Two new species of Velutinidae Gray, 1840 (Gastropoda) from the North Pacific with a preliminary molecular phylogeny of the family

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ABSTRACT

Two recent collections of shelled gastropods in the temperate North Pacific have identified two undescribed species of the family Velutinindae. *Marsenina zadei* new species is described from Port Townsend, Washington. *Onchidiopsis clarki* new species is described from Pribilof Island, Alaska, Bering Sea. Both species are barcoded with sequences of the mitochondrial COI and 16S genes. Additionally, a preliminary phylogeny for the Velutinidae based on these two genes is provided.

Additional Keywords: Velutinidae, Lamellariidae, *Marsenina*, *Onchidiopsis*

INTRODUCTION

Current classifications place lamellarid gastropods in the family Velutinidae Gray, 1840 (Bouchet and Rocroi, 2005). These are caenogastropods with an internal shell that have been traditionally neglected. The species-level taxonomy of this family has been in disarray. It is not the intention of this paper to review the higher taxonomic nomenclatural problems, so we follow the most recent review of species presented in Gulbin and Golikov (2001, but see 1997, 1998, 1999, 2000). Prior to that, the most comprehensive treatment of species from the North Pacific Ocean was given by Behrens (1980), which, while focusing on species known from the eastern Pacific (Alaska to Mexico), differentiated members of the genera *Lamellaria*, *Marsenina*, and *Marseniopsis*.

The main objective of this paper is to describe two new species of the genera *Marsenina* and *Onchidiopsis* (subfamily Velutininae Gray, 1842). Species of *Marsenina* are distinguished by a radula having a formula of 2.1.1.1.2 (two outer teeth are present on each side), being hermaphroditic, having a fissure or pore in the mantle exposing the shell and permitting the retraction of the mantle, and having a small, smooth foot that remains hidden under the mantle. Species of *Onchidiopsis* has the same radular formula as *Marsenina*, and are hermaphroditic, but are distinguished in that they have an internal shell fully enveloped by the mantle that is not retractile, and a long foot with a distinctive rugose or nodular edge.

In order to allocate these two species within the phylogeny of the group, preliminary molecular phylogenetic analyses (based on two mitochondrial genes) were conducted, comprising the two new species and other members of the Velutinidae for which sequences are available in GenBank.

MATERIALS AND METHODS

Collection and Preservation: Specimens were collected by scuba and trawl, respectively. All collected specimens were fixed and preserved in 95% ethanol to facilitate genetic analyses. All specimens were catalogued and deposited in the Invertebrate collection of the Natural History Museum of Los Angeles County (LACM).

Morphological Examination: Preserved specimens were dissected and the internal features were examined using a dissecting microscope. The buccal mass of one individual of each species was removed and dissolved in 10% sodium hydroxide until the radula and jaw were isolated from the surrounding tissue. The radula and jaw were then rinsed in water, dried, mounted, and sputter-coated for examination under a scanning electron microscope (SEM) Hitachi S-3000N at the LACM. The anterior end of the body, including the head and the penis, where dissected and chemically dried with hexamethyldisilazane for SEM examination.

DNA Extraction, PCR, and Analyses: DNA extraction was performed using a hot Chelex® protocol with approximately 1–3 mg of the foot cut into fine pieces. The tissue was rinsed and rehydrated using 1.0 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 20 minutes. A 10% (w/v) Chelex® 100 (100-200 mesh, sodium form, Bio-Rad) solution was prepared using TE buffer. After rehydration, the tissue mixture was then centrifuged, 975.00 µL of the supernatant was removed, and 175.00 µL of the Chelex® solution was added. Samples

were then heated in a 56°C water bath for 20 minutes, heated in a 100°C heating block for 8 minutes, and the supernatant was used for PCR. Universal16S rRNA primers (16S ar-L 5′-CGCCTGTTTATCAAAAACAT-3′, 16S br-H 5′- CCGGTCTGAACTCAGATCACGT-3′ developed by Palumbi, 1996) and universal CO1 primers (LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′, HCO2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ developed by Folmer et al., 1994) were used to amplify the regions of interest for all specimens.

