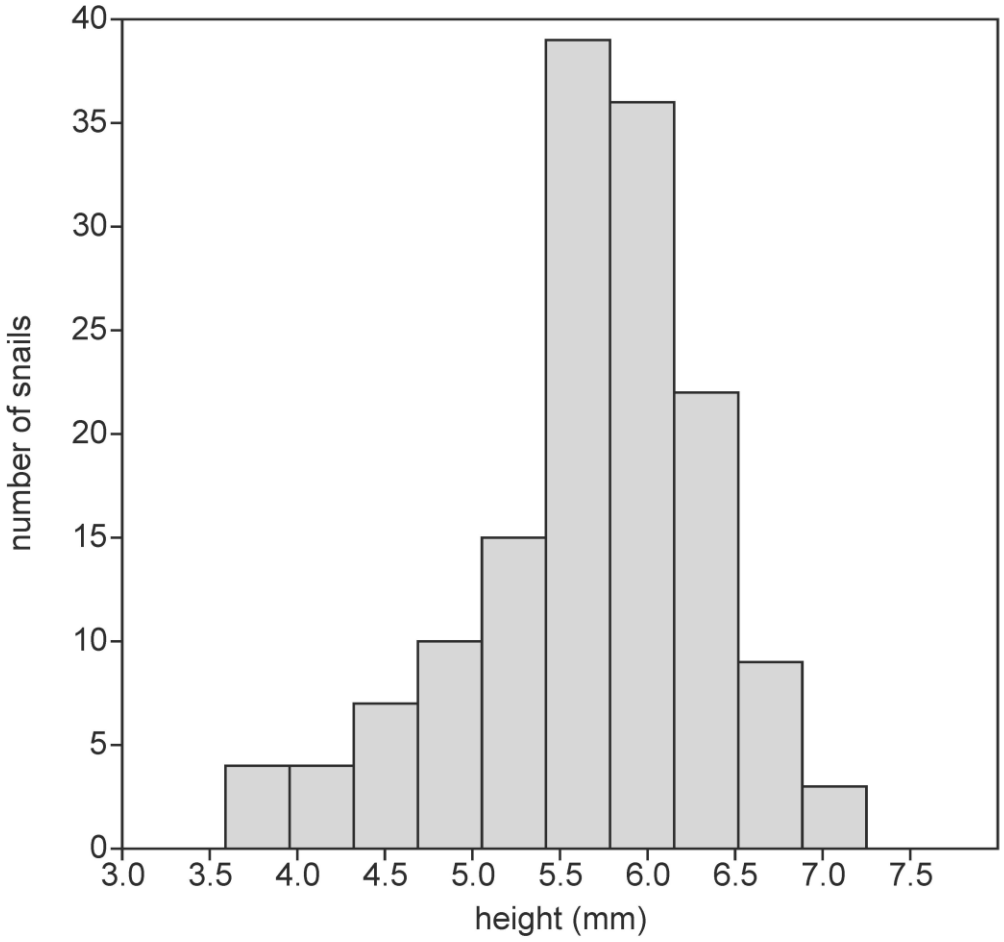

Figure 5. Distribution of *V. demissus* shell height frequency.

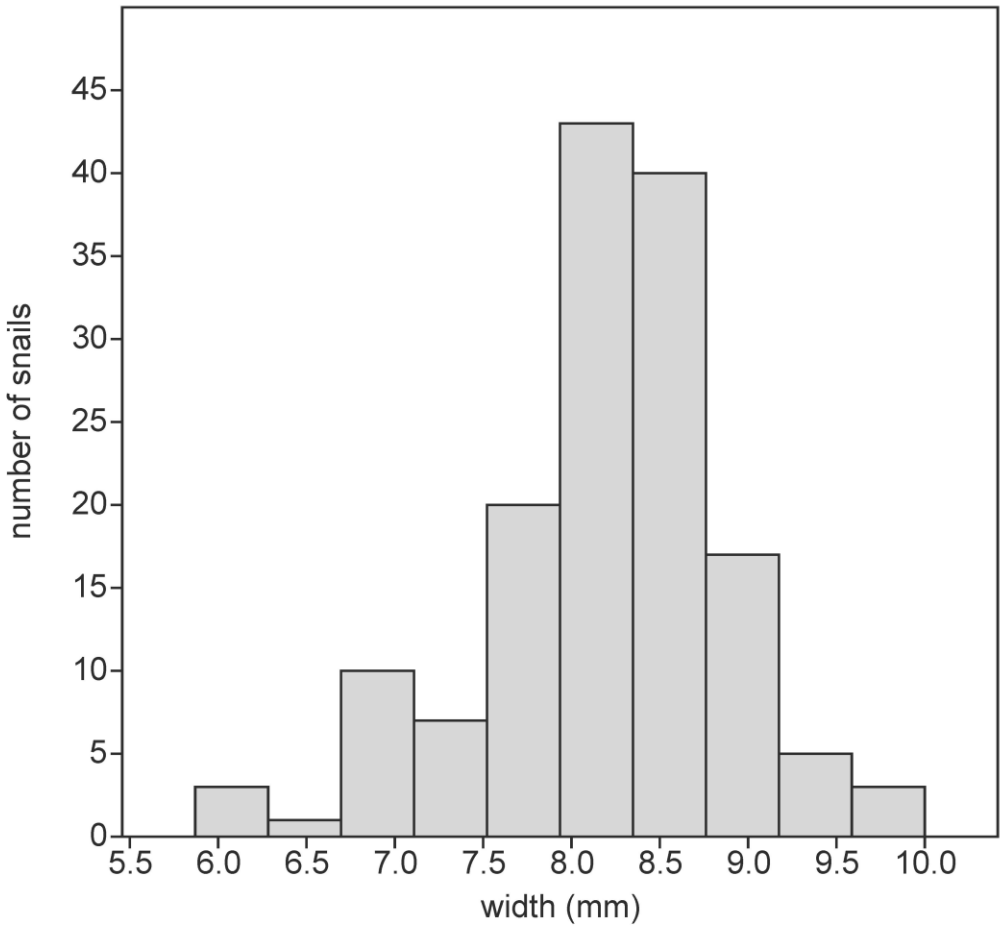

Figure 6. Distribution of *V. demissus* shell width frequency.

