STUDENT PROJECT FINAL REPORT TO THE UNIVERSITY OF HAWAIʻI MARINE OPTION PROGRAM

Evidence for a new genus of Triphoridae in Hawaiʻi based on scanning electron micrographs of the protoconch

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Abstract: Triphoridae are marine gastropods that feed on sponges and have tall multispiral shells with three openings, an incurrent siphon, excurrent siphon, and the aperture. Many of the Triphoridae have distinctive color patterns, teleoconch facies, shell shape, and siphon and shell size, which have been used for the classification of these animals. However, it has been argued that the protoconch microstructure should be the primary factor in defining a genus. The purpose of this project was to further investigate the use of protoconchs as the primary defining feature of a genus and to determine the classification of two unclassified shells. Triphoridae shells were collected over a 20-year period on the island of Hawaii in Hawaii, USA from varying depths. Micrographs were taken of the uncoated protoconch with a scanning electron microscope (Hitachi S3400-II, variable Pressure) at the University of Hawaii at Hilo. Microstructure of the protoconch was analyzed for several species (N=12) found in Hawaii. Two unclassified shells were also included to determine if they belong to genus *Viriola* as suggested by another scientist or if they belong to a different or new genus. Results indicate that the two unclassified samples may be a new genus and the protoconch and teleoconch structures are evidence for this. *Pilulaefusus* is the proposed new genus name and the two species also have proposed species names.

Introduction

Class gastropoda is diverse group containing both terrestrial and marine organisms, and they have a large visceral mass on top of the foot that is either spirally coiled or flat. Triphoridae are marine gastropods that feed on sponges and have tall multispiral shells with three openings, an incurrent siphon, excurrent siphon, and the aperture (Kay 1979). These marine gastropods are unusual in the fact that the majority of species are sinistral (Sasaki 2008; Marshall 1983; Kay 1979). Many of these organisms have distinctive color patterns and teleoconch facies and these characteristics of the teleoconch have been used for classification in the Triphorids. However, Marshall (1983) suggests that the classification system should analyze only the protoconch because there are some shells that appear to have identical teleoconchs but their protoconchs are different indicating a different genus. "Together with color and color pattern, protoconch features are unquestionably the most valuable for species discrimination. At this point I cannot emphasize too strongly that under absolutely no circumstances should further new species be proposed unless a complete, unworn protoconch can be illustrated. Protoconch should always be illustrated by scanning electron micrographs because certain important or potentially important details cannot be clearly resolved by conventional light microscopy (Marshall 1983)."

Scanning electron microscopes (SEM) are used to attain a high-resolution view of the topography of a specimen. The microscope uses beams of electrons that interact with the electrons in the sample. This produces a signal that contains information about the sample's topography and composition. SEMs can provide a resolution of a sample better than one nanometer and magnify up to six orders of magnification. The SEM also has the capability to view the specimen in different conditions, which allows the SEM to be useful in many different ways.

Using scanning electron micrographs of triphorids, the fine structure of the protoconch can be analyzed (Marshall 1983). The protoconch is the first few whirls that the animal makes of its shell while in the juvenile planktonic stage (Sasaki 2008; Ponder and Lindberg 1997). Protoconch coiling can occur in three different directions. One is planispiral where it is aligned with the anterior-posterior plane of the aperture and the other two is where it is offset either to the left (sinistral) or to the right (dextral). The surface of the protoconch can have many different facies made up of beads, keels, or both and the possibilities are unlimited, however they are the same intragenera (Marshall 1983, Kay 1979). Protoconch shape can be categorized into two different types; caplike where it is wider than long, and tubular when it is longer than wide. Kay (1979) states that there are four main types of protoconchs among the Hawaiian triphorids; "an acuminate conical form with spiral carinae or keels overrun by axial threads; a dome-shaped paucispiral type with axial ribs; an immersed form in which the protoconch consists of a single small whorl projecting from the beaded apical whorl of the teleoconch; and an acuminate, conical or blunt-tipped type with microscopic wavy spiral threads."

In this study, scanning electron micrographs will be taken of protoconchs of different Triphoridae. An analysis and comparison of the micrographs, color photographs of the whole shell, the description of the protoconch in Kay (1979), and the current classification will be carried out. This will determined if the fine structure of the protoconch defines a genus and supports Marshall (1983) or if a suite of characters defines it. Two unclassified species will also be analyzed to determine if they belong to an existing genus, such as *Viriola* as suggested by another scientist, or if they make up a new genus.

