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Functional Morphology of the Luminescence System of *Siphamia versicolor* (Perciformes: Apogonidae), a Bacterially Luminous Coral Reef Fish

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ABSTRACT Previous studies of the luminescence system of *Siphamia versicolor* (Perciformes: Apogonidae) identified a ventral light organ, reflector, lens, duct, and a ventral diffuser extending from the throat to the caudal peduncle. The control and function of luminescence in this and other species of *Siphamia*, however, have not been defined. Morphological examination of fresh and preserved specimens identified additional components of the luminescence system involved in control and ventral emission of luminescence, including a retractable shutter over the ventral face of the light organ, contiguity of the ventral diffuser from the caudal peduncle to near the chin, and transparency of the bones and other tissues of the lower jaw. The shutter halves retract laterally, allowing the ventral release of light, and relax medially, blocking ventral light emission; topical application of norepinephrine to the exposed light organ resulted in retraction of the shutter halves, which suggests that operation of the shutter is under neuromuscular control. The extension of the diffuser to near the chin and transparency of the lower jaw allow a uniform emission of luminescence over the entire ventrum of the fish. The live aquarium-held fish were found to readily and consistently display ventral luminescence. At twilight, the fish left the protective association with their longspine sea urchin, *Diadema setosum*, and began to emit ventral luminescence and to feed on zooplankton. Ventral luminescence illuminated a zone below and around the fish, which typically swam close to the substrate. Shortly after complete darkness, the fish stopped feeding and emitting luminescence. These observations suggest that *S. versicolor* uses ventral luminescence to attract and feed on zooplankton from the reef benthos at twilight. Ventral luminescence may allow *S. versicolor* to exploit for feeding the gap at twilight in the presence of potential predators as the reef transitions from diurnally active to nocturnally active organisms. J. Morphol. 272:897–909, 2011.

KEY WORDS: Apogonidae; bioluminescence; light organ; *Siphamia*; symbiosis

INTRODUCTION

Cardinalfish (Apogonidae) are a species-rich group of small, mostly tropical and subtropical coral reef-dwelling fish. As nocturnal zooplanktivores, cardinalfish typically remain in protective associations with echinoderms and corals during the day. Most or all species are paternal mouth brooders. Members of certain apogonid genera, *Archamia*, *Jaydia*, and *Rhabdamia*, are autogenously bioluminescent, producing light from their own luciferase and using luciferin apparently acquired in the diet. Light organs of these luminous apogonids are protrusions of the intestine or form from pyloric caeca (Kato, 1947; Iwai and Asano, 1958; Eibl-Eibesfeldt, 1961; Breder and Rosen, 1966; Tsuji and Haneda, 1966; Allen, 1972; Herring and Morin, 1978; Thresher, 1984; Gon, 1996; Nelson, 2006; Thacker and Roje, 2009; Froese and Pauly, 2010).

Unique among luminous apogonids, however, are members of *Siphamia*, which in contrast to autogenously luminous species use luminescent bacteria for light production. The examined species of *Siphamia* bear a ventral light organ, anterior to the pelvic fins, that contains a large population of luminous bacteria. The blue-green light produced by the bacteria is dispersed over the ventrum of the fish via translucent musculature (Eibl-Eibesfeldt, 1961; Tominaga, 1964; Haneda, 1965; Yoshiba and Haneda, 1967; Iwai, 1971; Herring and Morin, 1978; Fishelson et al., 2005; Nelson, 2006; Thacker and Roje, 2009; Froese and Pauly, 2010). A second light organ, at the anterior tip of the buccal cavity, has been reported for *Siphamia permutata*, *Siphamia cephalotes*, and *Siphamia cuneiceps* and is thought to function as a lure during feeding (Fishelson et al., 2005; Thacker and Roje, 2009). In *Siphamia versicolor*, development of the

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ventral light organ begins in larvae within a day after their release from the male's mouth; tissues making up the light organ arise from a proliferation and differentiation of intestinal epithelial cells (Leis and Bullock, 1986; Dunlap et al., 2009). The bacteria colonizing the ventral light organ of *S. versicolor*, which are extracellular and readily grow and luminesce in laboratory culture, have been identified by DNA sequence-based phylogenetic analysis as *Photobacterium mandapamensis* (Yoshiba and Haneda, 1967; Herring and Morin, 1978; Leis and Bullock, 1986; Wada et al., 2006; Kaeding et al., 2007).

Through the work of Iwai (1958, 1959, 1971) and others (Tominaga, 1964; Haneda, 1965; Fishelson et al., 2005), substantial descriptive information is available on the structure of the *Siphamia* luminescence system. The ventral light organ, a small, disc-shaped set of tissues, is composed primarily of chambers formed by epithelial cells and within which the symbiotic bacteria are housed. A reflector covers the dorsal surface of the light organ and is composed of an opaque layer of tissue containing iridiophores with guanine crystals and an outer layer of connective tissue containing melanophores. Passing dorsally from the light organ through the reflector and connecting posteriorly to the intestine is a duct composed of multiple tubules. Also passing through the reflector are blood vessels leading to a network of capillaries in the light organ. Ventral to the light organ is a pair of elipsoid bundles of transparent muscle tissue, referred to as a lens, and silver-white ventral translucent musculature making up a diffuser, which runs from the throat region to the caudal peduncle. The diffuser is composed of longitudinal muscle bundles sheathed in an epimysium of opaque fibrous connective tissue; it disperses the light from the light organ over the ventrum of the fish.

Despite the structural information available, key aspects of the luminescence system of *Siphamia* are not well understood. These include how the fish controls light emission and the function of luminescence. Control of light emission is thought to involve contraction and expansion of chromatophores in the skin covering the ventral diffuser, and reports of luminescence in *Siphamia* are limited to brief mention of ventral luminescence while swimming or being handled and buccal luminescence while feeding (Haneda, 1965; Fishelson et al., 2005). In this regard, examination of adult specimens of *Siphamia versicolor*, during an analysis of the brooding and development of their larvae (Dunlap et al., 2009), revealed the presence of previously unrecognized structural components of the luminescence system. In the course of that work it also became evident that the aquarium-held fish readily and consistently display luminescence. We therefore undertook and report here a more detailed analysis of the structural components of the luminescence system of *S. versicolor* involved in the production and control of emission of ventral luminescence and its function as assessed through extended observations of live, light-emitting fish.

