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Three New Species of *Gymnodoris* Stimpson, 1855 (Opisthobranchia, Nudibranchia) from the Philippines

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Three new species of nudibranchs in the genus *Gymnodoris* are described. All three have radulae typical of the genus, with no rachidian tooth and featuring hook-shaped, pointed lateral teeth. *Gymnodoris brunnea*, sp. nov. is translucent with a brownish tinge, covered in small, deep-orange, conical pustules and has gills arranged in a linear row. It can also be distinguished from all other *Gymnodoris* species that possess a linear gill by a short, broad, triangular second lateral tooth with a bifid cusp. *Gymnodoris tuberculosa*, sp. nov. differs in pigment from all previously described *Gymnodoris* species by its uniformly translucent white coloration, including the tubercles that cover the body and a gill arranged in a complete circle. *Gymnnodoris pseudobrunnea*, sp. nov. externally resembles *G. brunnea*, sp. nov., with small, deep-orange, conical pustules, and gills arranged in a linear row, though it does not always have a brownish tinge. These two species can be differentiated externally by the shape of a white patch seen dorsally through the skin, the outline of which is more irregularly shaped in *G. pseudobrunnea*, sp. nov. than in *G. brunnea*, sp. nov., and by the dark intestine that can be seen through the skin in *G. brunnea,* but not in *G. pseudobrunnea*, sp. nov.. Internally, they are differentiated by the radula, the radula of *G. pseudobrunnea*, sp. nov. lacks a bicuspid second inner lateral tooth, and by the position of the receptaculum seminis duct,which enters the middle of the vagina in *G. pseudobrunnea*, sp. nov. rather than the at the proximal end as in *G. brunnea*, sp. nov. Additionally, *G. brunnea*, sp. nov. specimens sequenced for a fragment of the COI gene differed from a *G. pseudobrunnea*, sp. nov. specimen by an uncorrected p-distance of 10.3% to 11.1%, further supporting these as two distinct species. All three species are found in the Anilao region of the Philippines, while *G. tuberculosa*, sp. nov. has been additionally recorded from Madang, Papua New Guinea and the Marshall Islands.

KEY WORDS: *Gymnodoris*, Gymnodorididae, new species, Philippines

Following the trend of many marine taxa, the opisthobranch diversity of the Coral Triangle in the Indo-Pacific is exceptionally rich, with the Philippines being home to over 700 species (Gosliner *et al*. 2008). New species continue to be found in this region. On the recent Hearst Philippine Biodiversity Expedition to the "Anilao" region of the Philippines, the team of scientists found 25 species of "opisthobranchs" that are new records for the region, and at least 50 additional species that are new to science. A taxon that was especially well-represented by collections on the expedition was the nudibranch genus *Gymnodoris*.

There has been some disagreement over the placement of *Gymnodoris*, whether it belongs in an independent family, Gymnodorididae and whether this family should be treated as a subfamily, Gymnodoridinae, within Polyceridae (summarized in Macnae 1958). The current generally accepted classification recognizes Gymnodorididae Odhner, 1941 (Bouchet and Rocroi 2005) including *Gymnodoris* along with several other genera (Bouchet 2014; McDonald 2009), though there has been no comprehensive review of the family. A recent molecular phylogenetic study found that *Gymnodoris*, represented by *G. alba* and *G. striata*, nests within the polycerid subfamily Polycerinae with high support (Palomar et al. 2013). However, the study also suggested that additional taxa need to be sampled to clarify the issue, and no taxonomic change was made. The taxonomic status of this family is beyond the scope of this paper, and until there is a formal change in the taxonomy, we follow the current accepted taxonomy here.

With respect to its taxonomy and identification, *Gymnodoris* is a particularly challenging genus of nudibranchs. Plagued by inadequate descriptions and little variation of external color patterns, the true diversity of this group has remained poorly documented in the literature.

We have found that molecular techniques have been useful in identifying putative undescribed species and have further highlighted the difficultly of reconciling old descriptions with the true diversity of this group of voracious predators. While there are approximately 30 described species of *Gymnodoris*, several of these have descriptions that are inadequate for matching species to them, and most of these species names likely lack associated type material. Few *Gymnodoris* species have been described in recent years. Most recently, Hamatani and Osumi (2003) described *Gymnodoris inariensis*, a small, blackish, gill-less species from Japan, and Swennen (1996) described *Gymnodoris pattani*, a translucent greenish species from Thailand. Here we describe three new taxa and compare them to known species of *Gymnodoris*. Because two of these new taxa were difficult to tell apart in the field, in addition to looking at morphological details, we sequenced specimens for a fragment of the cytochrome oxidase subunit I (COI) gene and calculated uncorrected p-distances to see if this was consistent with the differences seen in morphology.

The material was deposited in the Department of Invertebrate Zoology and Geology of the California Academy of Sciences, San Francisco (CASIZ), and sequences for the COI gene were uploaded to GenBank.

Methods

Morphological Examination.— We subsampled many of the specimens for DNA prior to morphological analysis or measurement, resulting in lengths that are smaller than the intact preserved specimens.

We dissected specimens by making an incision with forceps near the foot. Reproductive structures were drawn initially using a drawing tube attached to a dissecting microscope and subsequently scanned, traced and rendered digitally using Adobe Illustrator CS4 or CS6 and Photoshop CS4 or CS6.

To examine the radulae, we placed the buccal masses of specimens in a 10% NaOH solution until the tissue surrounding the radulae had dissolved sufficiently. We subsequently placed these radulae in deionized water to remove residual salt and mounted them on SEM stubs using double sided adhesive or no adhesive at all. The radulae were imaged using a scanning electron microscope (LEO 1450 VP). To assess whether the penis of specimens was armed with spines, the distal portion of the vas deferens was excised and cleared and stained by first dehydrating in a series of increasing concentrations of ethanol, then placed in acid fuchsin stain, then placed in xylene, removed and mounted on a glass microscope slide. The slides were photographed using a Spot FLEX Mosaic Model 15.2 digital camera mounted on a Leica DMRB microscope. Scale bars were applied in SPOT Software 5.0.25. Adobe Photoshop CS4 was used to get a rough approximation of the length and width of penial spines, by measuring and averaging the lengths of four different visible spines andwidths of four visible spine bases using the scale bar for measurement calibration.

Molecular Methods.— In this paper we were interested in comparing COI sequences for two taxa that had a similar external appearance that were difficult to tell apart in the field. We subsampled several specimens for DNA analysis by taking a small piece of tissue from the foot or tip of the "tail." We were careful to avoid subsampling digestive tissue since some gymnodorids are known to eat other nudibranchs including congeners and conspecifics, in some cases.