Methods and Materials

Dr. Don Hemmes collected Triphoridae shells over the past 20 years on the big island of Hawaii and they were sorted by genus and species according to the facies of the teleoconch. The specimens used in this study were from this collection and selected because their protoconchs appeared to be in good condition. Several species of five different genera were analyzed by a scanning electron microscope uncoated (SEM; Hitachi S3400-II, variable Pressure SEM with an Oxford x-Max 80 SDD detector) at the University of Hawaii at Hilo. The genera analyzed were *Viriola*, *Triphora*, *Mastoniaeforis*, *Mastonia*, and *Cautor*. Two unclassified species nicknamed "false fallax" and "rusty fused bead" were also analyzed. Three uncoated specimen of a species were analyzed in the SEM and confirmed that they are the same species, and a micrograph of the best protoconch was taken. After the images from the SEM were collected, the protoconch micrograph was compared to the description published in Kay (1979). The micrographs were then compared to the color images of the whole shell and the classification of the species was analyzed. If it was correctly classified according to the parameters set forth for the genus, then it was compared it to other similar looking genera and determine if the genus could be defined by the protoconch alone or if a suite of characteristics defines it.

Results

The protoconchs of the analyzed species did have the same microstructure intragenus (Figure 1). When *Cautor intermissa* and *Mastonia troglodytes* are compared they appear to be very similar but their protoconchs are very different. The teleoconch of both have beads and the shell shape and siphon sizes are also very similar but *M. troglodytes* is bicarinate and has a blunt rounded tip where *C. intermissa* has no keels and the tip is different (Figure 2). The protoconch can look very similar between two different genera but the teleoconch looks very different. *Mastonia quadrimaculata* and *Viriola abbotti* are examples of this because both protoconchs are blunt tipped, the first two whorls are unicarinate, the second two whorls are bicarinate, and there are axial threads. However, *M. quadrimaculata*'s teleoconch has beads where *V. abbotti* has smooth keels. The siphon sizes and shell

shape are also different (Figure 3). The protoconchs of the two unclassified shells are very similar, but are very different from a *Viriola* protoconch. *Viriola* has a smooth blunt tip, two whorls unicarinate, two whorls bicarinate, and axial threads. The two unclassified shells protoconchs have a flat tip, the whorls are bicarinate, and have axial threads (Figure 4). This protoconch microstructure does not match any other protoconch microstructure described in Kay (1979).

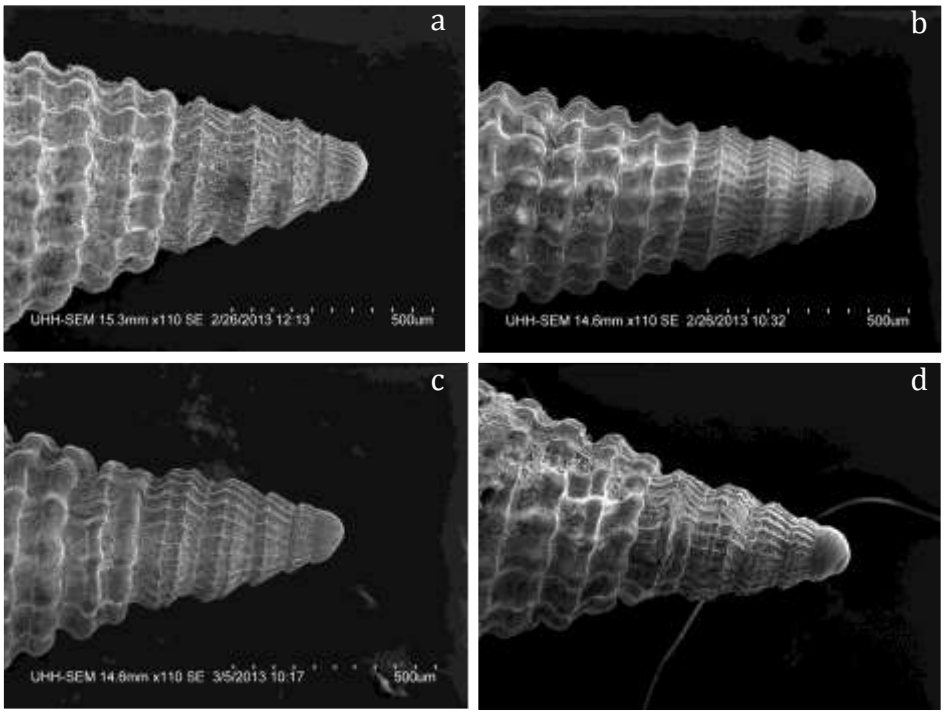

Figure 1. Micrographs of (a) *Triphora laddi*, (b) *T. pallida*, (c) *Mastonia quadrimaculata*, and (d) *M. gracilis* demonstrating protoconch similarity within a genus