The master mix was prepared using 34.75 µL H_2O, 5.00 µL Buffer B (ExACTGene, Fisher Scientific), 5.00 µL 25 mM $MgCl_2$, 1.00 µL 40mM dNTPs, 1.00 µL 10mM primer 1, 1.00 µL primer 2, 0.25 µL 5 mg/mL Taq, and 2.00 µL extracted DNA. Reaction conditions for 16S were as follows: an initial denaturation for 2 min at 94°C, 30 cycles of 1) denaturation for 30 sec at 94°C, 2) annealing for 30 sec at 50°C, and 3) elongation for 1 min at 72°C, and a final elongation for 7 min at 72°C. Reaction conditions for CO1 an initial denaturation for 3 min at 95°C, 35 cycles of 1) denaturation for 45 sec at 94°C, 2) annealing for 45 sec at 45°C, and 3) elongation for 2 min at 72°C, and a final elongation for 10 min at 72°C.

PCR products yielding bands of appropriate size (approximately 475 bp for 16S and 700 bp for CO1) were purified using the GeneJet PCR Purification Kit (Thermo Scientific). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 2.0 pmol/µL to send out for sequencing with the PCR products. PCR products were diluted to 7.5 and 11.5 ng/µL for 16S, and CO1, respectively. Samples were sequenced at Source Bioscience (Santa Fe Springs, CA).

For the phylogenetic analyses, sequences of the following species were obtained from GenBank: *Coriocella nigra* Blainville, 1824 (16S: AY161381, COI: AY161614), *Lamellaria* sp. 1 (16S: AY161382, COI: AY161615), *Lamellaria* sp. 2 (16S: AY161383, COI: AY161616), and *Marseniopsis mollis* (COI: GU227110). The triviid species *Triveilla millardi* (Cate, 1979) (16S: AY161389, COI: AY161622) was selected as the outgroup based on recent phylogenetic analyses (Meyer, 2003). Sequences for each gene were assembled and edited using Geneious Pro 4.7.4 (Drummond et al., 2010). Geneious was also used to extract the consensus sequence between the primer regions, to construct the alignment for each gene using the default parameters and to concatenate the alignments. The sequences were trimmed after alignment. A total of 473 bp for 16S, and 614 bp for CO1 were used for the phylogenetic analyses.

The phylogenetic analyses were conducted for both genes concatenated. The Akaike information criterion (Akaike, 1974) was executed in MrModeltest (Nylander, 2004) to determine the best-fit models of evolution for each gene (GTR+I+G for COI and GTR+I for 16S). The Bayesian analysis was executed in MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001), partitioned by gene (unlinked). The Markov chain Monte Carlo analysis was run with two runs of six chains for ten million generations, with sampling every 100 generations. The default 25% burn-in was applied before constructing majority-rule consensus tree/s. The maximum likelihood analysis was conducted with the program GARLI v0.96b8 (Zwickl, 2006). Default parameters were used to run three different GARLI searches of 10 replicates each, and a total of 2,000 bootstrap replicates were performed to assess the robustness of each clade (Felsenstein, 1985).

SYSTEMATICS

Family Velutinidae Gray, 1840
Subfamily Velutininae Gray, 1840

Genus *Marsenina* Gray, 1850

Type Species: *Lamellaria prodita* Lovén, 1846 (= *Oxynoe? glabra* Couthouy, 1838)

Marsenina zadei new species

(Figures 1–4, 7–9)

Description: External Morphology: Mantle color makes this species difficult to find on its substrate (Figure 1). Mantle color dusky-white to tan to orange, with a sprinkling of black specks. A dark brown horseshoe mark present posterior-medially in some specimens. Mantle with a dorsal slit or fissure, which may be retracted exposing shell. Mantle with an anterior and right lateral fold creating incurrent and excurrent siphons, respectively, circulating water over gills. Surface of the mantle covered with a pattern of spots resembling atrial siphons of ascidian host. Some specimens with radiating ridges on mantle (Figure 2). A most distinctive mantle feature is a series of tubercles within the dark horseshoe (Figures 2–4).