MATERIALS AND METHODS

Collection and Maintenance of Fish

Specimens of *Siphamia versicolor* were collected in association with the longspine sea urchin, *Diadema setosum*, from coral reefs at 2- to 4-m depth in the Motobu Peninsula area of northern Okinawa main island, Okinawa, Japan, using snorkeling and scuba. The fish were transferred with their urchin to 60-l glass aquariums with flowing natural seawater and aeration and were maintained under ambient natural light and temperature conditions in a tall, panoramically windowed aquarium building. Aquarium seawater temperature and salinity ranged from 25°C to 30.5°C and 34 to 35 ppt, respectively. Adult and juvenile fish were fed daily on small crustaceans, fish, and other zooplankton obtained by plankton tow. Brooding males were recognized by the swollen, distended lower jaw. Survival of adult and juvenile *S. versicolor* under the conditions used was >95% for up to 2 months. Collection, care, and handling of fish were carried out in conformance with the University of the Ryukyus Guide for Care and Use of Laboratory Animals (Dobutsu Jikken Kisoku, version 19.6.26). Fish were anesthetized with 2-phenoxyethanol (ca. 0.2 ml l^{-1}) or by placement in crushed ice for 5 min. The work reported here was conducted in the spring and summer months of 2008, 2009, and 2010. We note here that although *S. versicolor* is commonly associated with the longspine sea urchin, *D. setosum*, we also collected this fish in the Motobu Peninsula area of Okinawa in association with the similar appearing but short-spined diademid urchin, *Echinothrix calamaris*.

Species Identification

The fish examined in this study were identified as *Siphamia versicolor* based on data provided by Tominaga (1964) and on information provided by Froese and Pauly (2010) (see also Haneda 1965; Iwai 1971). Taxonomy of the genus *Siphamia*, however, is currently under revision (Gon et al., 2009), and the species epithet used here might change. For future taxonomic reference, specimens of the fish described here were deposited in the fish collection of the University of Michigan Museum of Zoology under catalog number UMMZ 248762 and the vertebrate collection of the Scripps Institution of Oceanography under catalog number SIO 10-98.

Morphological Analysis

Freshly sacrificed specimens and alcohol-stored specimens that had been preserved in 10% neutral buffered formalin in seawater were dissected and examined by light microscopy. For each of the following procedures, multiple specimens were examined; the structures and characteristics described were routinely and consistently observed. Some features of the luminescence system, however, were less readily evident in preserved material. For visual assessment of luminescence from fresh specimens and material, observations were made in a photographic darkroom after the observer had dark-adapted for 10 min or longer. For histological examination, specimens were fixed in standard Bouin's solution. Embedding, sectioning (3–5 μm), and staining with haematoxylin and eosin followed standard histological protocols; sections were examined with an Olympus BX50 microscope mounted with an Olympus DP70 digital camera. For thin sections (1 μm), specimens were fixed in a solution of 2% paraformaldehyde, 2.5% glutaraldehyde, and

0.1 mol l^{-1} sodium cacodylate buffer, embedded in resin, sectioned, and stained with toluidine blue. Tissues for examination by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were preserved in Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde, 0.1 mol l^{-1} sodium phosphate buffer; EM Sciences, Hatfield, PA) and stored at 4°C. For TEM, fixed tissues were washed in phosphate buffer, postfixed in buffered osmium tetroxide (1%) for 1 h, and then rinsed, dehydrated in ascending strengths of ethanol, infiltrated with propylene oxide, infiltrated with polyembed 812 epoxy resin, and polymerized. Ultrathin sections were mounted on slotted grids with a supporting membrane, double stained with lead citrate-uranyl acetate, and examined with a Philips CM-100 transmission electron microscope. For SEM, tissues were handled similarly through dehydration and then were treated with hexamethyldisilazane, allowed to dry, and then were mounted, sputter coated with gold and viewed on an Amray 1910 FE field emission scanning electron microscope at 5 KV. Digital images were collected with a Semicaps 2000A Imaging System. To test the effect of norepinephrine on retraction of the shutter, typically 20 μl of Ringer's solution (Young, 1933) containing norepinephrine at concentrations of 1, 10, and 100 μg ml^{-1} was topically applied to the exposed light organ; to remove the applied norepinephrine, preparations were subsequently rinsed with 0.5 ml of Ringer's solution. Most of the specimens examined in this study were adults, 20-mm standard length (SL) or greater, with reproductively mature gonads (Tominaga, 1964). Some juveniles (13.4- to 16.1-mm SL, immature gonads) were also examined; the luminescence systems of the smallest juvenile fish collected to date from association with *Diadema setosum* and *Echinothrix calamaris*, 13.4-mm SL, and all larger juvenile specimens were fully formed and appeared identical in structure and function to the luminescence system of adult fish (Dunlap et al., 2009).

Microbiological Analysis

Apical tips of the ventral diffuser were aseptically dissected from the fish, rinsed in buffered (25 mmol l^{-1} HEPES, pH 7.25) 70% seawater (BSW-70, filter-sterilized), and homogenized in 0.5 ml of BSW-70 in a sterile, hand-held tissue grinder. The homogenate was then spread or streaked onto plates of a nutrient seawater agar medium, LSW-70 agar (Kaeding et al., 2007), which contained per liter 10 g tryptone, 5 g yeast extract, 700 ml seawater, 300 ml deionized water, and 40 g of agar. The inoculated plates were incubated at room temperature (27°C–29°C) for 12–18 h and then examined in the dark for luminous colonies. Individual fecal strands, ca. 0.25 to 0.5 cm in length, freshly voided by the fish, were collected and either homogenized, diluted, and spread or directly streaked onto LSW-70 agar (40 g^{-1} agar).

Behavioral Observations

Behavioral observations were carried out on several (>25) independently collected groups of fish collected from the wild with their urchin and maintained in separate aquarium tanks. The luminescence displays and feeding activity described here were routinely and consistently observed after the fish had acclimated to the aquarium tanks for 1–2 days following their collection; observations were continued for up to 10 days. No behavioral differences were noted among the groups or between the behavior of juvenile and adult fish, except that smaller individuals sometimes left the urchin at dusk sooner than larger individuals. Fish were maintained under ambient natural light at all times except as noted below. Observations were made from 1 h before dusk to 2 h after sunset (circa 1 h after complete darkness), periodically during nighttime hours, from 1 h before dawn to 1 h after dawn, and periodically during daylight hours. To test the effect of changes in ambient down-welling light on the luminescence emitted by the fish, a weak light was reflected off of the gray-white cement ceiling (height of 6.4 m) of the aquarium building under otherwise dark conditions; for some observations a second somewhat stronger light was also used.

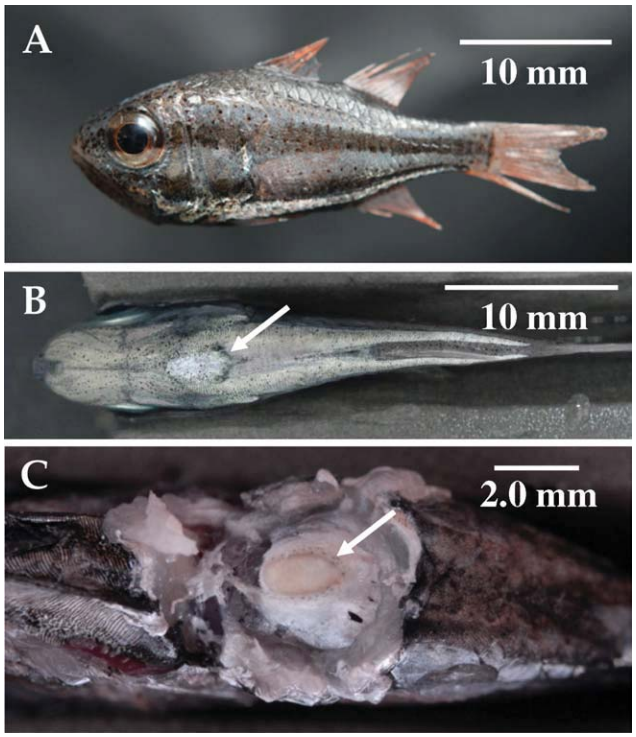

Fig. 1. The ventral light organ of *S. versicolor*. **A:** Lateral view of a 30-mm SL specimen. **B:** Ventral view of a similarly sized specimen; arrow indicates location of the light organ. **C:** Ventral view of a similarly sized specimen after removal of scales, skin, muscle, and other tissue ventral to the light organ to reveal the exposed (shutter mostly retracted) ventral surface of the light organ (arrow).