Discussion

Within analyzed genera, the protoconchs appeared to be the same. Marshall (1983) has also demonstrated this and used this information to propose that the protoconch should be the primary defining feature of a genus. This study provides support for the proposal because when two shells from different genera that have teleoconchs that suggest they belong to the same genus were compared, the protoconchs were very different. When investigating whether the protoconch could be the only defining feature of a genus it was found that two protoconchs can appear similar if not analyzed carefully and incorrect classification can occur due to the misjudgment. Using a suite of characteristics to define a genus would avoid this problem because the teleoconchs were very different for the two species with

similar protoconchs. These other characteristics may include, teleoconch microstructure, shell shape, siphon size, and even coloration to a degree.

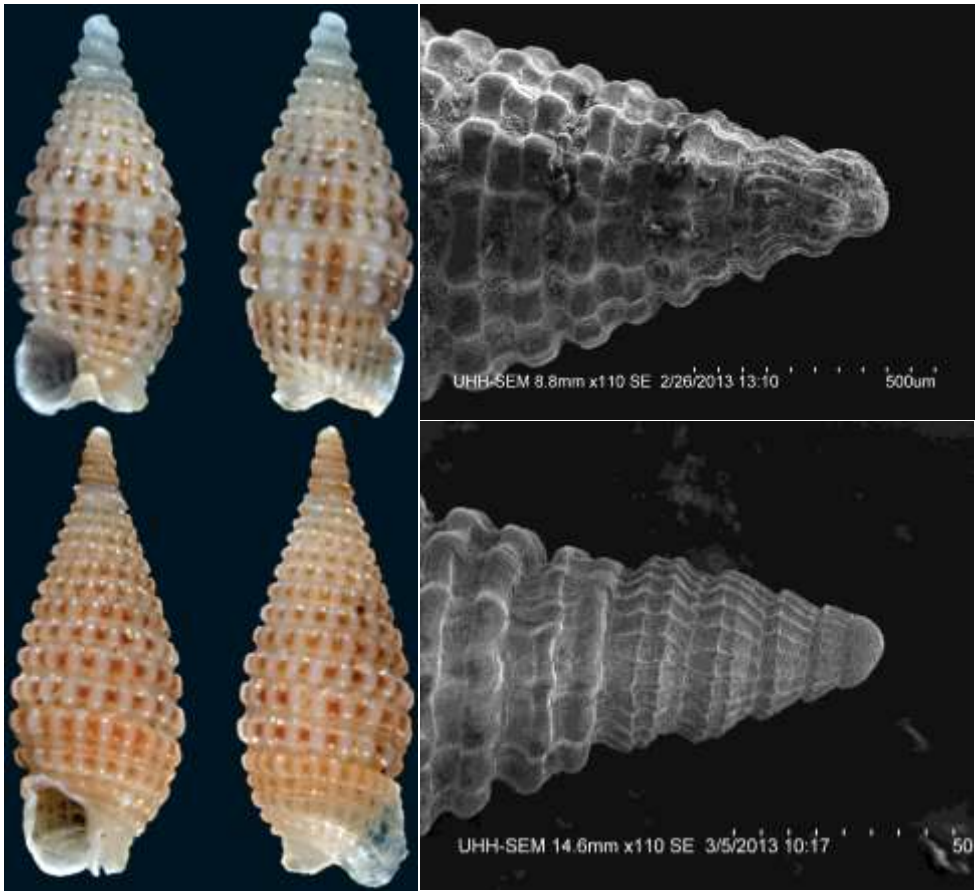

Figure 2. *Cautor intermissa* (top) and *Mastonia troglodytes* (bottom) displaying similar teleoconchs, but different protoconch microstructure.