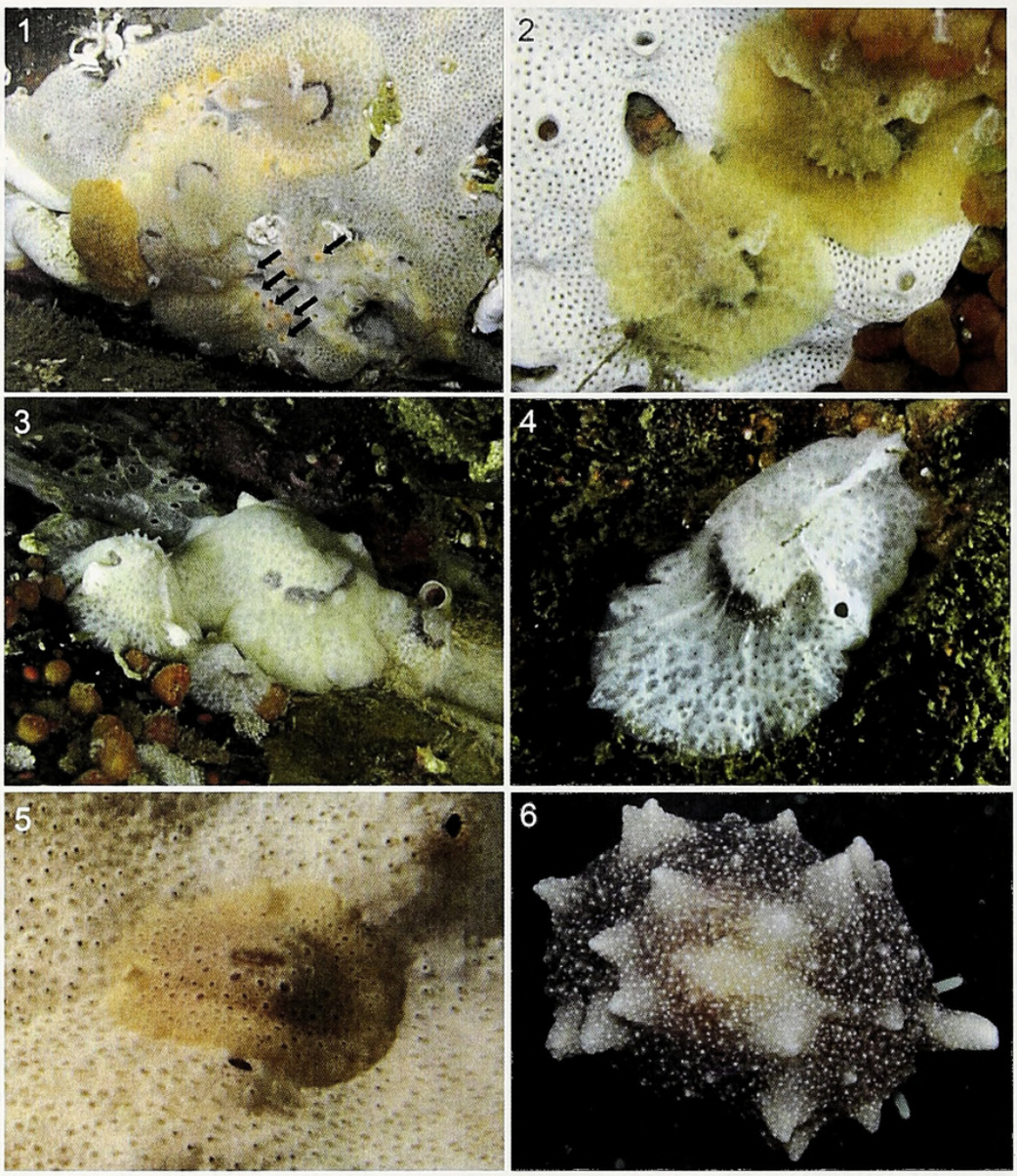
Shell: Shell oval, translucent-white, with a number of growth lines. Protoconch situated on posterior right side of shell, partially engulfed by teleoconch. Protoconch large, elongate, about 600 µm × 1 mm with 1.1 whorls (Figure 8).

Radula and Jaws: The radular formula is 98 × 2.1.1.1.2. The rachidian tooth bears 1 to 2 strong denticles to each side of the central cusp (Figure 7). The inner lateral teeth bear a single, strong denticle to each side of the central cusp. The outer lateral teeth are smooth and hamate. Masticatory border of the jaw (Figure 9) with a series of uniform denticles.