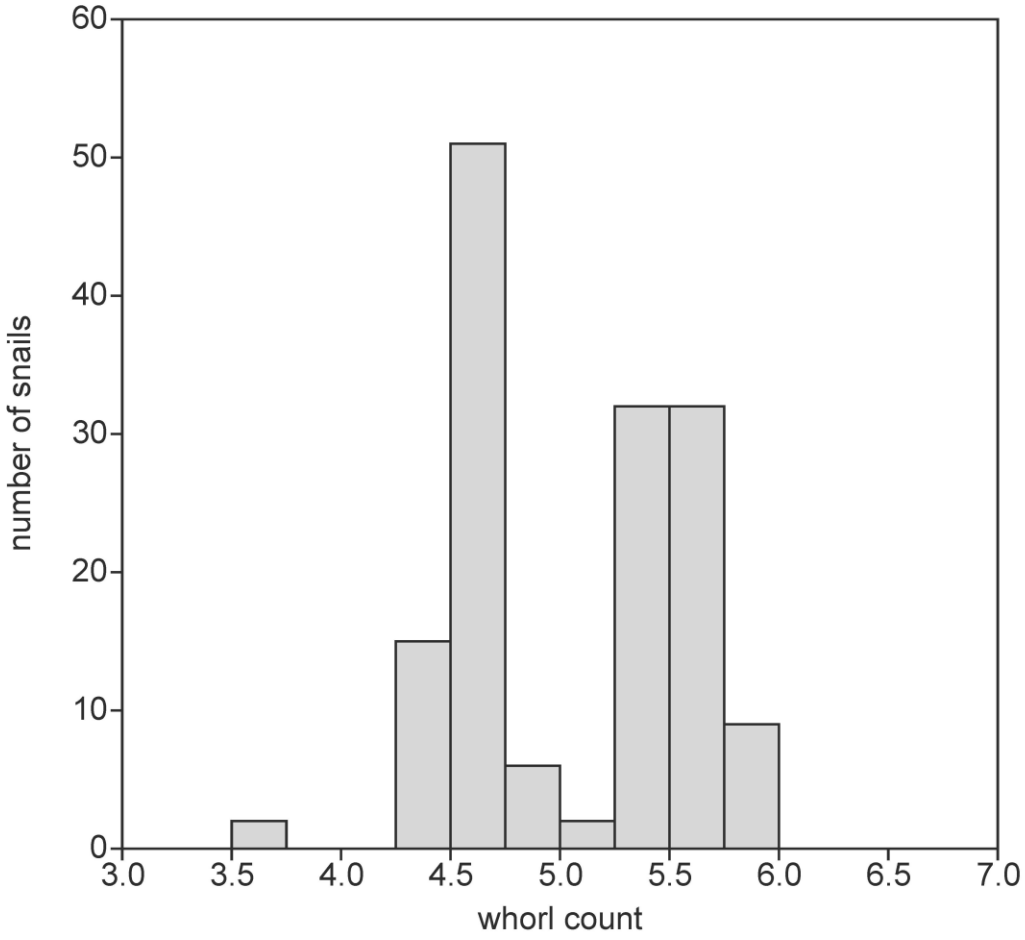

Figure 7. Distribution of *V. demissus* shell whorl count frequency.

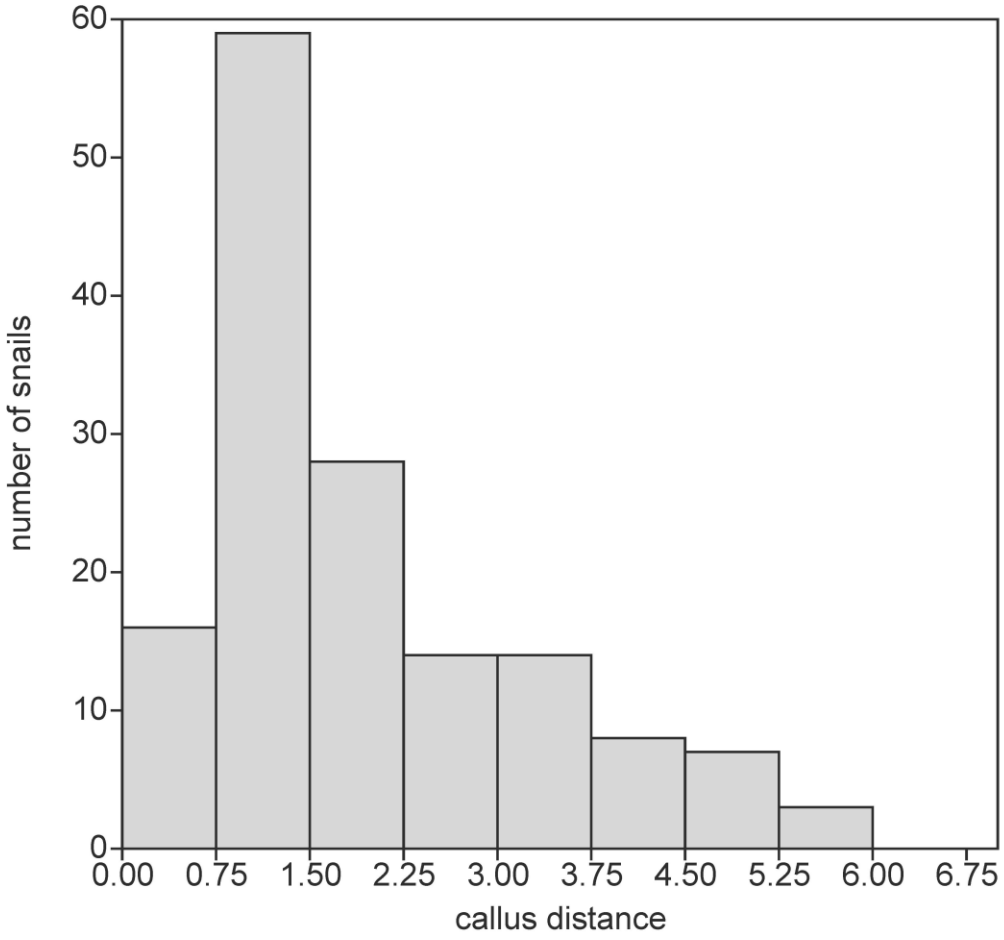

Figure 8. Distribution of *V. demissus* aperture to callus distance frequency.