After analyzing the two unclassified shells, it is clear that they do not belong in genus *Viriola*. Literature on the classification of Triphoridae in Hawaii did not have any existing genera that the two unclassified shells match the description for. Therefor a new genus is proposed for these two species called *Pilulaefusus*. This translates to fused bead and was chosen because that is a common feature of both species. The keels on each shell are not smooth as seen in *Viriola* but are bumpy demonstrating that keels are fused beads. Species names are proposed for both shells as well. For "rusty fused bead" *ferrugineae* was chosen because it means rusty and the shell is a rusty color, and *hemmesi* was chosen for "false fallax" to honor my mentor, Dr. Don Hemmes.

Classification of Triphoridae should involve analysis of the protoconch as the primary defining feature but include a suite of characteristics to define the genus. I

propose a new genus of Triphoridae, *Pilulaefusus*, which includes the two unclassified species in this study. The protoconchs of all Triphoridae in Hawaii should be analyzed to ensure proper classification and analysis of the DNA should be undertaken in order to compare phenotypic classification with genotypic classification. To date, there has been little effort put into collecting and studying these animals and there are many more new species to be discovered and classified.

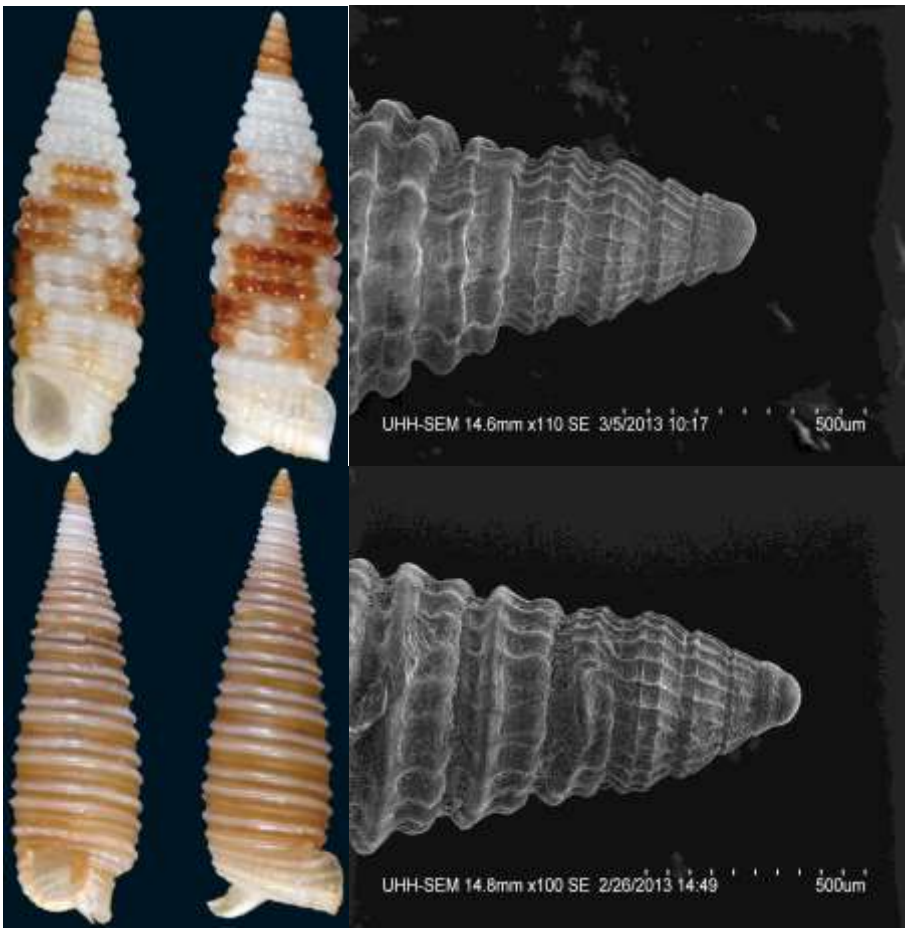

Figure 3. *Mastonia quadrimaculata* (top) and *Viriola abbotti* (bottom) demonstrating similar protoconchs but different teleoconchs.