Polymerase chain reaction (PCR) was used to amplify a partial COI gene fragment using the standard "Folmer" COI primers (Folmer et al. 1994). Each 25 μL reaction contained: 15.0 μL ster-

ile water, 2.5 µL 10X USB buffer, 1.0 µL 25 mM $MgCl_2$, 1.0 µL each of 10 µM forward and reverse primer, 0.5 µL 10 mM dNTPs, 1.5 µL, 10 mg/mL BSA, 0.5 µL 1.25 units/µL HotStart Taq and between 2 µL to 5 µL of template DNA, depending on the concentration of the sample. Cycling parameters were generally as follows for COI: an initial denaturation step at 94°C for 3 min, followed by 94°C for 30 s, 47°C for 30 s, 72°C for 60 s for 35 cycles and a final extension step at 72°C for 10 min. Following PCR, we visualized products on 1% agarose gel containing ethidium bromide. For reactions that successfully amplified the target gene fragment, we used an Exo-SAP-IT protocol ExoSAP-IT (usb.affymetrix.com) to purify products for cycle sequencing.

Purified PCR products were cycle-sequenced using a ~10 µL reaction that contained 5.7 µL sterile water, 1.5 µL 5X Big Dye buffer, 0.3 µL 10 µM primer, 0.5 µL Big Dye 3.1, and 1–5 µL of purified PCR product, depending on the sample's PCR band brightness during visualization. We cycle-sequenced all genes using a STeP Program (Platt et al. 2007) using an annealing temperature of 50°C.

Cycle-sequencing products were precipitated using a standard ethanol precipitation protocol and resuspended them in formamide. Samples were sequenced on the ABI Prism 3130 x l Genetic Analyzer at the Center for Comparative Genomics and the California Academy of Sciences.

Sequence trace files were then assembled into contigs and edited them using the program Geneious version 6.0 (Drummond et al. 2011*).*We aligned the resulting consensus sequences in Geneious using the MAFFT Multiple Alignment v1.1 (Biomatters Ltd) plugin with default settings. Geneious was then used to calculate the uncorrected p-distances between all the sequences.

The COI sequences were uploaded to GenBank and the accession numbers appear in Appendix, Table 1.

Results

We were able to get COI sequences for 8 specimens of the two similar-looking taxa, 7 individual sequences for one taxon and one of the second taxon. The uncorrected COI p-distances for the individuals within one taxonranged from 0.0% to 0.5%. Only one specimen amplified for the other taxon. The p-distances between this specimen and the other specimens ranged from 10.3% to 11.1%.

Species Descriptions

Gymnodorididae Odhner, 1941

Genus *Gymnodoris* Stimpson, 1855

***Gymnodoris brunnea* Knutson and Gosliner, sp. nov.**

Figures 1A-B, 2–4

Material examined.— Holotype: CASIZ 185943, one specimen, Anilao Pier, Anilao Harbor, Balayan Bay, Batangas Province, Luzon Island, Philippines, 13°45′35.78″N 120°55′33.56″E collected on April 30, 2011 by the members of the Hearst Philippine Biodiversity expedition, preserved specimen 9.5 mm length. Paratypes: CASIZ 185946, one specimen, subsampled for DNA, preserved specimen 7.0 mm length with part of the "tail" missing, CASIZ 185968 one specimen dissected, subsampled for DNA, preserved specimen 9.2 mm length with part of the "tail" missing, CASIZ 185971, one specimen, preserved specimen 5.0 mm length, CASIZ 185972, one specimen, subsampled for DNA, preserved specimen 6.5 mm length with part of the "tail" missing, CASIZ 185973, one specimen, dissected, CASIZ 185976, one specimen subsampled for DNA, preserved specimen ~8.0 mm length with part of the "tail" missing, all collected at the Anilao Pier, Anilao Harbor, Balayan Bay, Batangas Province, Luzon Island, Philippines, 13°45′35.78″N 120°55′33.56″E on April 30, 2011 by the members of the Hearst Philippine Biodiversity expedition.CASIZ 185979, one specimen, Matotonngil Point, Balayan Bay, Batangas Province, Luzon

Island, Philippines, 13°45′18.997″N 120°54′24.19″E collected May 1, 2011 by Vanessa Knutson and Michele Weber, subsampled for DNA, preserved specimen 5.2 mm length with part of the "tail" missing. CASIZ 185963, one specimen, dissected, subsampled for DNA, CASIZ 185966, one specimen dissected subsampled for DNA, preserved specimen 6.0 mm length with part of the "tail" missing, CASIZ 185975, one specimen, subsampled for DNA, preserved specimen 5.0 mm length with part of the "tail" missing, all collected on May 04, 2011 at the Anilao Harbor, Anilao, Balayan Bay, Batangas Province, Luzon Island, Philippines, 13°45′35.70″N 120°55′34.20″E by Michele Weber.

There are a couple of photos on the Nudipixel website of this species (<*http://www.nudipixel.net/photo/00034049*/> and <*http://www.nudipixel.net/photo/00034646/*>). These photos were taken from Anilao within approximately one month of when the specimens described here were collected.

Geographical distribution.— Currently known only from Balayan Bay, in the Anilao region of the Philippines.

Etymology.— *Gymnodoris brunnea* is named for its brownish coloration.

External morphology.— Several preserved specimens were subsampled for DNA before being measured and ranged between 5.0 and 8.0 mm in length with a subsection of the posterior end of the "tail" missing. Two intact preserved specimens were about 9.5 mm in length. The living animals (Fig. 1A, B) are translucent with a brownish tinge. Upon close inspection of the preserved specimens, this brownish hue appears to come from elongate dark particles present throughout the tissue of the animal. These dark particles appear to be generally concentrated on the dorsal side of the animal. The sides and dorsum of the body are covered in deep orange, conical pustules. These pustules are present on the anterior portion of the notal margin. Starting from the anterior margin, these pustules are situated in two rows following the mantle margin posteriorly and converge to a point roughly halfway between the gills and the tip of the posterior end of the foot. From here, the pustules continue in a row to the tip of the foot. Pustules can also be found between the rhinophores. In some of the preserved specimens, the very tip of the foot is keeled.

The foot is broad, widest anteriorly beneath the rhinophores. The foot has a thick anterior margin with a deep groove that separates the foot from the rest of the head. The anterior margin is tinged in deep orange. The rhinophores are perfoliate with approximately 8–9 lamellae and are tipped with orange. In some specimens, the orange runs down the anterior portion of the rhinophores, and wraps slightly around the sides, where it gradually fades. In some of the preserved specimens, the rhinophores were partially retracted and thus some lamellae were not visible.

The genital opening is located on the right side of the body, just anterior to the gill plume. The gill branches are arranged in a linear row or slight arc with between 4-7 unipinnate filaments. The filaments are tinged with a deep orange color with deeper pigmentation apically, when present. This coloration varies between specimens. In some specimens, the orange pigment is quite concentrated and may cover much of the gills; in others, the orange pigment covers only the apical portion of the gills. One specimen had a negligible amount of orange on the gills. The length of the gill filaments also varies.