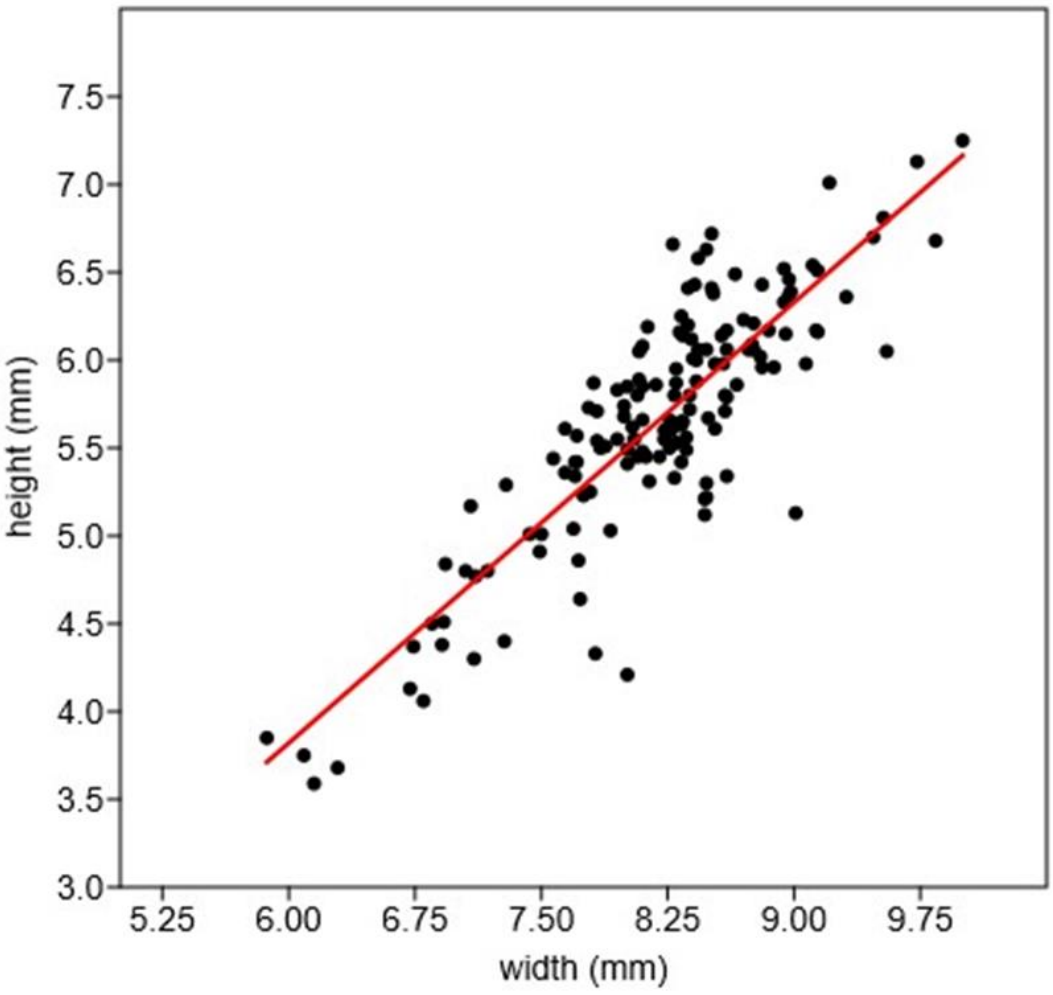

Figure 9. Plot of shell height (mm) versus shell width (mm) with best fit line in red. The relationship between measures was significant ($R^2 = 0.73$, $p<0.05$).

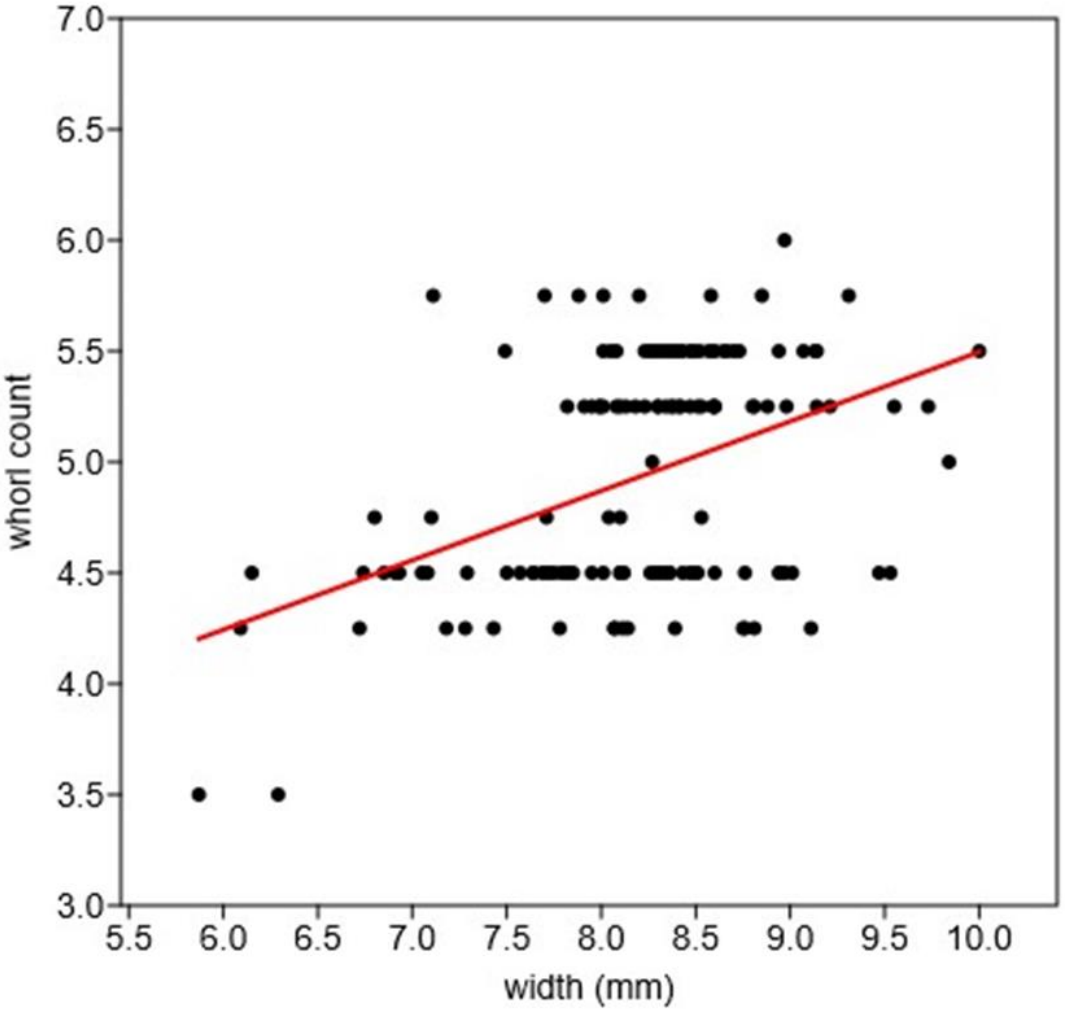

Figure 10. Plot of whorl count versus shell width (mm) with best fit line in red. The relationship between measures was significant ($R^2 = 0.18$, $p<0.05$).