Penis: The penis (Figure 10) is flat, branching into three blunt apices distally.

Molecular Data: Sequences of this species are available in GenBank. Molecular phylogenetic analyses place this species as sister to *Onchidiopsis clarki*, but with limited support in the Bayesian analysis (Figure 14).

Biology: All specimens were collected on an encrusting compound ascidian, tentatively identified as *Tridemnum*



Figures 1–6. Living animals of *Marsenina* and *Onchidiopsis* species. **1–4.** *Marsenina zadei* new species. Port Townsend, Washington, black arrows indicate the salmon-orange egg capsules. Photos by Rick Zade. **5.** *Marsenina stearnsii* (Dall, 1871). Keysone Jetty, Whidbey Island, Washington. Photo by Jan Kocian. **6.** *Onchidiopsis clarki* new species. Bering Sea, NNE of Pribilof Island, Alaska. Photo by Roger Clark.

opacum (Ritter, 1907), at depths from 3 to 15 m. Collected with the specimens, and buried in the tunic of the ascidian, were salmon-orange egg capsules (Figure 1). Upon dissection the orange color within some of the capsules was determined to be the color of the developing larvae.

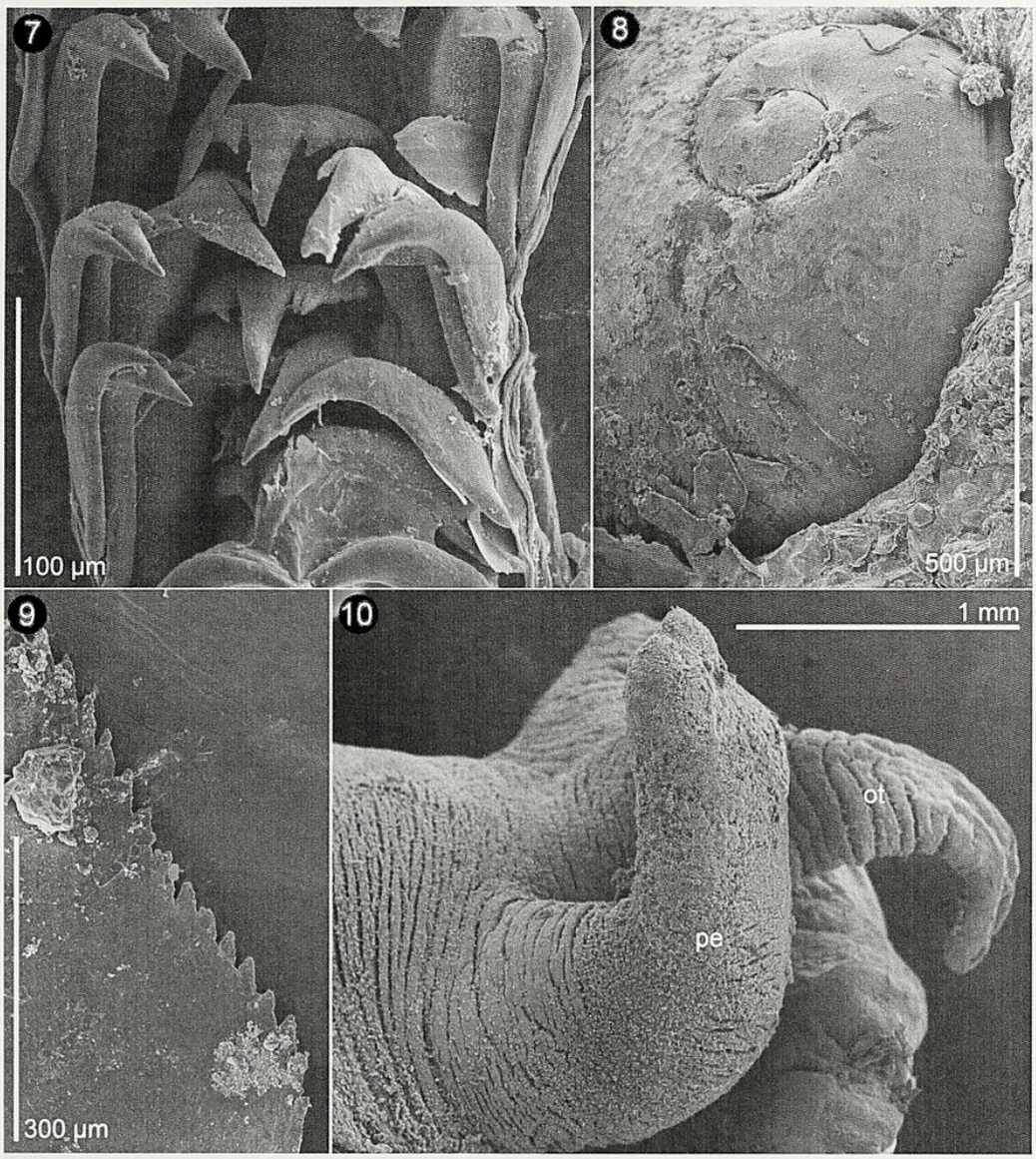
Type Material: Holotype, LACM 3280, 15 mm preserved length; Paratypes, LACM 3281, 11 specimens, 6–15 mm preserved length, all Richard Zade coll., 8 February 2010, from type locality.