Due to the translucent nature of the skin, the internal organs can be viewed externally. There is a white-colored patch visible just below the skin that extends forward and behind the gill, covering a portion of the dorsal side of the digestive gland. The function of this white patch is unknown. The ovotestis appear to be a light orange in color and appear to be located in various parts of the animal, including a section towards the posterior tip of the foot, approximately halfway between the gills and tip of the foot. The intestine is also visible through the skin as a dark colored arch anterior to the gill.

Internal Anatomy.— Gland-like structures are present at the base of the gill (one specimen had 6 of them), which may be defensive glands. In the preserved material, we were unable to locate the white patch that was visible in the living specimen.

Radular morphology: As is typical within *Gymnodoris*, the radula lacks a rachidian row of teeth (Fig. 2A). This species has very large, broad, inner lateral teeth, roughly hook-shaped, with very long bases (Fig. 2B). The inner lateral teeth are much larger and longer than the rest of the teeth. The second inner lateral teeth are much shorter in length than the first inner laterals (Fig. 2B). The second inner lateral teeth are broad and bicuspid with the second cusp smaller than the first and situated on the outer side of the main cusp. In a couple of specimens, a few of the inner laterals have the second cusp reduced to a small bump. There is a notch in the middle of the base of the second inner laterals as well as the other laterals. The mid and outer lateral teeth are much narrower than the second inner laterals and come to a sharp point. The outer laterals are gradually reduced in size approaching the outer margin of the radula. In some specimens, the third inner lateral teeth are slightly differentiated from the rest of the outer laterals and appear to have a wider base. In one specimen, one side of the radula had third inner laterals that were bicuspid, though this is likely a developmental abnormality. The outermost laterals are straighter in shape and the shortest of all of the teeth (Fig. 2C). The radular formulas of four specimens were counted as follows: 17 x 21.1.1.0.1.1.21 (CASIZ 185966), 19 x 21.1.1.0.1.1.21 (CASIZ 185968), 18 x 20.1.1.0.1.1.20 (CASIZ 185973), 22 x 21.1.1.0.1.1.21 (CASIZ 185963).

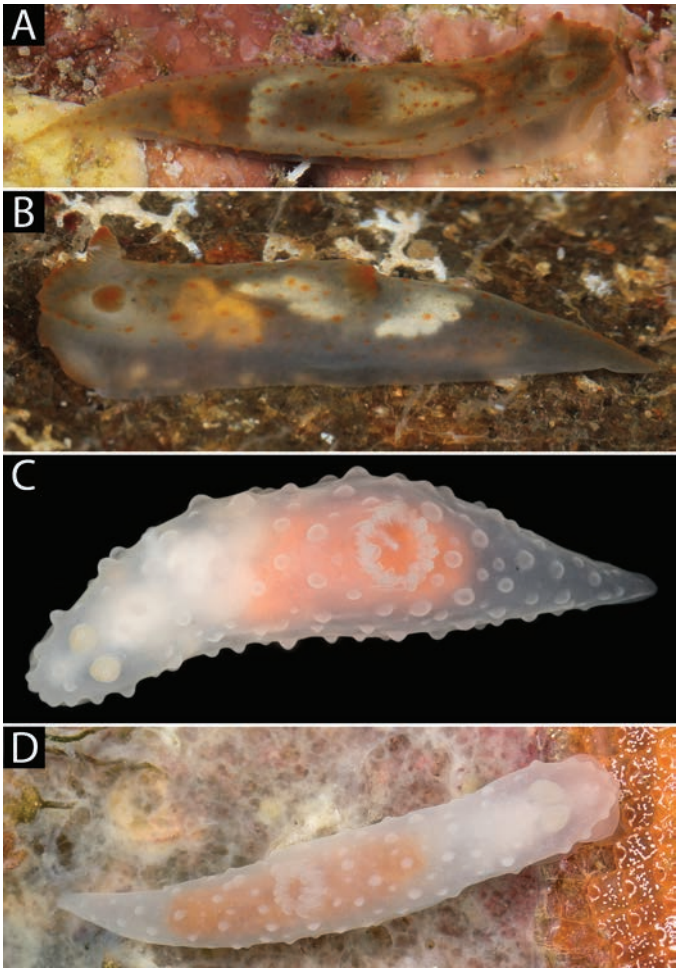

Figure 1. Photographs of living animals. A.- B. *Gymnodoris brunnea* sp. nov. C. *Gymnodoris tuberculosa* sp. nov. CASIZ 191397 D. *Gymnodoris tuberculosa* sp. nov. CASIZ 177268.

Reproductive system: There are four primary ovotestis follicles, three of which are united posteriorly with a single mass situated anteriorly. A hermaphroditic duct extends from the posterior mass of ovotestis follicles and unites with the hermaphroditic duct from the anterior mass (Fig. 3). Just anterior to their junction, the hermaphroditic duct widens into the curved ampulla. The ampulla then bifurcates into a short oviduct which enters the albumen gland within the female gland mass. The second branch widens into the curved prostate, which surrounds the bursa copulatrix and

then narrows into the muscular vas deferens. The vas deferens consists of several convolutions before entering the penis, adjacent to the vagina. The uterine duct emerges from the female glands near the oviduct and enters the small pyriform receptaculum seminis. The receptaculum duct enters the vagina just below the large, spherical, thin-walled bursa copulatrix. The vagina is curved and opens into the genital aperture. The albumen and membrane glands are small and not well differentiated. The mucous gland comprises the majority of the female gland mass. The penis of one specimen (CASIZ 185973) was cleared and stained (Fig 4A). The penis is densely armed with long spines, roughly 40 μm length, with a base of approximately 11 μm width.