RESULTS

Morphological Components of the *Siphamia versicolor* Luminescence System

Light organ. The structural and functional core of the *Siphamia versicolor* luminescence system is the light organ, a disc-shaped set of tissues located just anterior to the pelvic fins (Figs. 1 and 2), above the ventral musculature and below the liver and other organs of the abdominal cavity (Iwai, 1958, 1971; Haneda, 1965). Evidence presented below (see Shutter section) indicates that the light organ sits on the dorsal surface of the tissue lining the ventral wall of the abdominal cavity and does not penetrate through the abdominal cavity lining. The light organ dissected from freshly sacrificed specimens (e.g., ~2.0 mm anterior to posterior, 1.8 mm left to right, and 0.2–0.3 mm dorsal to ventral in a 30-mm SL adult specimen) emits a uniform and intense blue-green light (Fig. 3A,B). The light organ is composed primarily of cuboidal epithelial cells forming columnar chambers (Fig. 3C,D). The chambers typically extend dorsoventrally and are

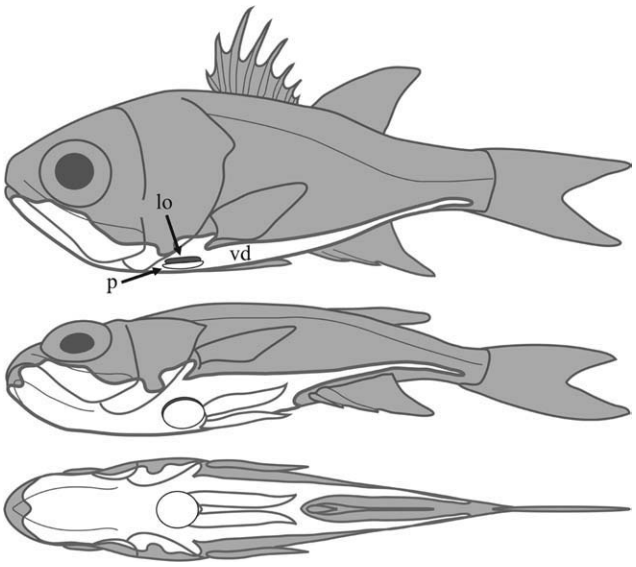

Fig. 2. Anatomical relationships of main components of the *S. versicolor* luminescence system. Shown are the light organ (lo), the transparent primary diffuser (p), and the ventral diffuser (vd). The ventral diffuser is contiguous from the caudal region to near the tip of the lower jaw. Not shown in this sketch are the reflector, which covers the dorsal face of the light organ, and the retractable shutter, which covers the ventral face of the light organ. The muscle tissue of the primary diffuser (see also Iwai, 1971) is similar to that of the ventral diffuser but appears somewhat less striated.

~200–300 μm in height and 20- to 30-μm in diameter. Several hundred chambers form the bulk of the light organs in adult fish. Many chambers join with adjacent chambers; i.e., they often are not completely separate from each other. Dorsally, the chambers coalesce into tubules that form the duct (see below under Reflector and Duct sections). The chamber lumina, ~14–21 μm in diameter, contain masses of bacterial cells (for bacterial light organ population sizes, see Dunlap et al., 2009). Chambers at the periphery of the light organ, however, which appear to be newly forming, often lack bacteria. A network of capillaries runs through the light organ (Fig. 3E,F). The ventral face of the light organ is covered by a thin, smooth, completely transparent membrane of connective tissue that is somewhat difficult to perforate. This membrane forms the ventral-most surface of the light organ; penetration through it allows access to the light organ chambers and capillaries, as evidenced by the release of bacteria and blood when the membrane is ruptured.

Reflector. As reported by Iwai (1958, 1971) for *Siphamia versicolor* and in greater detail for other *Siphamia* species by Fishelson et al. (2005), the dorsal face of the light organ of *S. versicolor* is covered by a cap-like structure, the reflector (Fig. 4A), which is composed of multiple layers. Directly over the dorsal face of the light organ is a thick silver-white layer containing iridiophores and masses of elongate, flat crystals. Dorsal to this layer is a silvery-white layer, spotted with black chromatophores and rich in needle-shaped crystals. Although the dorsal surface of the reflector sits flush with and looks identical to the dorsal-most lining of the abdominal cavity (Fig. 4B), the reflector remains attached to the light organ dissected from the fish and therefore is separate from the lining of the abdominal cavity (Fig. 4C). When the dissected light organ with the reflector attached is viewed from the dorsal perspective, no light is seen, whereas light is seen when the light organ is viewed from below (with the shutter, described below, retracted or removed). Therefore, the function of the reflector is to block dorsal emission of light from the light organ and redirect light ventrally.

Duct. The dorsally coalescing chambers of the light organ form into tubules that traverse through the reflector at approximately its midpoint and group together to form the duct (Fig. 5A). The duct then extends posteriorly to connect to the intestine. The duct appears mostly transparent or translucent, but the exterior surface of some portions is black due to the presence of melanophores (see Fig. 4B,C). Blood vessels that lead to the net-

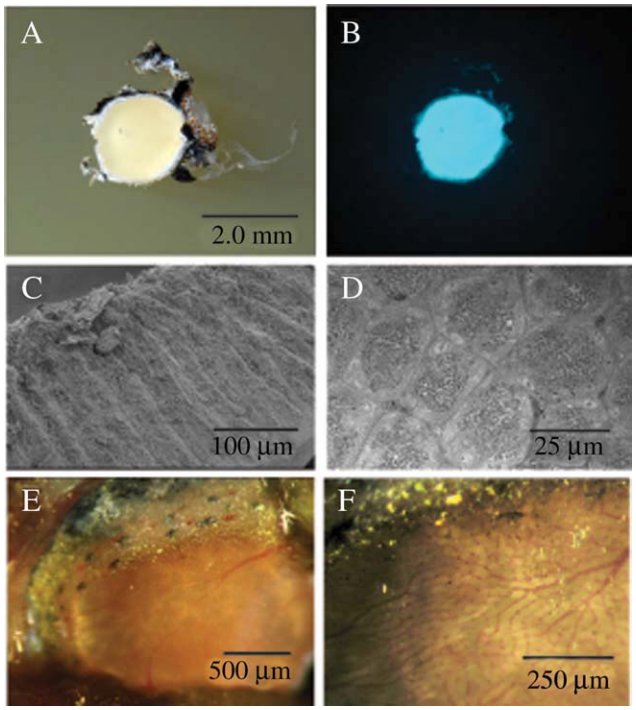

Fig. 3. Light organ of *S. versicolor*. The light organ, dissected from a freshly sacrificed specimen and photographed in the light **(A)** and in the dark **(B)**, emits a uniform and intense blue-green light. The light is produced by symbiotic luminous bacteria (see also Fig. 6D). The light organ is composed of cuboidal epithelial cells forming columnar chambers (**C**) [SEM]; (**D**) [TEM]. The chamber lumina, ~14–21 μm in diameter, contain masses of bacterial cells. A network of capillaries **(E,F)** [**F**, close up of **E**] runs through the light organ among and between the columnar chambers.