Type Locality: Hudson's Point (48°6.949N, 122°44.999W), Port Townsend, Washington State, USA, 15 m depth.

Distribution: Port Townsend, Washington (present study); Ten Mile Point, Greater Victoria, British Columbia, Canada (photo by James Hester); Pigeon Point, San Mateo County, California (photos by Gary McDonald and Doug Mason); Carmel Point, Monterey County, California (photo by Gary McDonald).

Etymology: This species is named after Richard Zade, the collector of the type specimens.

Remarks: *Marsenina zadei* differs significantly from the two other described species from the North Pacific, both internally and externally. As in *M. zadei*, the mantle of *Marsenina stearnsii* resembles the encrusting tunicate, *Tridemnum opacum*, but *M. stearnsii* lacks any dark markings or black specks, and has a smooth mantle



Figures 7–10. *Marsenina zadei* new species, Port Townsend, Washington. **7.** SEM of a section of the radula, showing rachidian, lateral, and marginal teeth. **8.** SEM of protoconch. **9.** SEM of masticatory border of jaw. **10.** SEM of penis (pe) and oral tentacle (ot).

lacking tubercles (Figure 5). Dall in Orcutt (1885) and Smith (1948) state that *Marsenina stearnsii* var. *orbiculata* is not a valid taxonomic entity. Ghiselin (1964) and Behrens (1980; 1984) describe the morphology of the mantle and are the only known published photographs of living specimens of *M. stearnsii*. In *M. stearnsii*, the mantle coloration is white to creamy white with slightly elevated darker cream colored spots resembling the atrial siphons of its host ascidian (Figure 5). Numerous photos were submitted by colleagues and more found on the web that are attributable to *M. zadei*, but those had been idntified as *M. stearnsii*. Bsed on these photos, it is possible extend the geographical range of *M. zadei* south to Carmel Pt. California, December 2, 1971 (photo by G. McDonald).

Of the internal anatomy, only the radula and shell have been described for *M. stearnsii* and *M. rhombica*. Dall (1871, 1885) reported the distinguishing characteristics of the shell surface of *M. stearnsii* to be microscopic fine revolving striulae. However, such striulae were not observed on the shell a specimen of *M. stearnsii* from Marin County, California (LACM) examined for this study. The main differences between the shells of *M. stearnsii* and *M. zadei* is the protoconch morphology; in *M. stearnsii* the protoconch is more circular in shape and has nearly 2 whorls, whereas the protoconch of *M. zadei* is more oval and has 1.1 whorls.

In most cases, as in other genera of this family, only the penis has been described for *Marsenina*. The penis of *Marsenisn zadei* is not similar to any of those species for which descriptions are available. The penis of co-occuring *Marsenina rhombica* is figured and described by Gulbin and Golikov (2000) as horn-shaped, not flattened and slightly bifurcate as described for *M. zadei* herein.