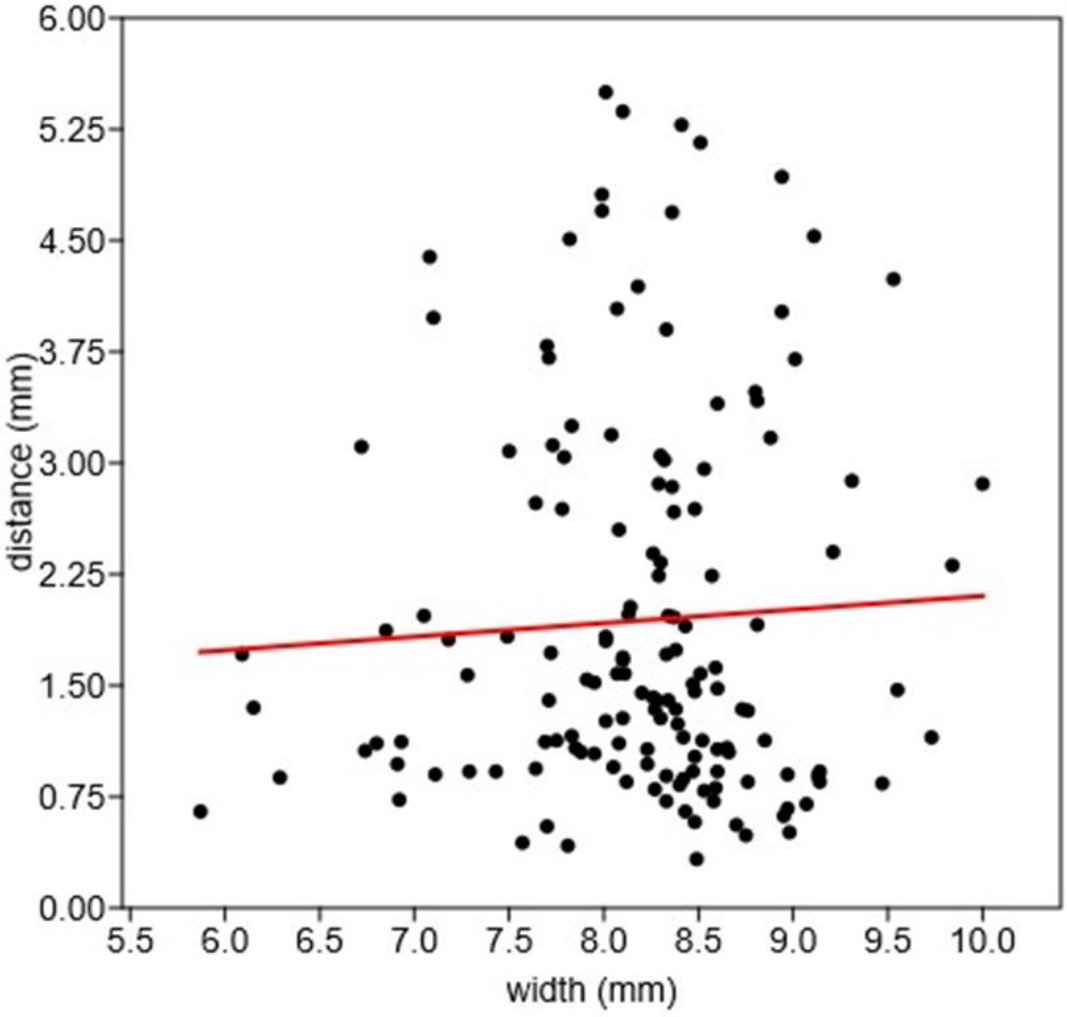

Figure 11. Plot of lamellar ridge distance from aperture (mm) versus shell width (mm) with best fit line in red. The relationship between measures was not significant (p=0.53).

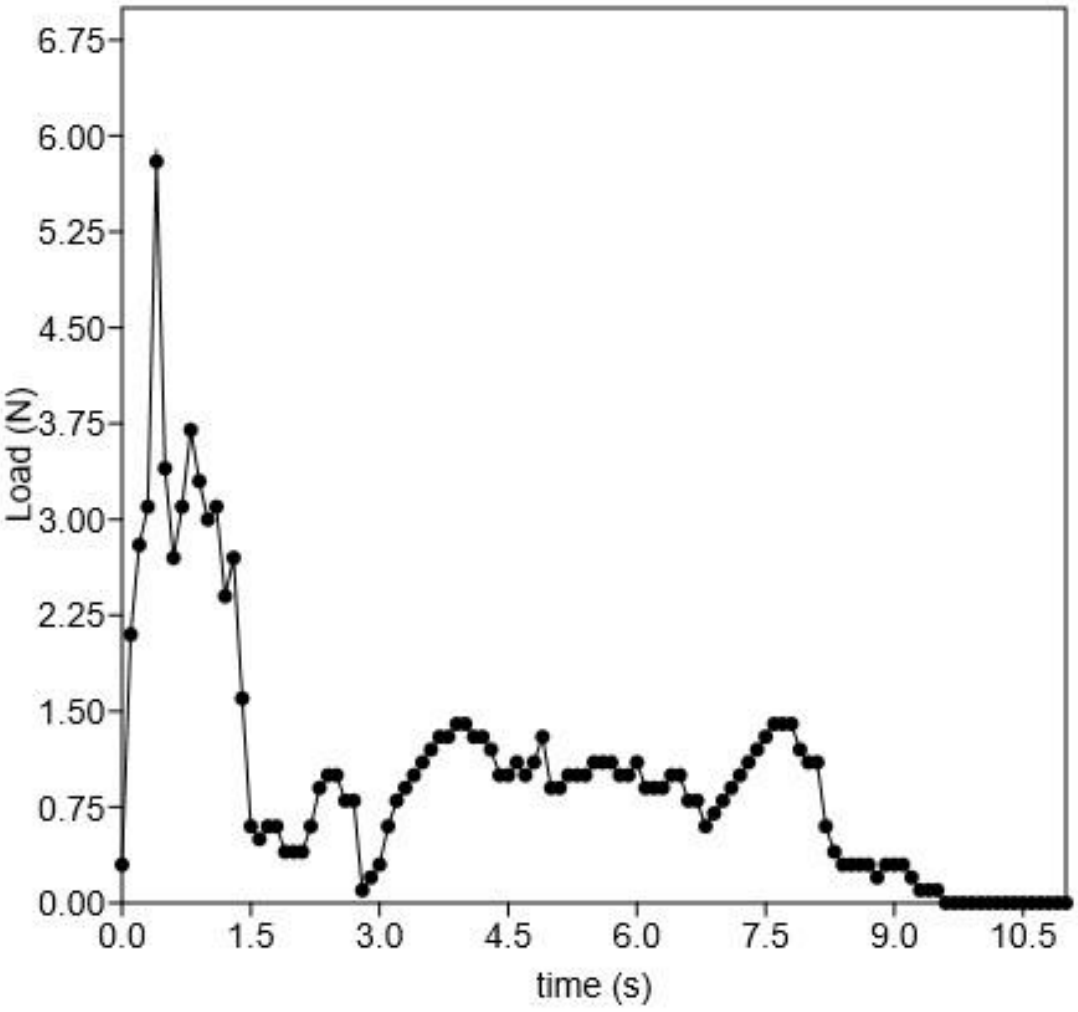

Figure 12. Shell crushing data from one *V. demissus* shell. First failure of the shell occurred with 5.8 N of force.