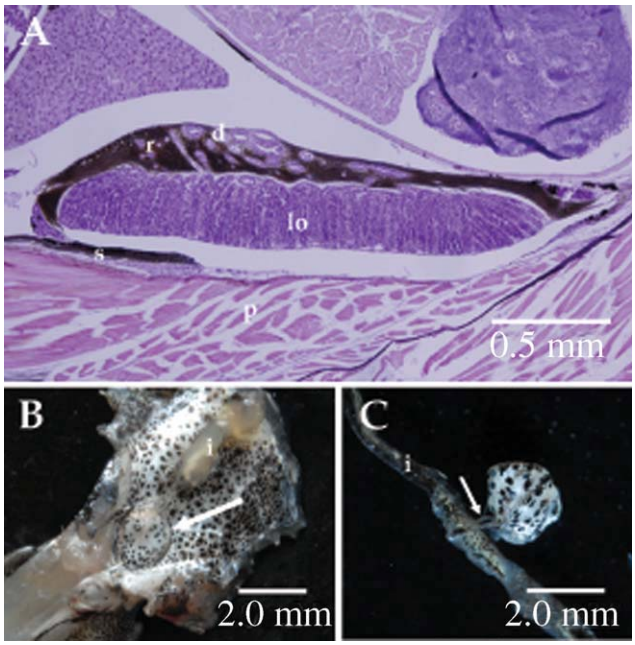

Fig. 4. Light organ reflector. **A:** Sagittal histological section, anterior to the right, through the light organ (lo) to show the reflector (r), which covers the dorsal surface of the light organ, the duct (d), which passes through the reflector, the partially retracted shutter (s), which covers the ventral face of the light organ, and the striated transparent muscle tissue of the primary diffuser (p) directly ventral to the light organ. **B:** View of the abdominal cavity lining from the dorsal perspective, showing the location of the light organ (arrow) with its silvery white cap-like reflector (i, intestine). Note the similarity in appearance of the dorsal lining of the abdominal cavity and the dorsal surface of the reflector. **C:** Relationship between the light organ and the intestine (i) and the connection between them via the duct (arrow), also visible in **(B)**, to show the separate nature of the reflector from the abdominal lining.

work of capillaries in the light organ also pass through the reflector (Fig. 5A,B). Nine to twelve tubules were observed in the *Siphamia versicolor* duct (Fig. 5B); each tubule therefore apparently forms from a coalescence of the lumina of many light organ chambers. As noted here and elsewhere (Iwai, 1959, 1971), the tubules are composed of cuboidal epithelial cells (Fig. 5B); they appear very similar to the cells making up the light organ chambers. Along with the tubules, the duct is composed of a matrix of epithelial cells different in structure from cells forming the tubules (Fig. 5B). Bacterial cells are present in tubules of the duct (Fig. 5B,C); the tubules therefore apparently function as conduits for the release of excess bacteria from chambers of the light organ into the intestine. Consistent with this function, freshly voided feces of the fish are strongly luminous (Fig. 6A,B) and contain high numbers of the symbiotic bacteria (Fig. 6C,D).

Shutter. Covering the ventral face of the light organ and sliding over the smooth ventral surface of the transparent membrane is a delicate layer of tissue, an eyelid-like shutter (Fig. 7A). The thinness of the shutter tissue and its delicate nature makes it easy to destroy during dissection. The shutter is composed of two equal halves that meet at the midline of the long, anterior to posterior, axis of the light organ. The shutter halves retract laterally, exposing the ventral face of the light organ, and relax medially, meeting at the midline of the light organ long axis and completely covering its ventral face. The ventral, outer-facing surface of the shutter is golden and silvery, due to the presence of reflective material, presumably guanine, embedded in the thin tissue, along with some orange and red chromatophores and black melanophores (Fig. 7A). Peripheral to the golden-silvery area, the shutter tissue is densely black. The inner,

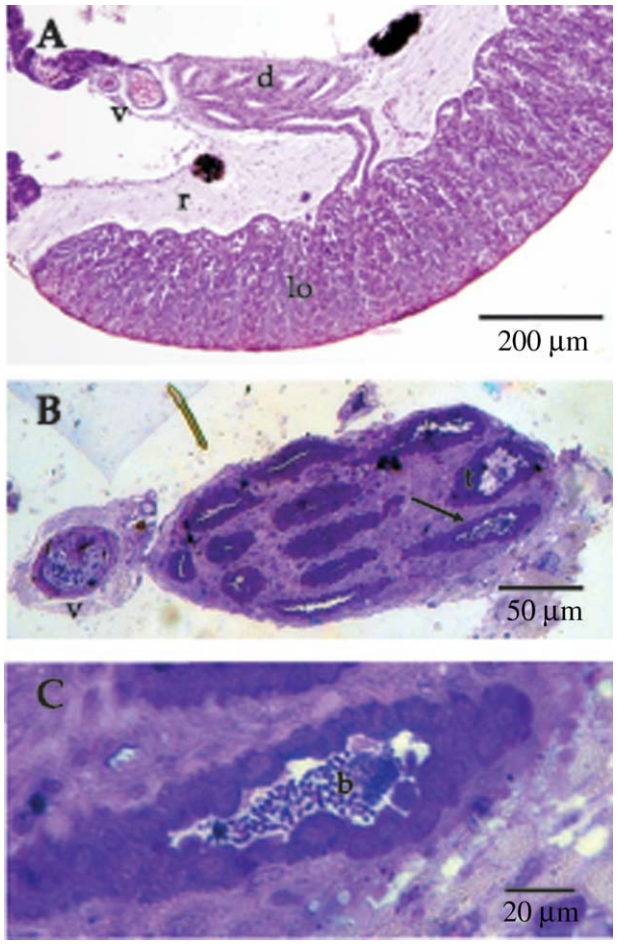

Fig. 5. Light organ duct. **A:** Histological section through light organ to show the duct as it exits the reflector (lo, light organ; r, reflector; d, duct; v, blood vessel). **B:** Histological section through the duct and blood vessel (v) at approximately the midpoint of the duct between the light organ and intestine. Twelve tubules (t) are evident, one of which contains a mass of bacteria (arrow). The tubules are composed of cuboidal epithelial cells that appear similar to those forming the chambers within the light organ. Note that in addition to the tubules, a matrix of epithelial cells forms the bulk of the duct. **C:** Close up of tubule showing the bacterial cells (b).