Behrens (1980) described the radulae of *M. stearnsii* and *M. rhombica*. While the number of rows of teeth in

the radula was not given for either of the north Pacific *Marsenina* species, the morphology of the teeth was found to be identical between the two and differs slightly from that of *M. zadei*. *Marsenina stearnsii* is reported to have a rachidian tooth with zero or one denticle flanking the central cusp, while the rachidian of *M. zadei* bears 1 to 2 strong denticles to each side of the central cusp. The inner and outer lateral teeth are similar in all three species.

Genus *Onchidiopsis* Bergh, 1853

Type Species: *Onchidiopsis groenlandica* Bergh, 1853

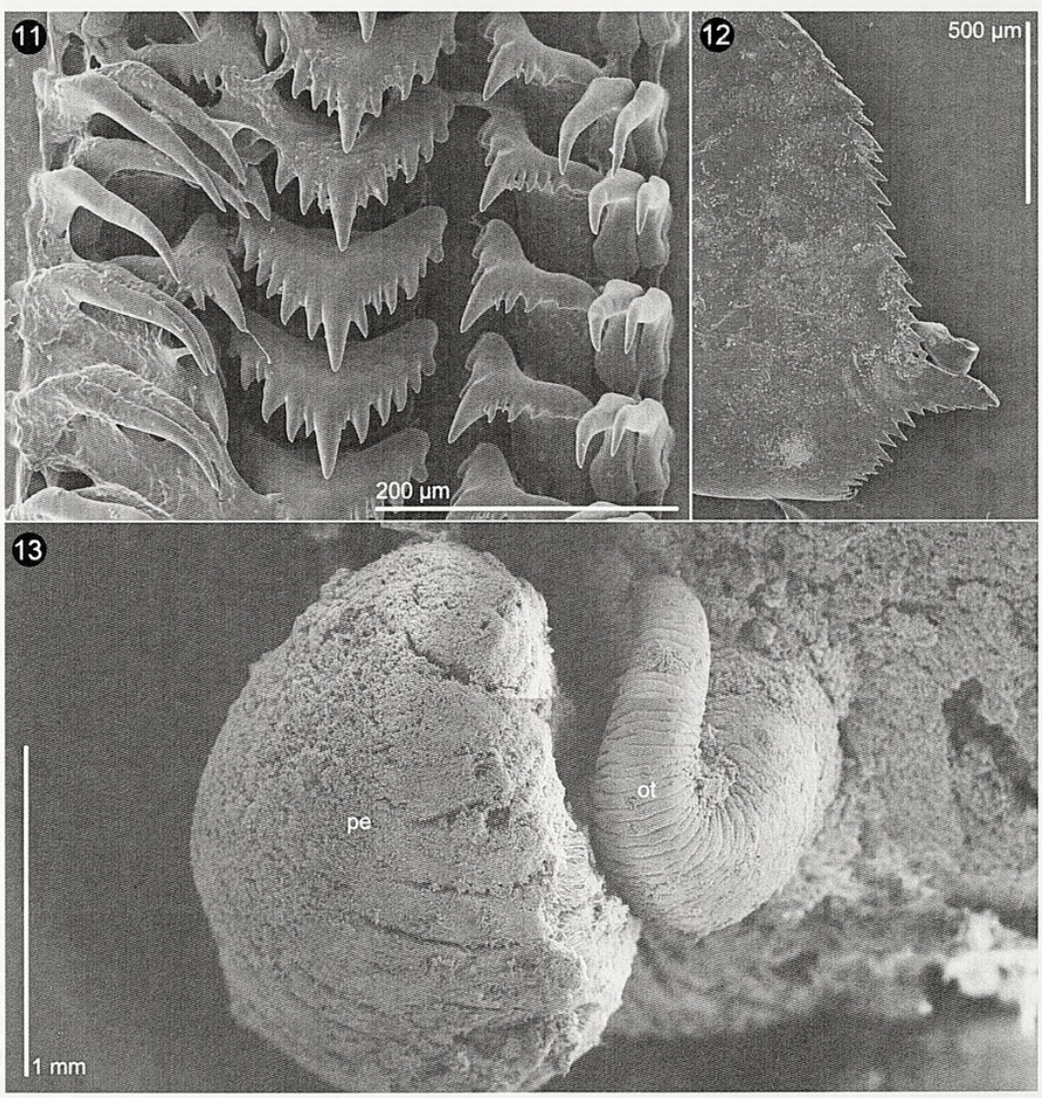
Onchidiopsis clarki new species
(Figures 6, 11–13)

Description: External morphology: Mantle completely covers shell. Mantle covered with randomly spaced, large triangular tubercles (Figure 6). An anterior fold gives origin to incurrent siphon. Oral tentacles protruding from under mantle. Oral tentacles long, slender, and tapering (Figure 13). Specimens brown with uniformly distributed white specks. Brown ground color fades to off-white on tubercles and siphon.