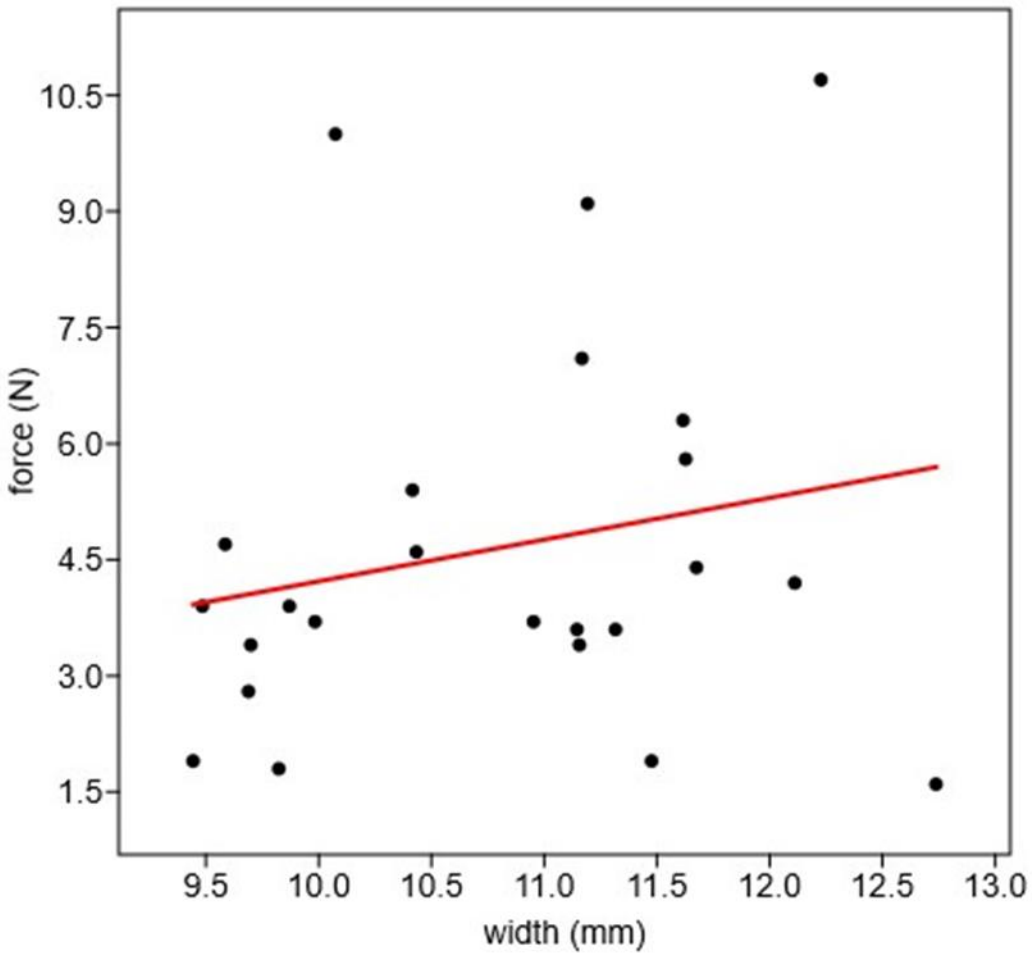

Figure 13. Plot of crushing force (N) versus shell width (mm) with best fit line in red. The relationship between measures was not significant (p=0.32).

Reproduction

I observed one incident of mating between two *V. demissus*. The two individuals were positioned facing opposite one another. 2 reproductive structures, one from each snail, were present at one time suggesting the reproductive method of *V. demissus* is simultaneous as opposed to unilateral. The structures appeared as a thin white extensions that retracted and extended to the body of the other snail (Figure 14). This activity continued for at least 15 minutes at which point I continued to gather other data. A short movie clip of the mating can be found on YouTube (https://youtu.be/cat3DNqtCd0). I also observed a single incidence of egg laying (Figure 15). The individual was positioned aperture up, and four eggs were laid while I observed. Other eggs had been laid recently (Figure 16), but I could not determine if they came from the individual I was watching.

Figure 14. Mating observed between two *V. demissus* in laboratory conditions.

Figure 15. *V. demissus* laying four eggs in laboratory aquaria.

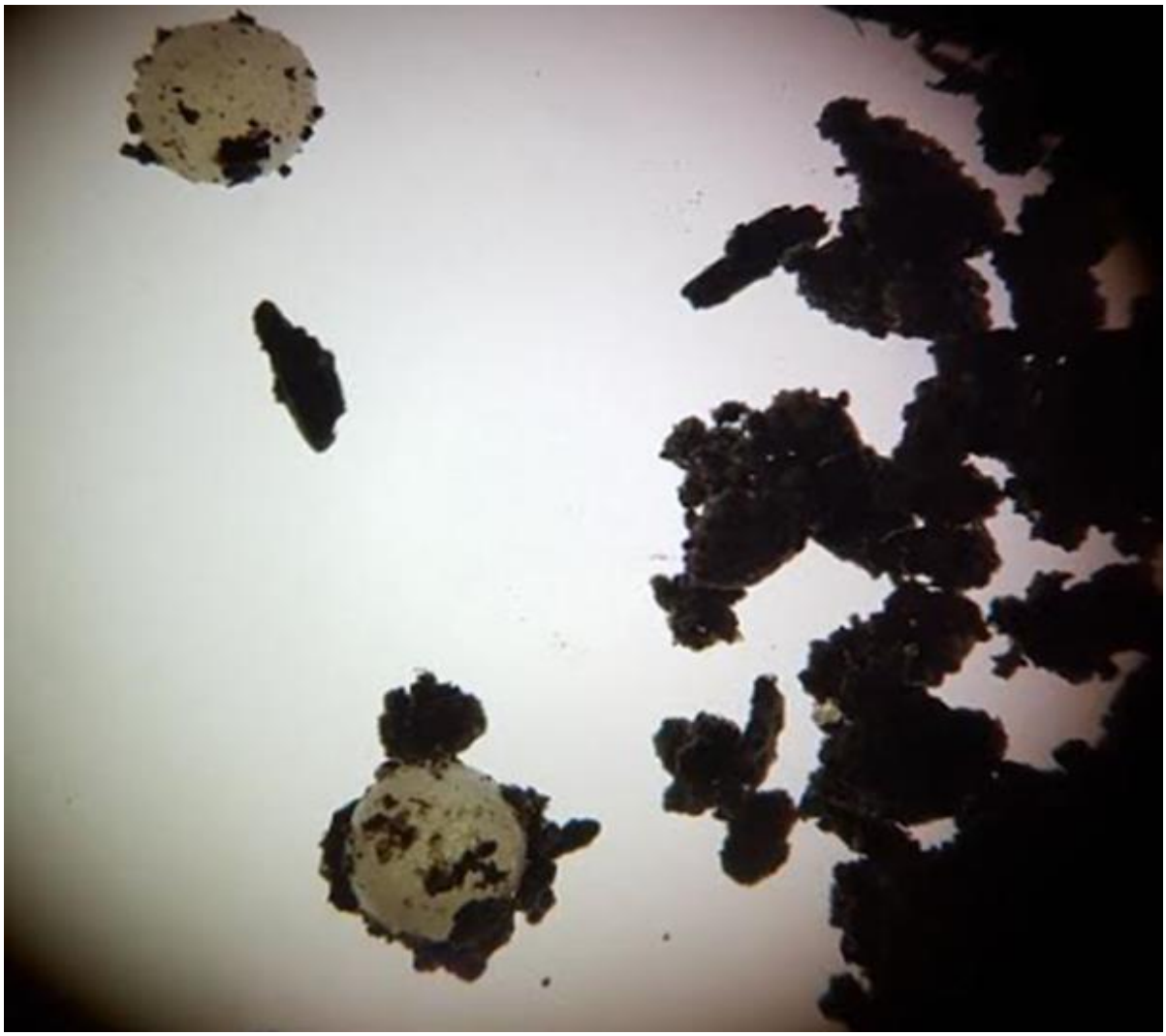

Figure 16. Two *V. demissus* eggs underneath a disecting microcope.

Egg Size

Average egg width (*n*=321) was 1.53 mm ± 0.12 mm; minimum egg width was 1.21mm and maximum egg width was 1.97 mm. Egg width data were normally distributed with a passing skewness test value of 0.118 and kurtosis value 0.145. The skewness and kurtosis tests for egg width data pass according to passing values of [-1,1] (Groeneveld and Meeden 1984). Figure 17 displays egg width distribution.