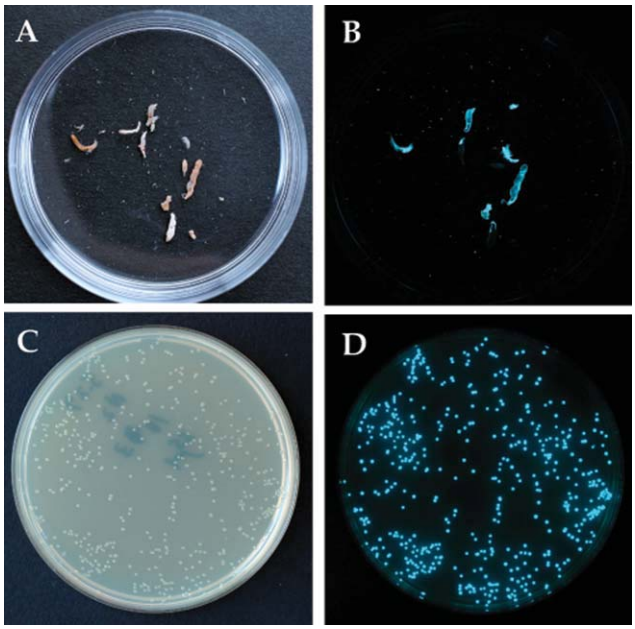

Fig. 6. Symbiotic bacteria in feces of *S. versicolor*. Intact fecal strands, collected shortly after they were voided from the fish, were photographed in the light **(A)** and the dark **(B)** [plate diameter, 50 mm]. Bacterial colonies, photographed in the light **(C)** and the dark **(D)** [plate diameter, 85 mm], arising on a seawater-based agar growth medium after circa 12 hours incubation at room temperature from the plating of a portion of one homogenized, serially diluted fecal strand. The luminous colonies arising from feces typically were identical in growth and luminescence characteristics to bacteria cultured from the *S. versicolor* light organ. Many hundred additional bacterial colonies, which were not luminous, arose on the plate within a further 24 hours of incubation.

dorsal-facing surface of the shutter is similar in appearance but with more black melanophores (Fig. 7B). With careful dissection, the shutter was found to be continuous with the silvery-white layer that covers the ventral surface of the abdominal cavity; the shutter therefore appears to be a localized modification of this tissue layer. This relationship indicates that the light organ does not penetrate through the lining of the abdominal cavity.

Examination in the dark of freshly sacrificed specimens of the fish in which the ventral face of light organ had been exposed in situ by removal of the tissues ventral to it (i.e., scales, skin with chromatophores, thin silvery-white membrane, layers of translucent muscle tissue) revealed that the relaxed, i.e., closed, shutter occludes the light from the light organ, whereas removal or manual retraction of the shutter halves allows the ventral emission of the light. Furthermore, topical application of norepinephrine (at concentrations of 1, 10, and 100 $\mu g\ ml^{-1}$) in fish Ringer's solution to the exposed light organ and surrounding tissue resulted in lateral retraction of the shutter halves (lower concentrations were not effective); complete opening took ~1–3 s depending on the preparation. Subsequent rinsing of the preparation with fish Ringer's solution without norepinephrine led to relaxation of the shutter; complete closing took ~3–5 s depending on the preparation. The tissue identified here as the shutter therefore functions to control the ventral release of light from the light organ, and its operation apparently is under neuromuscular control. A more detailed analysis will be necessary to determine if the tissue making up the shutter itself is contractile or if instead the retraction and relaxation of the shutter halves are controlled by attached muscle fibers.

Primary diffuser. Directly ventral to the shutter are two small gel-like masses of transparent muscle tissue. This tissue apparently is that referred to by Iwai (1958) as a lens; it functions, however, not to focus light but to disperse it, and it is therefore referred to here as the primary diffuser (Figs. 2 and 4A). Although in direct contact with muscle tissue of the ventral diffuser

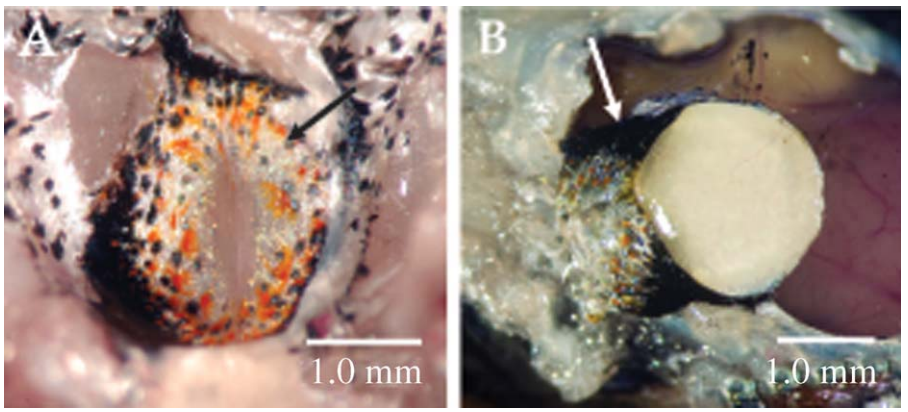

Fig. 7. Light organ shutter. **A:** Shutter (arrow) with tissues (scales, skin, primary diffuser) ventral to it removed. The shutter halves retract laterally to expose the ventral face of the light organs. **B:** Shutter pulled away from the light organ and inverted to show its dorsal face (arrow; anterior is to the right).

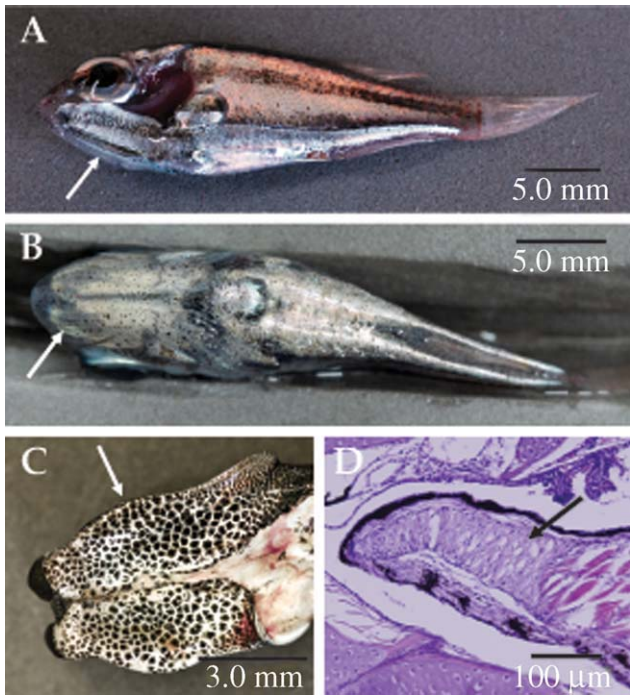

Fig. 8. Ventral diffuser. **A:** Contiguous nature of the ventral diffuser (arrow), which runs from near the chin posteriorly to near the caudal peduncle. **B:** Transparency of tissues forming the lower jaw. The anterior portion of the ventral diffuser, which runs along the floor of the mouth, can be seen through the transparent mandible (arrow) and other transparent tissues of the lower jaw. **C:** Anterior portion of ventral diffuser (dorsal view) dissected from the fish, showing the black apical tips and the flanges of tissue (arrow) that, within the buccal cavity, slide up to abut the ventromedial edge of the eye. **D:** Sagittal section through one apical tip, showing the transparent striated muscle tissue, which composes the bulk of the ventral diffuser, and the open lattice of tissue of the anteriormost tip of the ventral diffuser (arrow). No masses of bacteria are evident in this tissue.