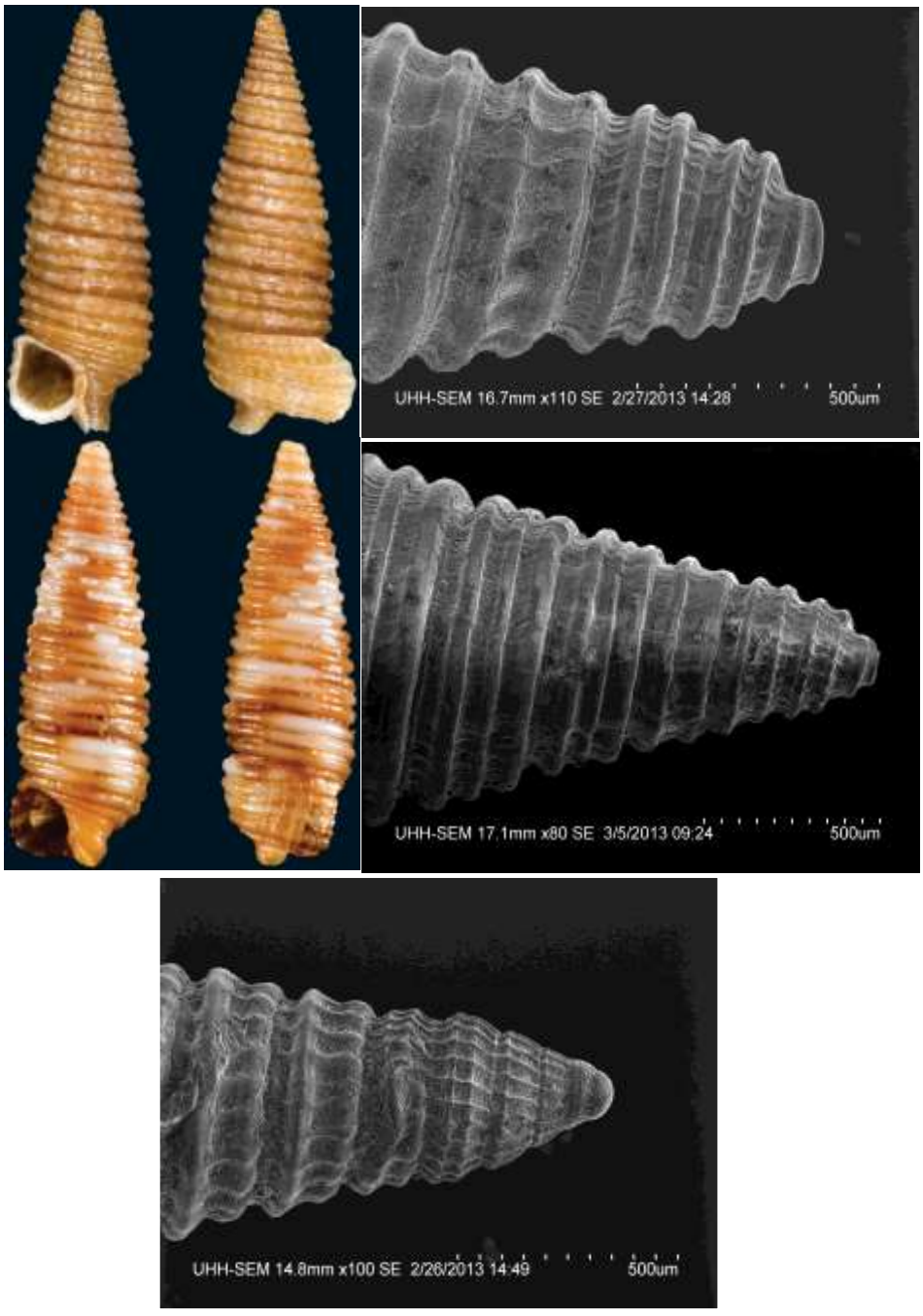

Figure 4. "Rusty fused bead" (top) and "false fallax" (middle) protoconchs suggesting that they are the same genus, but do not belong to genus *Viriola* because their protoconchs are very different from the protoconch of *Viriola abbotti* (bottom).

Acknowledgements

A sincere thanks goes to Dr. Don Hemmes for being my mentor and aiding me with this project. Another sincere thanks to the University of Hawai'i Marine Option Program for funding this project. Also thanks to Dr. Jason Turner for being my MOP advisor and Mrs. Jen Turner for helping with MOP as well. Thank you to John Coney for teaching me how to use the SEM and Mike Severns for allowing me to use his photographs of the Triphoridae.

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