Shell: The shell is an un-calcified, soft plate without any recognizable characteristics in the specimens examined.

Radula and Jaws: Radular formula is 74 × 2.1.1.1.2 (Figure 11). Rachidian bears 5–7 irregularly sized denticles on each side of central cusp. Inner lateral teeth bear 3–5 dissimilarly sized denticles on each side of the central cusp. Pair of outer lateral teeth simple, hooked, lacking denticles. Masticatory margin of jaws with a series of nearly uniform denticles, posteriorly with a large denticulate flange (Figure 12).

Penis: The reproductive system is typical of members of the genus, *Onchiopsis*, from what little information we could find. The penis (Figure 13) is thickened, highly twisted, with a blunt truncated end.



Figures 11–13. *Ochidiopsis clarki* new species, Alaska, Bering Sea. **11.** SEM of a section of the radula, showing rachidian, lateral and marginal teeth. **12.** SEM of masticatory border of jaw. **13.** SEM of penis (pe) and oral tentacle (ot).

Molecular Data: Sequences of this species are available in GenBank. Molecular phylogenetic analyses place this species as sister to *Marsenina zadei*, but with limited support in the Bayesian analysis (Figure 14).

Biology: Both specimens were collected in the same trawl over a mud bottom at a depth of 87 m. There were no indications of which of the other organisms collected in the trawl might be this species prey.

Type Material: Holotype, LACM 3282, specimen 28 mm preserved length; Paratype, specimen 26 mm preserved length, LACM 3283, all trawled by the R/V ARTURUS, leg. Roger Clark, from type locality.

Type Locality: NNE of Pribilof Island (58°00.79N, 170°58.18W), Bering Sea, Alaska, Bering Sea, USA, 87 m depth on mud, bottom temperature 4.0°C (NMFS 88-2003-1-138).

Etymology: This species is named after Roger Clark, the collector of the type specimens.

Remarks: The genus *Onchidiopsis* is found in both the cold temperate Atlantic and Pacific Oceans. Both faunas are poorly known. Depending on which database we examined, numbers of species varied around 15 for the North Atlantic, while 10 are reported from the North Pacific (Gulbin and Golikov, 2001). Several of these are reported from throughout the Arctic Sea, reaching into both oceans.

Although Balch's (1910) original description of *Onchidiopsis corys* from Newfoundland and Labrador, North Atlantic, describes the notum as smooth on the top and sides, with wrinkles and folds elsewhere, photos on the web (http://eol.org/pages/72611/overview and http://eol.org/pages/593815/overview) of recent specimens "thought to be" *O. corys* bear some external similarity with *O. clarki*, having triangular tubercles on the mantle. These specimens have lighter color and their mantle surface is knobby and granular between the tubercles. Balch's description of the internal anatomy is vague and could apply to any species in the genus.

The most obvious Circumboreal species to be considered here are *Onchidiopsis glacialis* (Sars, 1850) and *Onchidiopsis groenlandica* Bergh, 1853. These species seem to have been maintained as separate in the literature (Bergh, 1886; MacGinitie, 1959; Gulbin and Golikov, 2001) even though there appears to be adequate arguments to synonymize the two (Balch, 1910; Thorson, 1944; Macpherson, 1971). Where the mantles of these two very similar species are discussed, descriptions vary from rugose and wrinkled (Gulbin and Golikov, 2001) to convoluted (brain-like) (Macpherson, 1971). We have found no mention of specimens with triangular tubercles.

Nowhere in the literature can we find a North Pacific species with large triangular tubercles seen in *O. clarki*.