(described below), the more gel-like, less distinctly striated nature of the primary diffuser tissue distinguishes it somewhat from the ventral diffuser. The position of this tissue, directly ventral to the light organ, and its transparency gives the primary diffuser tissue a slightly yellowish appearance when viewed from the ventral perspective. The function of the primary diffuser presumably is to capture ventrally emitted light from the light organ and disperse it into the surrounding, anteriorly extending and posteriorly extending musculature of the ventral diffuser.

Ventral diffuser. In *Siphamia versicolor*, the ventral diffuser, composed of transparent striated muscle tissue, extends over the entire ventrum of the fish, from near the tip of the chin at the floor of the buccal cavity posterior to the vent, where it then separates into two parallel extensions that reach the caudal peduncle (Fig. 8A,B). Previous reports for *S. versicolor* (Iwai, 1958, 1971) incorrectly show the ventral diffuser tissue extending anteriorly only as far as the throat region. The ventral diffuser extends anteriorly along the ventrum of the fish above the lower jaw nearly to the chin; the anterior part extends along the floor of the buccal cavity, with flanges that swell out near the anterior end at the position in the buccal cavity of the eyes (Fig. 8C). The muscle tissue of the ventral diffuser is essentially transparent, but it appears opalescent silvery-white due to its enclosure in a sheath (epimysium) of highly reflective silver-white translucent connective tissue. The muscle and sheath readily capture and translocate light. When white light is shined on muscle tissue exposed in the sheath, the light is transmitted and reflected, causing the tissue to glow an intense silver-white. The muscle and sheath tissue of the ventral diffuser make up a substantial portion of the body mass of the fish (Table 1).

Lower jaw. Because light was observed being emitted evenly over the entire ventrum of the fish (described below), from the caudal peduncle to the chin, we examined the bones and other tissues making up the lower jaw. The ventral diffuser, in extending anteriorly, runs above the lower jaw below the floor of the buccal cavity (Figs. 2 and 8). The mandible, the posterior-ventral part of the maxilla, the lower margin of the preopercle, the interopercle, and the branchiostegal membranes were found to be largely transparent in fresh specimens; the ventral diffuser is visible in the gular and chin regions from the flank and ventrum through these tissues (Fig. 8A,B). The transparency of the bones and other tissues of the lower jaw and gular region allows light from the ventral diffuser to shine ventrally from the fish in the area from the throat region to the chin.

Absence of a buccal light organ. A buccal light organ containing bacteria and formed by tissues at the apical tips of the ventral diffuser was reported for *Siphamia permutata* and *Siphamia cephalotes* (Fishelson et al., 2005). Apical tip tissues interpreted as a buccal light organ were described also for *Siphamia cuneiceps* (Thacker and Roje, 2009). To determine if a buccal light organ is present in *Siphamia versicolor*, we examined the apical tips of the ventral diffuser by various means. In fresh specimens, the apical tips, viewed from the front and above looking into the mouth of the fish, are black (Fig. 8C) and have an iridescent blue sheen under some lighting conditions. When examined in the dark, the tips exhibited no luminescence, either in the intact, freshly sacrificed fish or in freshly dissected material. Furthermore, when care was taken to avoid external contamination, no luminous bacteria were cultured from the fresh tissue. Histologically, the apical tip tissue is seen in sagittal sections to extend anteriorly from the ventral diffuser muscle as an open lattice of tissue (Fig. 8D) covered dorsally with a layer of black pigment. The appearance of this tissue is essentially the same as that shown for

TABLE 1. Relationship between body mass and mass of the luminescence system[a] in S. versicolor

Specimen[b]	SL (mm)	Body mass (g)[c]	Luminescence system mass (g)	Ratio (%)
1	28.4	0.641	0.061	9.5
2	29.6	0.805	0.065	8.1
3	32.0	1.056	0.087	8.2

[a]Primary and ventral diffusers (muscle and sheath) and light organ with reflector.
[b]Reproductively mature females.
[c]Average of three wet weight measurements; variation between measurements $< \pm 3\%$.

S. cuneiceps (Thacker and Roje, 2009). The layer of black pigment presumably functions to block the emission of light from the ventral diffuser into the buccal cavity. Neither chambers resembling those of the ventral light organ nor masses of bacterial cells indicative of a bacterial light organ were evident. Serial cross-sections from the tip of the jaw posteriorly confirmed this structure and the absence of bacterial cells. We conclude that this tissue is the anterior terminus of the ventral diffuser, not a light organ. Therefore, *S. versicolor* and other *Siphamia* species bear only a single, ventral light organ.

Novel structural modification of the eyes. Viewed from outside the head of the fish, the eyes of *Siphamia versicolor* appeared normal for teleost fish. The ventromedial portion of the eyes, however, visible within the buccal cavity of fresh specimens either by opening the mouth or by removing the lower jaw, exhibited a black-looking patch and a black-looking stripe (Fig. 9), referred to here as an ocular patch and stripe. At these locations, the normally present silver-white argenteum that covers the surface of the eye is absent. In formalin-preserved specimens, the ocular patch and stripe were difficult to discern. Careful examination of the buccal cavity of intact, fresh specimens through the mouth or through the operculum revealed that the lateral flanges of the ventral diffuser (Figs. 2 and 8C) slide up at the sides of the buccal cavity to a position that is at or close to the ventromedial surface of the eyes. This anatomical positioning places the edge of the flange close to the ocular patch and stripe. Consistent with the absence of the reflective argenteum, the ocular patch and stripe were found to be translucent, allowing the passage of light into the eye. The eyes of fresh specimens of nonluminous species of apogonids were found to lack the patch and stripe. We hypothesize that in *S. versicolor* light from the ventral diffuser, as a consequence of this positioning, shines directly into the ocular patch and stripe and allows the fish to detect and assess the intensity of its ventral light emission.

Luminescence Behavior of Siphamia versicolor. The extensive anatomical commitment of the fish to producing and controlling the emission of light described above suggests that luminescence plays a major role in the daily biology of fish. Previous reports of light emission in *Siphamia versicolor* and other *Siphamia* species, however, are limited to brief mention of ventral luminescence in the swimming fish or when the fish were handled and a report of buccal luminescence while feeding (Haneda, 1965; Fishelson et al., 2005). We noted in this regard that aquarium-held fish readily and consistently displayed luminescence. Therefore, to gain insight into how the fish uses the bacterial light produced in its light organ, we monitored the activity and behavior of aquarium-held adult and juvenile *S. versicolor* over several day–night cycles.