Growth

During the first week of life hatchlings were similar to average egg size of *V. demissus* ($\bar{x}$ =1.53 mm ± 0.12 mm) and growth rate of the cohort is represented by the equation of the best fit line y = 0.0632x + 1.4183. Snails grew another millimeter at about 14 weeks of life. Once the 2.5mm mark was reached at approximately 21 weeks the remaining snails died. Figure 18 shows snail hatchling growth over a 21 week period.

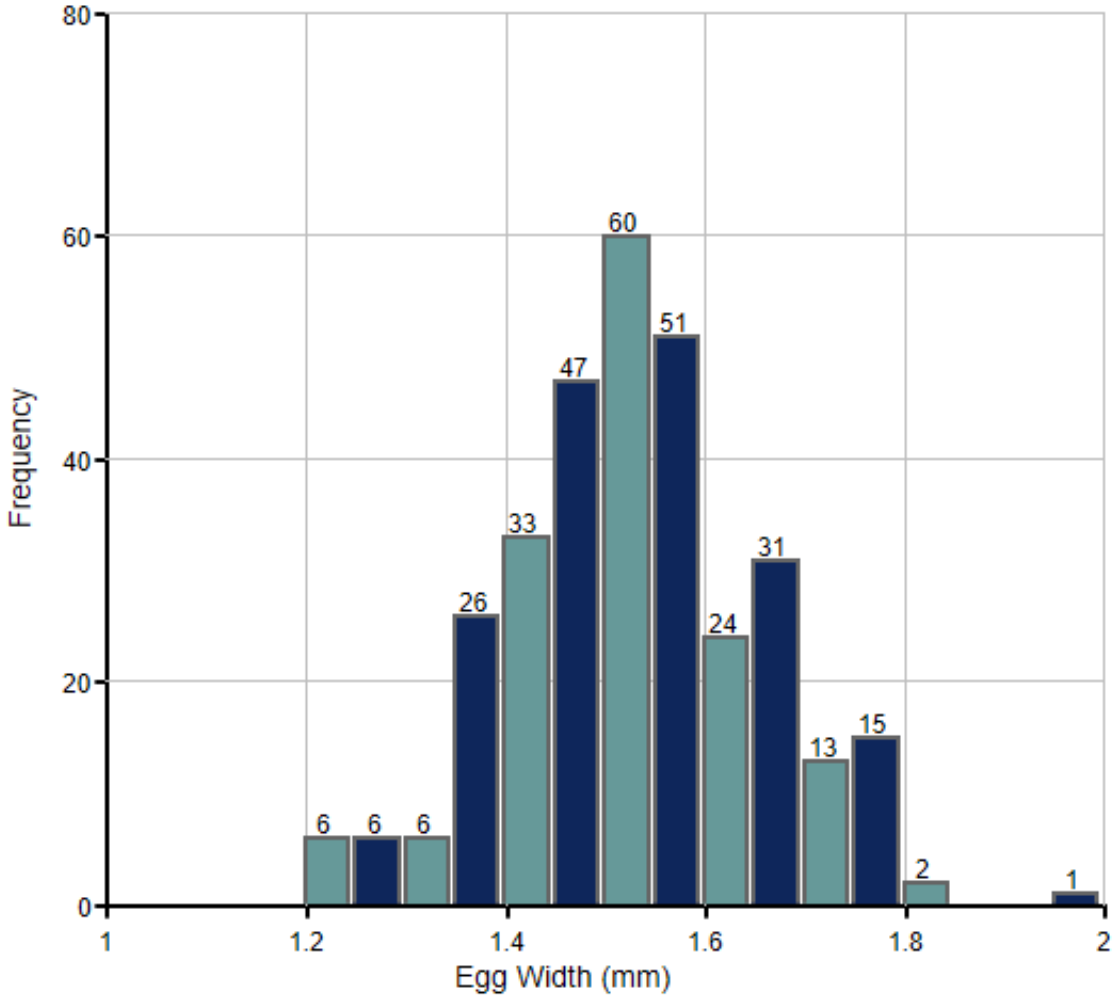

Figure 17. Distribution of egg sizes (n=321, $\bar{x}$=1.53 mm ± 0.12 mm) laid by *V. demissus* in a laboratory setting.

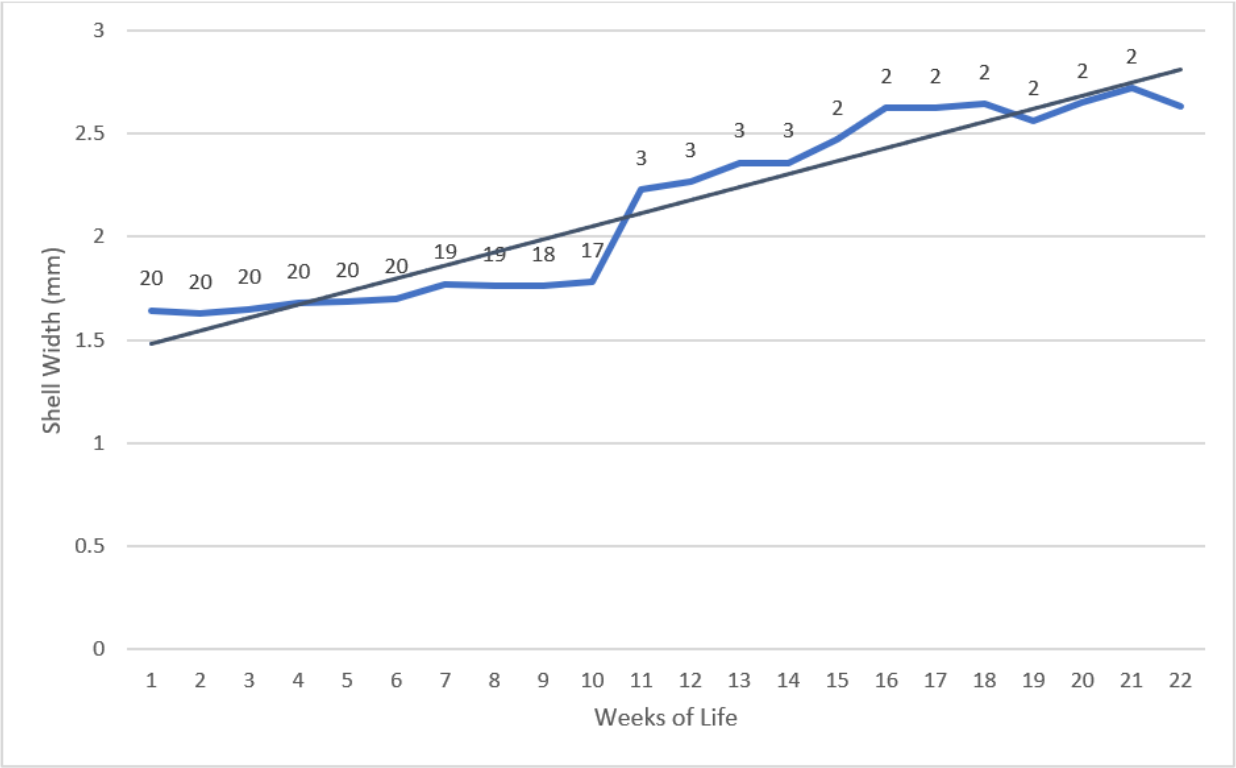

Figure 18. Hatching growth measured over a 22 week period in laboratory conditions. The equation of the best fit growth line was (y = 0.0632x + 1.4183), where y=shell width and x=weeks of life. Numbers above the data line indicate how many hatchlings were alive that week.