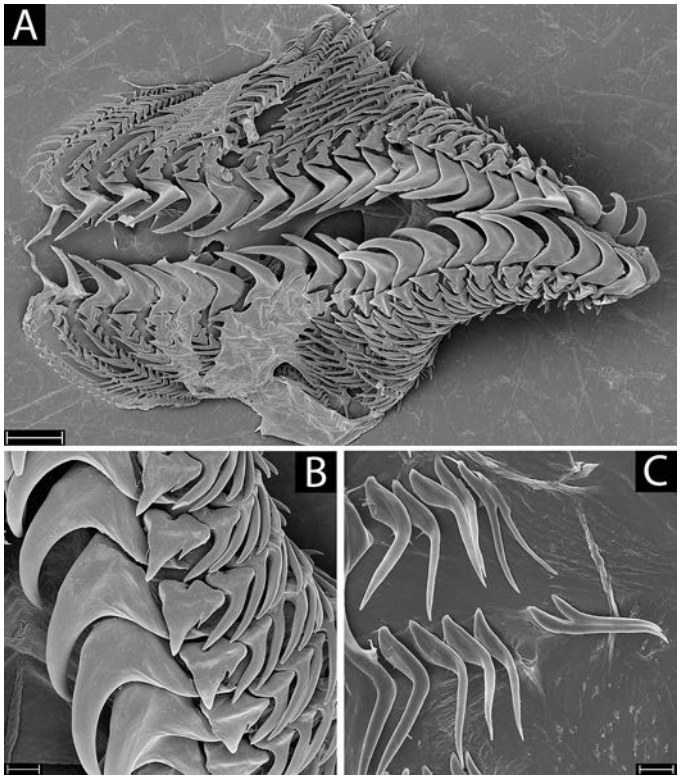

FIGURE 2. *Gymnodoris brunnea* sp. nov. scanning electron micrographs of the radula. A. View of entire radula. (Note that portionsof the radula on the right side of the image are folded underneath), CASIZ 185963, scale= 90 μm. B. Inner lateral teeth, note bicuspid second inner lateral, CASIZ 185963, scale= 20 μm. C. Outer lateral teeth, CASIZ 185968, scale= 10 μm.

NATURAL HISTORY.— Specimens were collected subtidally in 3-5 meters depth at night on a sandy bottom. Due to their sandy habitat, these animals may burrow into the sand.

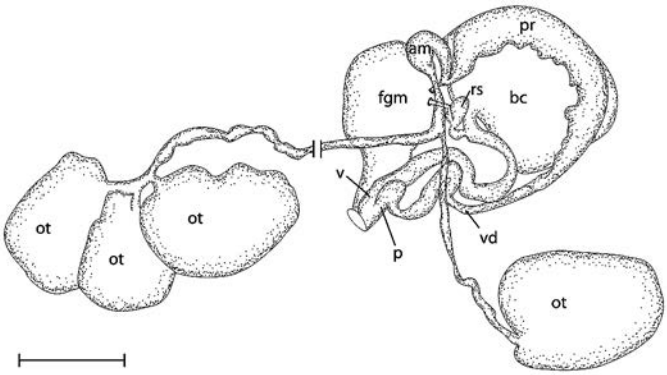

FIGURE 3. *Gymnodoris brunnea* sp. nov. reproductive system: scale bar = 1mm. Abbreviations: am, ampulla; bc, bursa copulatrix; fgm, female gland mass; p, penis; pr, prostate; rs, receptaculum seminis; v, vagina; vd, vas deferens; ot, ovotestis.

DISCUSSION.— Although some authors place gymnodorids that have a linear arrangement of gills into a separate genus, *Analogium,* Risbec 1928, (Hamatani 1995; Yonow 2011) there has never been a comprehensive phylogenetic study of this group to support the separation of these species into a separate genus (Jensen 1998). The radula of this specimen, along with the conical pustules that cover the body are more reminiscent of *Gymnodoris citrina* (Bergh, 1877) than of either of the two described species that have been placed in *Analogium*. Because of this, we place this species within *Gymnodoris*.

The overall body profile of *G. brunnea* is similar to the species depicted as *Gymnodoris marginata* (Odhner, 1917). *Gymnodoris marginata* also has gills arranged in a linear "bow." A major difference, however, is the position of the genital aperture, which is located well forward of that in *G. brunnea*. The radular teeth depicted in the *G. marginata* description are also very different in shape to those of *G. brunnea*, as the inner lateral of *G. marginata* looks nearly identical to the outer lateral depicted, whereas in *G. brunnea* the first and second inner lateral teeth are clearly differentiated from the outer laterals.

This species has only been documented from the Anilao region of the Philippines and it is unclear if it is restricted to this region or has a wider distribution. We have a photo of a *Gymnodoris* specimen from Japan that has a similar appearance to *G. brunnea*. This specimen appears to have a broader foot. It also does not seem to have the same dark brownish pigment to the skin and more subtle orange on the front margin of the foot. These animals would have to be further investigated via DNA or dissections to confirm if they belong to the same or a different, closely-related species.

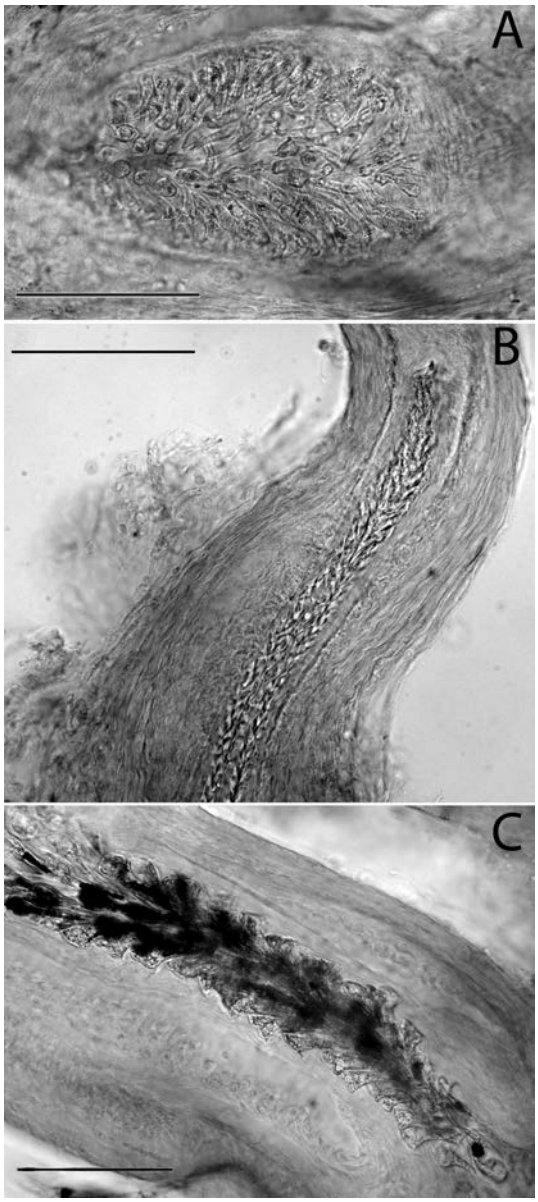

FIGURE 4. Light micrographs of the penial spines: scale bar=100 µm. A. *G. brunnea* sp. nov., CASIZ 185973B. *G. tuberculosa* sp. nov., CASIZ 185954. C. *G. pseudobrunnea* sp. nov., CASIZ 185967.

Gymnodoris tuberculosa Knutson and Gosliner, sp. nov.

Figures 1 C-D, 5, 6, 7

Gymnodoris sp. 3. Gosliner, et al., 2008: 149.