During daylight hours, the fish usually remained associated with the urchin (Eibl-Eibesfeldt, 1961; Tamura, 1982), facing inward toward the urchin test while holding position and moving about among the urchin's spines. The fish typically drew closer to the test, i.e., deeper into the spines, when the aquarium was approached. On infrequent occasions during the day, some individuals would leave the spines of the urchin for short distances and times, and in some of these instances, the fish were observed to capture and ingest prey (e.g., small fish) that had been swimming nearby.

Fig. 9. Modification of the argenteum of the eyes of *S. versicolor*. The lower jaw has been removed to allow a ventromedial view of the eyes. Upper arrow indicates the ocular stripe and lower arrow indicates the irregularly shaped ocular patch. The silvery argenteum, which otherwise covers the ventromedial surface of the eyes, is absent at the ocular patch and stripe.

They would quickly return to the urchin if the tank was approached.

The behavior of the fish changed with the onset of dusk. As ambient light began to decline, the fish more typically positioned themselves further out among the spines of the urchin, often facing away from the urchin test. At this time some individuals, typically the smaller individuals first, would hesitantly leave the urchin, moving a short distance out and then returning. As ambient light continued to decline, all the members of the group individually or together in small numbers would leave the urchin (with the exception of brooding males, which tended to remain among the spines of the urchin most or all of the time, day and night). At that time, the fish were observed to begin emitting light, a ventral glow. Initially, the ventral glow was difficult to discern and difficult to distinguish from the reflection of the weak ambient light from the somewhat silvery lower flanks of the fish. As the ambient light declined further, however, the ventrally emitted light became more distinct. Viewed from the side of the fish, the emitted light was seen to come from the ventrum and lower flank of the fish along its length, from chin to tail. When viewed from directly above the fish, in tanks having reflective bottoms, the dark silhouette of a fish swimming within a few centimeters of the tank bottom was seen to be surrounded by a luminous halo. When observed from below, the individual fish were seen as a uniformly luminous ellipse. Luminescence, although not continuous, generally was on during this time.

After leaving the urchin and while emitting ventral luminescence, the fish actively fed on live zooplankton (small crustaceans, fish, worms, etc.) provided in the aquarium tanks. The feeding behavior involved short runs (a few to several centimeters) and turns while taking prey items, with the ventral luminescence illuminating a zone below and around the fish. The fish mostly stayed within 2–15 cm of the tank bottom (water height in tanks ca. 30 cm), although they often moved higher up in the water column for brief periods to take prey. If the tank was approached or the observer moved, the fish tended to quickly go to the tank bottom or to reassociate with the urchin. The fish were observed to feed as individuals and often also in pairs or small groups swimming and luminescing together. Feeding and luminescence continued for ~45–90 min as ambient light continued to decline through twilight toward complete darkness. During this time, luminescence typically was emitted continuously, but often was turned off for one to a few seconds by individual fish. When darkness was near total, or shortly afterward, the fish tended to stop feeding, sometimes formed up in a closely associated group away from the urchin or reassociated with the urchin, and stopped luminescing.

Fish that had stopped emitting luminescence, however, could be stimulated to do so again and in the manner described above when a weak light was shown on them from above. Individual fish and groups of fish responded to the down-welling light with an onset of ventral luminescence that was essentially immediate. When the down-welling light ceased, the ventral luminescence of individual fish quickly declined and stopped over 1–3 s, and it resumed essentially immediately when the light was shown again. When the weak down-welling light was supplemented with a second somewhat stronger light for a few to several seconds, the ventral luminescence of the fish was seen to be stronger for a few seconds after this second light was switched off, and it then decreased back within a few seconds to the level initially seen with the single weaker light. The responsiveness of these fish to the amount of down-welling light generally disappeared after 20–30 min of complete darkness or was less evident or absent in fish that had reassembled with the urchin. The fish then typically remained dark for the rest of the night. During the night, however, some individuals were observed staying close to the tank bottom, away from the urchin. Weak down-welling light stimulated these individuals, which typically were dark, to emit ventral luminescence. As predawn ambient light began to increase, the fish reassembled with the urchin, if they had not already done so, and they typically stayed with the urchin for the remainder of the day. These observations suggest a strong relationship between ventral luminescence and feeding at twilight. No instances of buccal luminescence during feeding or at other times, no instances of flashing from the flank or ventrum, and no discrete ventral or flank spot of light on the fish were seen at any time.

DISCUSSION

Several newly identified morphological components contribute to the control and ventral emission of luminescence in the bacterially luminous apogonid fish *Siphamia versicolor*. In addition to the light organ, reflector, duct, primary diffuser (lens), and ventral diffuser previously identified in this and other *Siphamia* species (Iwai, 1958, 1959, 1971; Haneda, 1965; Tominaga, 1966; Fishelson et al., 2005), newly described here are the light organ shutter, the transparent membrane covering the ventral face of the light organ, the contiguous nature of the ventral diffuser, and the transparency of the tissues of the lower jaw. These components all work together to allow the fish to produce and control the emission of a uniformly even ventral luminescence. The substantial anatomical commitment of the fish to ventral light emission suggests that luminescence plays a major role in the daily life of fish. This view is supported by

observations of the luminescence behavior of the fish, which reveal a direct relationship between ventral luminescence and feeding at twilight. The structural and functional information presented here provides a foundation for studies of the behavioral ecology of this bacterially luminous coral reef fish and the ontogeny of its luminescence system.

Central to the control of light emission in *Siphamia versicolor* is the light organ shutter. The bacteria in the light organ apparently emit light continuously; the light organ dissected from the fish remains strongly luminous for hours, and no instance of a nonluminous light organ was encountered for this fish (personal observation). The opening and closing of the shutter therefore controls when and how much light the fish emits. A shutter has not been described for, but presumably also is present in, other species of *Siphamia*. The thin and delicate nature of the shutter, which is easily torn, retracted, or destroyed during routine dissection of the light organ, presumably accounts for it not having been identified previously. Haneda (1965) suggested that chromatophores in the skin covering the ventral diffuser, through their contraction and expansion, function to control light emission, but this apparently incorrect view was based on limited behavioral and anatomical observations of *S. versicolor* and on analogies with the luminescence systems of fish with light organs that lack shutters. In addition to *S. versicolor*, certain other bacterially luminous fish, i.e., leiognathids (Perciformes: Leiognathidae) and anomalopids (Beryciformes: Anomalopidae), bear light organs with shutters, the opening and closing of which is under neuromuscular control. Furthermore, the presence of shutters correlates with behavioral complexity in the use of the light, i.e., the ability in these fish to turn the emission of light on and off quickly and to adjust its intensity (Harvey, 1922; Hastings, 1971; Herring and Morin, 1978; Morin et al., 1975; McFall-Ngai and Dunlap, 1983, 1984; Dunlap and McFall-Ngai, 1987; Johnson and Rosenblatt, 1988; McFall-Ngai and Morin, 1991; Woodland et al., 2002; Sasaki et al., 2003; Sparks et al., 2005). The shutter in *S. versicolor* is contiguous with the tissue lining the ventralmost layer of the abdominal cavity; it therefore appears to be a localized modification of this tissue. One modification is the high density of silvery reflective material and the presence of orange and red chromatophores and black melanophores. Another is the division of this tissue into two halves that cover the ventral face of the light organ, together with the ability of each half to retract laterally to expose the ventral face of the light organ and to relax medially to occlude the light organ. The thin, transparent membrane covering the ventral face of the light organ provides a smooth surface over which the shutter halves glide. Preliminary observations suggest the shutter in *S. versicolor* is under neuromuscular control, but it is not yet clear if the shutter tissue itself is contractile or is pulled open by attached muscle fibers.