Gut microbial diversity

Figure 19 shows a rarefaction graph of OTU count versus number of reads. The shape of the curve, increasing without leveling off, suggested that all OTUs present in *V. demissus* were not sampled. For a proper rarefaction curve and more accurate OTU estimate to be produced more samples are needed. A list of all bacterial groups present at abundances greater than 1% is shown in Table 2. *Mycoplasma* spp. were the most abundant microbial group representing 52% of the total bacterial diversity.

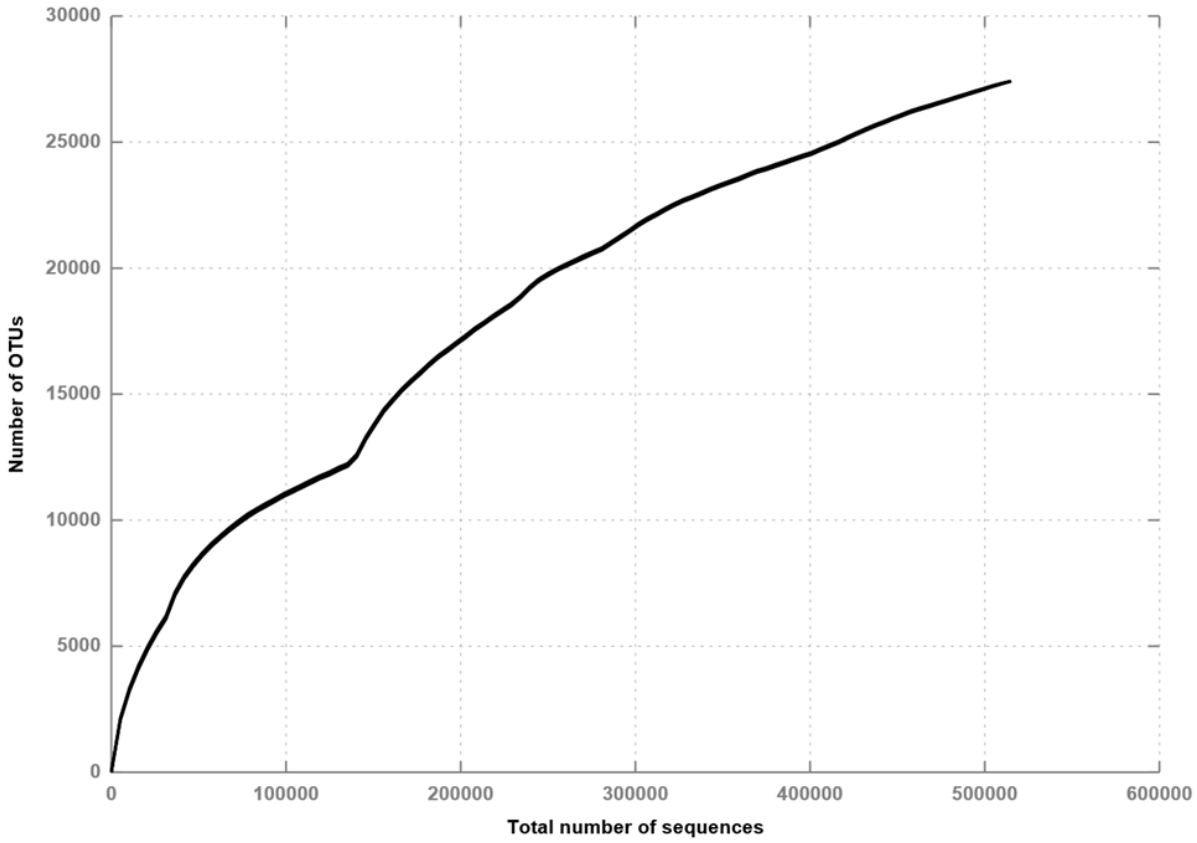

Figure 19. Rarefaction curve of bacterial richness found in the gut of V. demissus.

Table 2. Bacterial diversity sampled from *V. demissus* guts. Groups representing at least 1% of the total diversity are listed. Identifications are at the lowest taxonomic level resolved by SILVAngs.

Bacterial taxon	*Percent of total bacteria*
Mycoplasma	52%
Paenibacillus	11%
uncultured Simkaniaceae	10%
Enterobacter	9%
Rosenbergiella	3%
Klebsiella	2%
Buttiauxella	1%
uncultured Spirochaetes	1%
Raoultella	1%
Other	10%

Distribution of *V. demissus*

The distribution map of *V. demissus* based on museum collections suggest a species range from the northeastern Gulf Coast of Texas across the southeastern states and up through the Appalachian mountain range (Figure 20). Notable exceptions to that distribution exist in central Texas and Oklahoma, central Florida, and the Midwest (Michigan and Ohio).