TYPE MATERIAL.— HOLOTYPE: CASIZ 191190, one specimen, "Kranket Fish Market Cove," near Madang Resort, Madang Province, Papua New Guinea, 5°12′28.4148″S, 145°48′31.9896″E, collected at night, November 13, 2012 by Vanessa Knutson, preserved specimen 6.0 mm length. PARATYPES: CASIZ 083765, one specimen, dissected, 18.6 m depth, Bonito Island, Batangas, Luzon Island, Philippines, collected February 22, 1992 by T.M. Gosliner, preserved specimen 8.3 mm length. CASIZ 086418, one specimen, dissected, 5.2 m depth, S. side of Rasch Passage, Madang, Papua New Guinea, collected June 17, 1992 by T.M. Gosliner, preserved specimen 11.0 mm length. CASIZ 177268, one specimen, 14.2 m depth, Layag Layag, Caban Island, Maricaban Island, Batangas Province, Luzon, Philippines, collected March 16, 2008, preserved specimen 4.0 mm

length with part of the "tail" missing. CASIZ 185954, one specimen, dissected, Cemetery Beach, Maricaban Island, Batangas Province, Luzon, Philippines, collected May 19, 2011 by Ditto de la Rosa, preserved specimen 9.0 mm length with part of the "tail" missing. CASIZ 191207, one specimen, Sek Island, Madang Province, Papua New Guinea, collected November 14, 2012 by Vanessa Knutson, preserved specimen 6.0 mm length with part of the "tail" missing. CASIZ 191461, one specimen, 6m depth, Lighthouse Point, Madang Province, Papua New Guinea, collected at night, November 28, 2012 by Anthony Berberian, preserved specimen 6.0 mm length with part of the "tail" missing. CASIZ 191294, two specimens, near Madang Resort, Madang Province, Papua New Guinea, 5°12′27.63″S 145°48′32.45″E, collected at night November 17, 2012 by Vanessa Knutson, preserved specimen "A" 5.2 mm length with part of the "tail" missing, preserved specimen "B" 3.5 mm length. CASIZ 191397, one specimen, Madang Province, Papua New Guinea, 5°12′1.08″S 145°48′45.66″E, collected at night, November 22, 2012 by Vanessa Knutson, preserved specimen 5.0 mm length. CASIZ 191477, one specimen, Tab Island, Madang Province, Papua New Guinea, collected November 30, 2012, by Vanessa Knutson, preserved specimen 1.7 mm length. CASIZ 191094, one specimen, Kranket Fish Market Cove, near Madang Resort, Madang Province, Papua New Guinea, 5°12′28.4148″S, 145°48′31.9896″E, collected at night, November 9, 2012 by Anthony Berberian, preserved specimen 5.0 mm length with part of the "tail" missing. CASIZ 191518, one specimen, dissected,1.5 m depth, SW Kranket Island, Madang Province, Papua New Guinea, collected on December 4, 2012 by T.M. Gosliner, preserved specimen 8.0 mm length with part of the "tail" missing.

Etymology.— This species is named for the tubercles covering its body.

Distribution.— This species has been found in Papua New Guinea, the Philippines and documented from the Marshall Islands (Gosliner et al. 2008). There is also a photo on the Nudipixel website showing this species from Raja Ampat, Indonesia.

External morphology.— The length of the preserved specimens varies from 1.6 mm to 11.0 mm. Some of these were subsampled for DNA analysis prior to measurement (CASIZ 177268, CASIZ 185954, CASIZ 191207, CASIZ 191461, CASIZ 191294A, CASIZ 191094, CASIZ 191518). The living animals (Fig. 1C, D) are uniformly translucent white in color and covered in small, rounded tubercles. While these tubercles are obvious in the living animal, they are not always apparent in preserved specimens, depending on the method of preservation. The tubercles seem to preserve well when fixed with Bouin's solution, but not always when fixed with alcohol. The internal organs can be seen through the translucent skin. The color of the digestive gland varies and has been observed as bright orange to pink to brown, perhaps due to the diet of the animal.

The rhinophores are lamellate with between 8 and 13 lamellae and range in color from translucent white to cream or pale yellow. The gill branches are the same color as the body of the animal and consist of approximately 9–13 main branches arranged in a complete circle with the anus located at the center. They are located close to the center of the length of the animal in the posterior half of the body. In preserved specimens, the gill may appear to be in the posterior third of the body. The genital opening (Fig 5) is located on the right side of the animal approximately one third of the length of the distance between the back of the rhinophores to the gills. There is a deep groove in the anterior portion of the foot, ventral to the mouth.

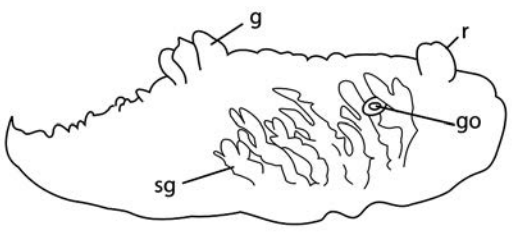

Figure 5. *Gymnodoris tuberculosa* sp. nov. arrangement and position of subcutaneous glands in preserved specimen, CASIZ 086418. Abbreviations: g, gills; go, genital opening; r, rhinophores; sg, subcutaneous glands.

The specimens examined have spicules

embedded in the skin. The spicules can be seen through the skin in some of the photos and are present in the preserved specimens. These spicules vary greatly, in one specimen, CASIZ 191461 there are spicules that have a pink tinge to them while others appear colorless. There also appears to be different morphologies for the spicules within a specimen. Some of the larger spicules were "T" or "Y"-shaped and approximately 0.2 mm long, others were smaller and rod-shaped.

Internal Anatomy.— On the ventrum and sides of the animal there are slightly branched, thickened glandular structures (Fig. 5). These structures are white to pale yellow in color and appear to originate at the foot and come up the sides of the animal. Although these structures are subcutaneous, they are clearly visible through the translucent skin in the living animal and in preserved specimens. These structures seem to be concentrated anterior to and at the level of the gills. The structures do not appear to be present in the posterior portions of the foot of the living animals. These structures are more pronounced in some specimens than in others. They resemble the subcutaneous glandular tubes described for *Gymnodoris aurita* (Gould, 1852), (Gosliner 1997), and presumably serve a defensive function.

Buccal and radular morphology: The buccal mass is muscular and has long strap-like salivary glands. Typical of the genus, the radula lacks a rachidian row of teeth (Fig. 6A). The inner lateral tooth (Fig. 6B) is a curved hook with a thickened, relatively straight base. The mid lateral teeth (Fig. 6C) are hook shaped with an elongate cusp. The outermost laterals (Fig. 6D) are thinner than the mid laterals and have a less pronounced base and a more elongate cusp. The radular formulas of four specimens were counted as follows: 20 x 20.1.0.1.20 (CASIZ 185954), 21 x 21.1.0.1.21 (CASIZ 191518), 21 x 20.1.0.1.20 (CASIZ 083765), 18 x 15.1.0.1.15 (CASIZ 086418).