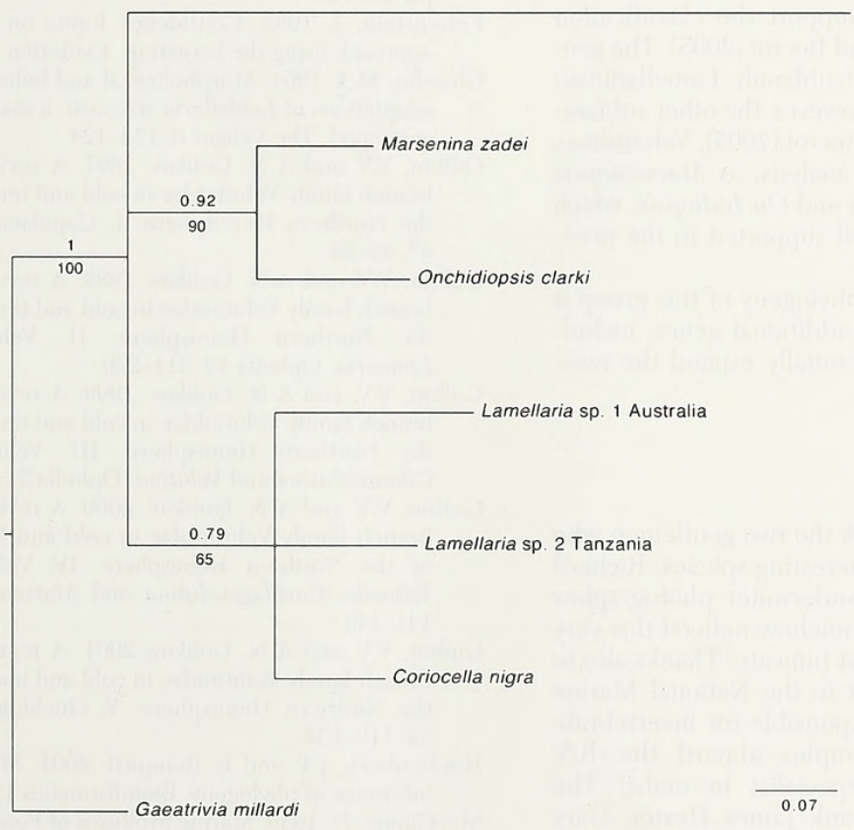


Figure 14. Bayesian consensus tree of the concatenated analysis including posterior probabilities and bootstrap values from the maximum likelihood analysis.

All of the species figured in Gulbin and Golikov (2001) appear to have smooth or slightly granular mantle surfaces, lacking tubercles.

We were unable to find any published description of the internal reproductive system, only that of the penis, which in the genus seems to be diagnostic. Of those species described by Gulbin and Golikov (2001), none are comparable to the penis of *O. clarki* described here as thickened, highly twisted, with a blunt truncated end. In *O. groenlandica*, they described the penis as having an ancillary appendage hanging over the penis. In *O. variegata*, they described the penis as long, cylindrical, its distal part thickened and curved, terminating in a swelling with a thin fold. In *O. zuchsi*, they describe the penis as highly characteristic with a large divided lobe. On the side it bears an extending papilla, rimmed with a thin fold. All members of the subgenus *Rostroonchidiopsis* have a long tapering, hook shaped penis with a crest at the bend, while members of the genus *Bulloonchidiopsis* have a flattened, hammer-shaped penis, the distal end of which is recurved upward.

DISCUSSION

In this paper we include the first, albeit very preliminary, molecular phylogeny of the Velutinidae, based on COI and 16S sequence data. The resulting tree lacks support for most branches, suggesting that the two genes sequenced are not adequate to recover the phylogeny of the Velutinidae. However, the general structure of the tree appears to partially support the classification scheme proposed by Bouchet and Rocroi (2005). The genera *Lamellaria* and *Coriocella* (subfamily Lamellariinae) are placed in the same clade. However, the other subfamily recognized by Bouchet and Rocroi (2005), Velutininae, is paraphyletic in the present analysis, as *Marseniopsis* does not cluster with *Marsenina* and *Onchidiopsis*, which form a monophyletic group well supported in the maximum likelihood analysis.

In order to reconstruct the phylogeny of this group it will be necessary to sequence additional genes, including nuclear markers and substantially expand the taxonomic coverage.

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