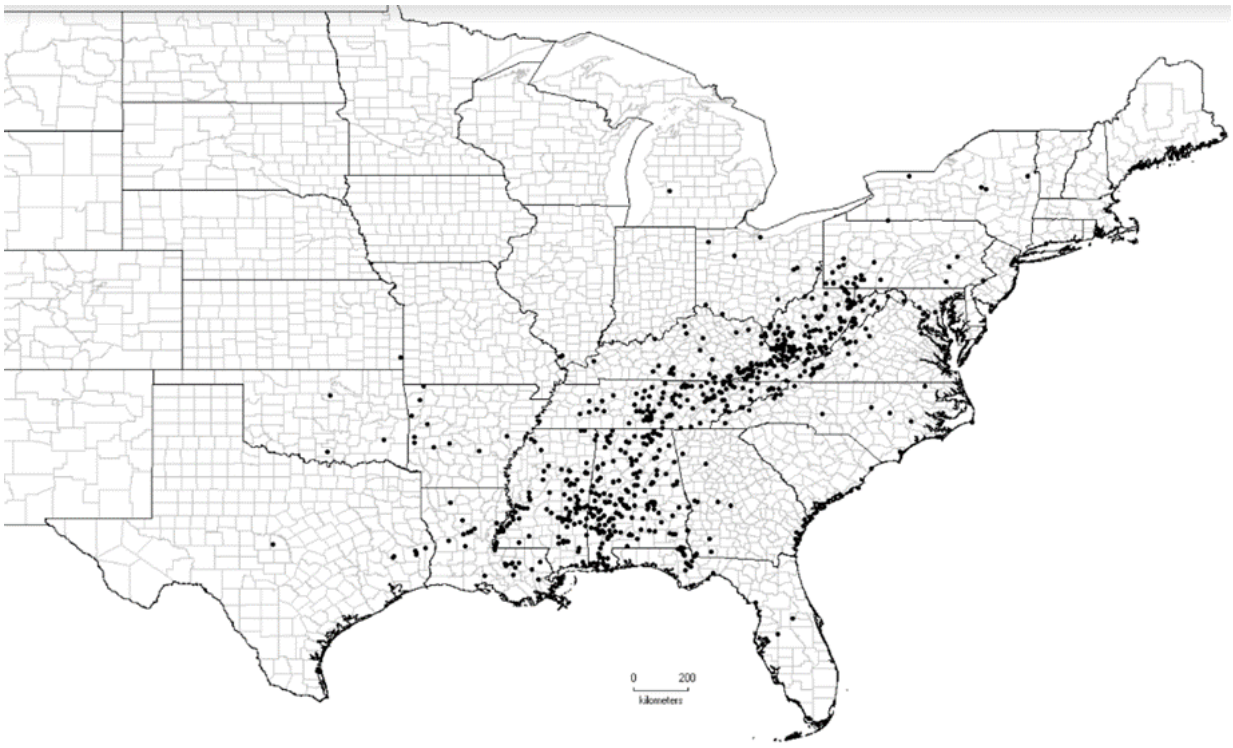

Figure 20. Point distribution map of *V. demissus* in the eastern United States based on museum records.

DISCUSSION

In this study I examined morphological variability, shell strength, reproductive behavior, egg size, hatchling growth, microbial gut content, and species distribution of *Ventridens demissus*. Initial observations on variables such as shell strength, hatchling growth and microbial content were recorded for *V. demissus*. Observations of morphological variability, reproductive behavior, egg size, and species distribution were updated and improved upon.

Morphology

In land snails, morphological traits (sculpture, shell and aperture size and shape, color, etc.) have proven crucial in regulating water and heat budget and to avoid desiccation (Cowie and Jones 1985; Cook 2001; Pfenninger et al. 2005). It is possible shell strength in *V. demissus* did not evolve against predatory pressures but more likely environmental pressures. A shell strength of 4.6N ± 2.5N is relatively weak and because many predators of small land snails enter from the aperture (Barker 2004), shell strength would not be primarily functional for predatory protection. Shell width, height, and whorl count measurements were similar to past smaller collection observations by Pilsbry (1946) from other parts of the U.S. and suggests that size among *V. demissus* throughout the U.S. does not have much variability. The distribution of whorl count frequency appeared bimodal suggesting two different cohorts of snails were collected at the collection site. Due to growth of snails slowing down in autumn and winter seasons I was

able to see a whorl count peak at 4.5 and 5.5 whorls in the histogram, possibly each representing a different cohort.

Egg Size

The egg size of *V. demissus* (n=321,$\bar{x}$ =1.53 mm ± 0.12 mm) was normally distributed in the laboratory setting. The texture was soft and the shape of eggs was spherical. Based on superficial observations it seemed that white eggs were viable and eggs that were brownish were no longer developing. This data along with clutch size and hatching success data we can gain a detailed more detailed insight about *V. demissus* reproductive life history strategies.

Mating

Mating success was observed in laboratory settings. Two perforate dome snails were found mating face-to-face simultaneously on a piece of lettuce that was provided as a food source. With reproductive structures visible from both individuals, we know that *V. demissus* does not mate unilaterally. This was the first recorded and documented mating occurrence of *V. demissus* and is now known to be successfully completed in laboratory conditions.

Hatchling Growth

Measuring growth of *V. demissus* in laboratory settings was challenging due to the unexpected rapid deaths of snail hatchlings. This may have been due to lack of

required environmental variables that are necessary for juvenile development. Growth of individuals can be variable in space and time, for example, as a consequence of variation in food availability, temperature, and precipitation, but also due to variation in genotype and phenotype among individuals (Schmera 2015). Although juvenile snails were difficult to house in laboratory conditions, adult snails collected from the field were easily kept alive with soil, food, and moisture. This leads me to believe juvenile *V. demissus* are sensitive at younger stages until adulthood is reached. Using the growth rate formula and mean shell width recorded from snails measured, it is estimated that *V. demissus* hatchlings reach adult size at 108 weeks. Although hatchlings did die off throughout the 21 week measurements, the growth rate ($y = 0.0632x + 1.4183$) is similar to the growth rates of other land snails.

Microbial Gut Content

The gut microbial diversity consisted of four large groups. *Mycoplasma* represented over half of the bacteria present. *Mycoplasma* is a pathogenic bacterium in many species of animals that lacks a cell wall. Bacteria phyla Proteobacteria and Firmicutes were also prominently found in the gut content of the invasive Giant African Snail, *Achatina fulica* (Cardoso 2012). *Paenibacillus* within Firmicutes is commonly found in soil and vegetative matter. Proteobacteria has been described as a diverse group of environmentally important and pathogenic bacteria. Chlamydiales affect many different host but its bacterial group *Rhabochlamydia* is predominately found in arthropods like woodlice and roaches (Niemczuk 2013). The bacterial group richness

observed in the gut of *V. demissus* is likely incomplete due to the shape of the rarefaction curve produced form my samples. The rarefaction curve shows incomplete growth, which estimates more bacterial OTU's may be present. In order for a more accurate OTU estimate to be produced more samples are needed.

Species Distribution

Distribution of a species not only shows us the range but can allow us to plan species survival evaluations and make better management decisions when a range map is produced (Wood et al. 2015). Distribution maps offer primary information on species and their habitat preferences. Our findings from the *V. demissus* distribution map show species distribution in the Eastern Temperate Forest and Great Plains Ecoregion according to the EPA Ecoregions of North America Level 1 Map (https://www.epa.gov/eco-research/ecoregions-north-america). A level 2 map allows for overview of national ecological patterns within level 1 regions. Level 2 regions that overlap with our point distribution map at the most concentrated sampling areas include the southeastern coastal plains, Ozark, Ouachita- Appalachian Forests, and the Texas – Louisiana coastal plains. In a habitat assessment of our collection site we observed that the community was healthy with an abundance of soil life including centipedes, wood louse, spiders, earthworms, springtails, snails, and other small arthropods. The map shows sampling areas of *V. demissus* that extend to where *V. demissus* was thought to be confined. Central Texas, Oklahoma, Kansas, and Michigan were not described in the previous species range. Assuming these samplings were not misidentifications, it is

possible *V. demissus* may be found throughout the U.S. in areas that contain wooded area habitat and lack of predators. The Appalachian region may be most favorable for the species but they may also tolerate less favorable, yet livable conditions throughout the U.S.

Future Studies

To improve this project I would suggest a proper clutch size observation in reproductive data. I would have liked to have more time allowed for growth and to keep hatchlings alive longer in more natural conditions. With this data I would have liked to include a life table to gain a better understanding of life expectancy and mortality of *V. demissus*. For the microbial gut content assessment, an increased sample size would be favorable if the budget allows. A microbial assessment of soil at the sampling site could be done to compare to gut content microbes to observe which gut microbes may have been contracted from the soil. Future studies may be able to focus on *V. demissus* diet specifically. Now that I have produced a species distribution map and provided biological data, a study of *V. demissus* parameters between different locations in the U.S. could be done to determine if parameters differ based on environment for this species. This study lays a foundation for future studies on snails in the Zonitidae family and allows reference for future studies to fill data gaps in scientific literature in land snail biology.

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