Reproductive system: There are two ovotestis masses located anterior to the digestive gland and posterior to the genital mass. A hermaphroditic duct extends from each of the ovotestis follicles (Fig. 7). Just anterior to their junction, the hermaphroditic duct widens into the ampulla. The ampulla then bifurcates into a short oviduct which enters the female gland mass. The second branch widens into the curved prostate, which surrounds the bursa copulatrix and then narrows into the muscular vas deferens. The vas deferens consists of several convolutions and coils before entering the penis, adjacent to the vagina. The uterine duct emerges from the female glands near the oviduct and enters the small pyriform receptaculum seminis. The elongate receptaculum duct enters the vagina at the beginning of a

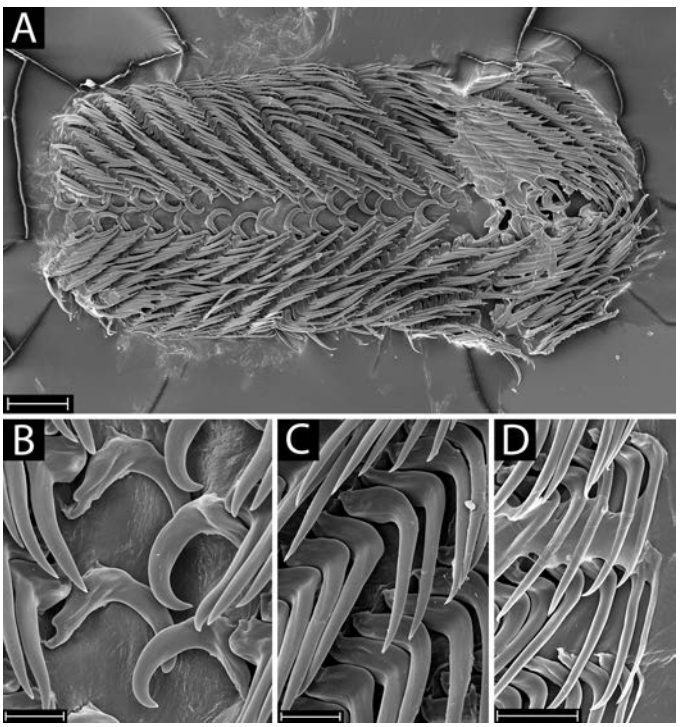

Figure 6. *Gymnodoris tuberculosa* sp. nov. scanning electron micrographs of the radula. A. View of the entire radula, CASIZ 185954, scale= 90 μm. B. Inner lateral teeth, CASIZ 185954, scale= 20 μm. C. Mid lateral teeth, CASIZ 185954, scale= 20μm. D. Outer laterals, CASIZ 086318 scale= 20μm.

greatly expanded, bulbous portion of the vagina just below the spherical, bursa copulatrix. The vagina again narrows and curves slightly before joining the genital opening. The albumen and membrane glands are small and not well differentiated. The mucous gland comprises the majority of the female gland mass. The penis of one specimen (CASIZ 185954) was cleared and stained (Fig 4B). The penis of this specimen is armed with small spines. The spines were difficult to distinguish due to their small size. These spines are estimated at a length of approximately 12 μm.

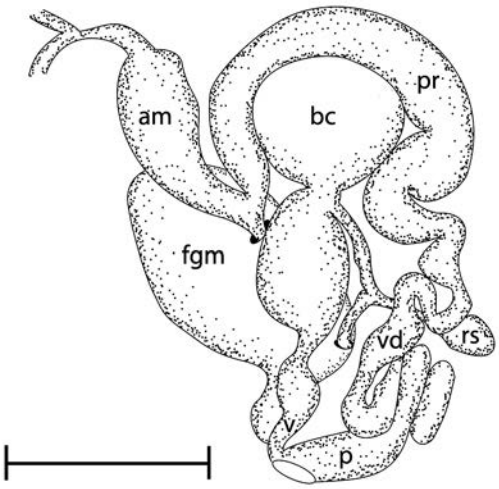

FIGURE 7. *Gymnodoris tuberculosa* sp. nov. reproductive system: scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; fgm, female gland mass; p, penis; pr, prostate; rs, receptaculum seminis; v, vagina; vd, vas deferens.

NATURAL HISTORY.— This species appears to be active at night, but is often found on the undersurface of coral rubble during the day.

DISCUSSION.— *Gymnodoris tuberculosa* differs from other described species of *Gymnodoris* by its general translucent white color, and the presence of translucent white tubercles. The other species of *Gymnodoris* that possess prominent tubercles have tubercles that are pigmented, often from yellow to orange to red in color such as *Gymnodoris aurita* (Gould, 1852), or *Gymnodoris ceylonica* (Kelaart, 1858). The vagina of this species has a wide bulbous portion similar to that depicted for *Gymnodoris inornata*, (Bergh, 1880). The depiction of part of the reproductive system of *Gymnodoris alba* (Bergh, 1877) has a smaller, less pronounced, but similar structure.

Gymnodoris pseudobrunnea **Knutson and Gosliner, sp. nov.**
Figures 8, 9, 10

TYPE MATERIAL.— HOLOTYPE: CASIZ 185974, one specimen, Anilao Harbor, Anilao, Balayan Bay, Batangas Province, Luzon Island, Philippines, collected May 4, 2011, by Michelle Weber, subsampled for DNA, preserved specimen 5.0 mm in length with part of the "tail" missing. PARATYPES: CASIZ 185960, one specimen, subsampled for DNA, preserved specimen 5.0 mm length with part of the "tail" missing, CASIZ 185962, one specimen, dissected, subsampled for DNA, preserved specimen 4.5 mm length with part of the "tail" missing, CASIZ 185967, one specimen, dissected, DNA subsampled, CASIZ 185969, one specimen, dissected, DNA subsampled, preserved specimen 6.2 mm in length with part of the "tail" missing, CASIZ 185981, one specimen, subsampled for DNA, preserved specimen 3.0 mm length with part of the "tail" missing, all collected at Anilao Harbor, Anilao, Balayan Bay, Batangas Province, Luzon Island, Philippines, May 4, 2011, by Michelle Weber, except CASIZ 185981, which was collected by Alexis Principe.

ETYMOLOGY.— This species is named for its similarity in appearance to *G. brunnea.*

GEOGRAPHICAL DISTRIBUTION.— Currently known only from the type locality, the Anilao region in the Philippines.

EXTERNAL MORPHOLOGY.— Preserved specimens were subsampled for DNA before they were measured and ranged 3.0–6.2 mm in size with a portion of the tail missing. Living *G. pseudobrunnea* (Fig. 8A,B) are translucent and may have a slight brownish tinge, but the brownish coloration is relatively subtle, if present. The foot is broad. The sides and dorsum of the body are cov-

ered in deep orange, conical pustules. These pustules are present on the anterior portion of the notal margin. Starting from the anterior margin, these pustules are situated in two diffuse rows following the mantle margin posteriorly and converge towards the posterior tip of the foot. It is difficult to tell from some of the photos of specimens where the pustules converge and it may be that the pustules do not converge in some of the specimens. None of the living specimens observed appeared to have pustules between the rhinophores, which have approximately 7–8 lamellae and vary in coloration. Some specimens had rhinophores with a brownish tinge with orange at the tip, others were orange on the upper half of the rhinophore or nearly the whole rhinophore. One specimen had rhinophores that were deep orange-brown.

FIGURE 8. Photographs of living animals. A–B. *Gymnodoris pseudobrunnea* sp. nov.

Specimens had between 7 and 11 unipinnate gill filaments arranged in a linear row or in as light arc anterior to the anus. The filaments may be tinged with a deep orange color, though this coloration varies between specimens. In some specimens, the orange pigment is highly concentrated and may cover much of the gills; in others, the orange pigment covers only the apical portion of the gills. In others, there is a negligible amount of orange on the gills. The length of the gill filaments also varies.

Due to the translucent nature of the skin, the internal organs are visible externally. A bright white patch can be viewed below the skin and is located from the gill to about halfway to the posterior end of the foot. The margin of this white patch is irregular in shape and varies between specimens. No dark brown intestine can be seen through the skin in this species. The genital aperture is located forward of the gills at approximately the same level as the pericardium.

INTERNAL ANATOMY.— *Buccal and radular morphology*: The buccal mass is muscular and the salivary glands are strap-like. Typical of the genus *Gymnodoris*, the radula has no rachidian row of teeth (Fig. 9A). The innermost lateral teeth are large and hook shaped with a long base (Fig. 9B). The second inner lateral is not differentiated from the rest of the mid-laterals, which are long and pointed (Fig. 9B). The laterals decrease in size very gradually and the outer laterals are the smallest of the teeth (Fig. 9C). The radular formulae of two specimens were counted and approximated as follows: 14 x 15.1.0.1.15 (CASIZ 185962), 16 x 14.1.0.1.14 (CASIZ 185969). A third radula was prepared, but not all of the rows were visible, at least 9 rows could be counted x 12.1.0.1.12 (CASIZ 185967).

Reproductive system: There are two large main ovotestis masses, one located ventral-laterally on the right side to the digestive gland and the other on the left, posterior to the genital mass. A hermaphroditic duct (Fig. 10) extends from each of the ovotestis follicles; these unite into a single duct that widens into the swollen ampulla. The ampulla bifurcates into a short oviduct that enters the female gland mass. The second branch widens into the curved prostate, which surrounds the large

bursa copulatrix and narrows into the muscular vas deferens. The vas deferens consists of a convolution where it widens and enters the penis, adjacent to the vagina. The uterine duct emerges from the female gland mass and joins with the pyriform receptaculum seminis. The receptaculum duct enters the vagina midway between the bursa copulatrix and the genital aperture. The albumen and membrane glands are small and not well differentiated. The mucous gland comprises the majority of the female gland mass. The penis of one specimen (CASIZ 185967) was cleared and stained (Fig. 4C) and is armed with densely distributed spines that have a broad base (roughly 19μm across). The spines appear to be longer at the base of the penis, roughly 67 μm, and shorter at the very tip, roughly 31μm.

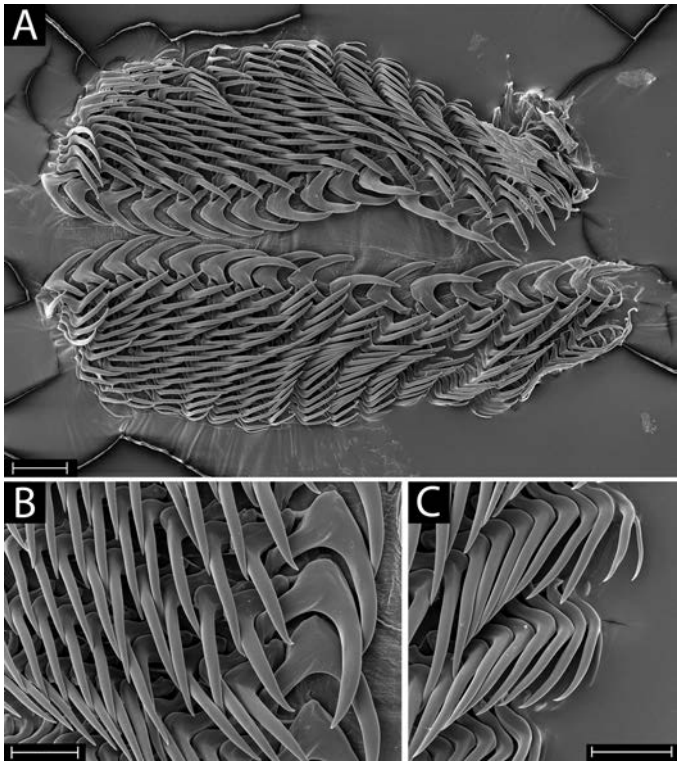

FIGURE 9. *Gymnodoris pseudobrunnea* sp. nov. scanning electron micrographs of the radula. A. View of the entire radula, CASIZ 185969, scale = 60 μm. B. Inner and mid lateral teeth, CASIZ 185969, scale = 30 μm. C. Outer lateral teeth, CASIZ 185969, scale = 30 μm.

NATURAL HISTORY.— This species was collected at night from a sandy bottom and likely burrows in the sand. One of the specimens dissected (CASIZ 185967) had a minimally digested nudibranch in its stomach. The nudibranch pulled from the stomach had a dark coloration concentrated on the dorsal side, consistent with *G. brunnea.* After imaging the radula of this nudibranch (Fig. 11) with a scanning electron microscope, it is clear that it is consistent with that of *G. brunnea* with large, hook-shaped inner laterals and distinctive bicuspid second lateral teeth. Due to the early stage of digestion of the stomach contents, it is possible that feeding took place following collection, if the two specimens were placed in the same container at the time of collection. If this is what occurred, one may

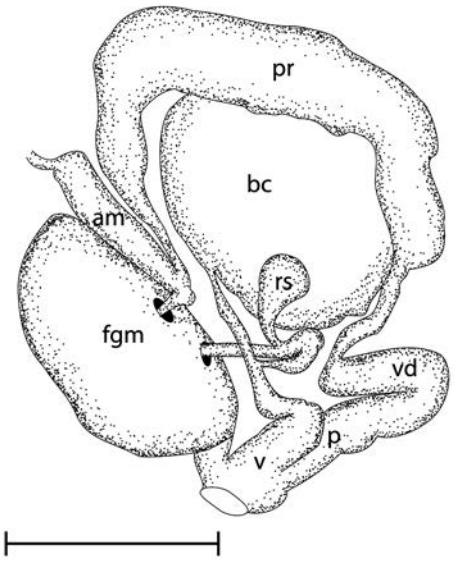

FIGURE 10. *Gymnodoris pseudobrunnea* sp. nov. reproductive system: scale bar =1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; fgm, female gland mass; p, penis; pr, prostate; rs, receptaculum seminis; v, vagina; vd, vas deferens.

speculate that perhaps the unnatural conditions led to *G. pseudobrunnea* feeding on *G. brunnea*; however, it is not unusual for gymnodorids to feed upon congeners and even conspecifics (Nakano 2011). Initially, it was thought that cannibalism in *Gymnodoris* was induced by unnatural laboratory conditions (Young 1967); however, gymnodorids have exhibited cannibalism in their natural habitat (Johnson and Boucher 1983; Johnson 1992; Nakano 2011). This demonstrates that unnatural conditions don't necessarily lead to abnormal feeding behavior. Further, in the field we were careful to keep *Gymnodoris* specimens separated from each other and other opisthobranchs to prevent loss of specimens. It is therefore likely that *G. pseudobrunnea* preys upon *G. brunnea* under natural conditions, and that this most likely occurred prior to collection.

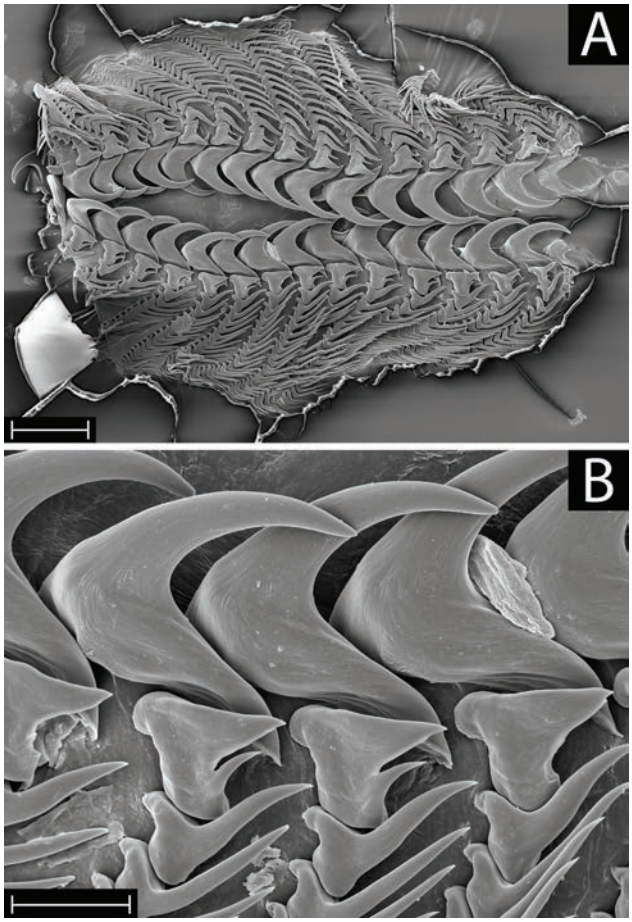

FIGURE 11. Scanning electron micrographs of the radula found inside of the digestive gland of a specimen of *G. pseudobrunnea* sp. nov. CASIZ 185967. A. View of the entire radula. B. Detail of the inner lateral teeth, note the bicuspid second lateral tooth.

DISCUSSION.— Externally, this species looks very similar to *G. brunnea*. Upon close examination, there are some subtle, but consistent differences in appearance between the two species. The features that best differentiate these species externally include the shape and position of the white patch visible through the skin on the dorsal side of the animals, and the external visibility of the intestine. The margin of the white patch is far more irregular in shape in *G. pseudobrunnea* than it is in *G. brunnea.* Also, in *G. pseudobrunnea,* the white patch appears at the gills and posteriorly, whereas in *G. brunnea,* the white can be seen forward of the gill and posteriorly. In *G. brunnea,* the intestine is visible through the skin as a dark arch; this does not appear to be visible in *G. pseudobrunnea.*

Internally, there are distinct radular differences and differences in the arrangement of reproductive organs. The inner lateral teeth of *G. brunnea* are much broader than those of *G. pseudobrunnea*. Also, the second inner lateral teeth of *G. brunnea* are broad and bicuspid, whereas those of *G. pseudobrunnea* are not differentiated from the rest of the middle and outer laterals. Further, in *G. pseudobrunnea*, the lateral teeth are longer relative to the inner laterals, than those in *G. brunnea*. The main difference between the reproductive systems is the position of the receptaculum seminis. In *G. brunnea*, the receptaculum seminis duct enters the vagina close to the bursa copulatrix, whereas in *G. pseudobrunnea* the receptaculum seminis enters the vagina approximately halfway between the bursa copulatrix and the genital aperture.

The morphological differences show clearly that these are two different species, and the molecular data presented here further corroborate their distinctiveness. The uncorrected COI p-distances within *G. brunnea* specimens ranged from 0.0% to 0.5%, representing intraspecific diversity. We were only able to successfully amplify the COI fragment for one specimen of *G. pseudobrunnea* and, although with only one specimen we cannot measure any intraspecific genetic variation for this species, this one specimen ranges from 10.3–11.1% different in the COI fragment from the *G. brunnea* specimens. This is well in line with the mean COI divergence of Herbert et al. (2003), which found a mean divergence of 11.1 ± 5.1% for congeneric pairs within Mollusca, and above the 5.5% minimum p-distance cutoff applied in a recent paper that delineated sister species of aeolid nudibranchs (Carmona et al. 2013).

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APPENDIX

TABLE 1. Specimens used to compare uncorrected p-distances based on the COI gene for *G. brunnea* and *G. pseudobrunnea*.

Species	**Voucher**	**GenBank Accession number COI**
Gymnodoris brunnea, sp. nov.	CASIZ 185963	KJ396778
Gymnodoris brunnea, sp. nov.	CASIZ 185966	KJ396779
Gymnodoris brunnea, sp. nov.	CASIZ 185968	KJ396780
Gymnodoris brunnea, sp. nov.	CASIZ 185973	KJ396781
Gymnodoris brunnea, sp. nov.	CASIZ 185975	KJ396782
Gymnodoris brunnea, sp. nov.	CASIZ 185976	KJ396783
Gymnodoris brunnea, sp. nov.	CASIZ 185979	KJ396784
Gymnodoris pseudobrunnea, sp. nov.	CASIZ 185967	KJ396785