The chambers of the light organ function to house the symbiotic bacteria, which are extracellular. The network of capillaries running through the light organ among the chambers presumably supplies the bacteria with nutrients for reproduction and oxygen for luminescence and removes waste products of bacterial metabolism, either directly or by transfer from and to the cells that form the chambers. At this time, no information is available on the physiological and nutritional conditions of the light organ experienced by the bacteria except that those conditions are suitable for continuous light production.

The duct, which is formed by tubules arising from a coalescence of the light organ chambers, traverses the multilayered reflector and apparently provides the symbiotic bacteria with access to and egress from the light organ at different stages in the life history of the fish. At the larval stage, the newly forming duct tubules presumably allow entry of the symbiotic bacteria from the intestine into the nascent light organ to initiate the symbiosis (Haneda, 1965; Leis and Bullock, 1986; Dunlap et al., 2009). Some evidence supporting this function has been obtained from wild-caught and cultured larvae of *Siphamia versicolor*, but the available information is not yet conclusive (Leis and Bullock, 1986; Dunlap et al., 2009). After the symbiosis is established, the duct tubules serve as a conduit for release of excess bacteria from the light organ into the intestine and from there into the environment. Evidence supporting this function includes the presence of bacteria in the tubules (Fig. 5) and the presence of the symbiotic bacteria in the intestine of the adult fish (unpublished data of G. S. Holland, J. R. Paxton, and J. L. Reichelt, cited in Leis and Bullock, 1986) and in freshly voided feces (Fig. 6). Reproduction of the bacteria in the light organ and secretions from the chamber-forming cells might passively push excess bacterial cells and fluid into and through the duct tubules. Alternatively, the duct might undergo peristaltic contractions that move bacteria through the tubules to the intestine. It is not yet known, however, if the release of bacteria from the light organ is sporadic or continuous, or if it might be diurnal, as seen for the squid *Euprymna scolopes* (e.g., Lee and Ruby, 1994). The light organ itself does not appear to be contractile.

The contiguous nature of the ventral diffuser in *Siphamia versicolor*, extending from near the tip of the lower jaw to the caudal peduncle, differs from initial descriptions of the luminescence system of *S. versicolor*, which do not show the anterior portion of the ventral diffuser (Iwai, 1958, 1971). It differs also from the situation in

Siphamia permutata, in which the ventral diffuser apparently is divided into two separate portions, an anterior portion, from the throat region to the chin, and a posterior portion, from the hypobranchial region to the caudal peduncle (Fishelson et al., 2005). Furthermore, the bones and other tissues of the lower jaw and isthmus of *S. versicolor* are transparent. Together, the contiguous nature of the ventral diffuser from the caudal peduncle to the chin and the transparency of tissues of the lower jaw account for the even emission of luminescence over the entire ventrum of the fish. The striated muscle tissue and sheath that form the ventral diffuser are remarkable for their ability to translocate and disperse light. The ventral diffuser appears to operate as a leaky fiber optic light guide, one in which there is linear transmission anteriorly and posteriorly, substantial scattering, and ventral release of light.

We noted also that, in contrast to the situation reported for *Siphamia permutata*, *Siphamia cephalotes*, and *Siphamia cuneiceps* (Fishelson et al., 2005; Thacker and Roje, 2009), the ventral diffuser in *Siphamia versicolor* does not end apically in a buccal light organ. No evidence, visual, microbiological, or histological was found for a buccal light organ in *S. versicolor*. Furthermore, the similarity between the apical tip tissues of *S. versicolor* (Fig. 6D) and *S. cuneiceps* (Fig. 2A of Thacker and Roje, 2009) indicates that also in *S. cuneiceps* this tissue is not a light organ. This view is supported by the dissimilarity of the apical tip tissue in *S. versicolor* and *S. cuneiceps* to chambers forming the ventral light organ in *S. versicolor* or to tissues harboring bacteria in light organs of other bacterially luminous fish (e.g., Ahrens, 1965; Bassot, 1968; Bassot, 1975; Kessel, 1977; Tebo et al., 1979; Dunlap et al., 2008). It is further supported by the inability to see bacterial cells in this tissue and by the inability to culture luminous bacteria from this tissue. Furthermore, despite several hours of observations of live, light-emitting, feeding specimens of *S. versicolor*, no instance of buccal luminescence was observed. We conclude that *S. versicolor* and other *Siphamia* species bear only a single, ventral light organ.

With respect to the function of luminescence in *Siphamia versicolor*, ventral luminescence was emitted at twilight during feeding and not at other times. This relationship strongly suggests that *S. versicolor* uses ventral luminescence to attract and feed on zooplankton from the reef benthos at twilight. Although the observations here were made on fish held under aquarium conditions, which could yield non-natural behaviors, the consistent, daily emission of luminescence by the fish at twilight and its correlation with feeding argue that this is the natural behavior of the fish. We propose that ventral luminescence allows *S. versicolor* to exploit for feeding the gap at twilight in the presence of potential predators as the reef transitions from diurnally active to nocturnally active organisms. Ventral luminescence apparently serves in this fish as a source of light sufficiently strong to stand out against the background of weak ambient down-welling light at twilight and to thereby attract and make visible benthic zooplankton, but not so strong as to be seen by or to attract predators. The ability of the fish to increase and decrease ventral luminescence in response to stronger and weaker down-welling light, respectively, is consistent with this view. Presumably, other species of *Siphamia* use ventral luminescence for the same purpose.

Alternatively, ventral luminescence might function in *Siphamia versicolor* for counterillumination, which is thought to be a function of ventral luminescence in many fish (Herring and Morin, 1978). In leiognathids, for example, an open coastal water schooling fish, uneven ventral luminescence, produced from an internal, circumesophageal light organ, provides camouflage from predators by disrupting the silhouette of the fish viewed from below (McFall-Ngai and Morin, 1991). This function seems less likely in *S. versicolor*, however, because the fish remains close to the benthic substrate, emits a uniformly even ventral glow, and uses the light during feeding. Another alternative is luminescence signaling in reproduction (e.g., Morin et al., 1975; McFall-Ngai and Dunlap, 1983, 1984; Sasaki et al., 2003; Sparks et al., 2005). Courtship and spawning have not been observed in *S. versicolor*, however, so there is no evidence at this time for or against luminescence signaling in reproduction in this fish. Externally, males and females do not appear to be sexually dimorphic, except for a small conical papilla just anterior to the vent in males (Tominaga, 1964). Discrete spots or flashes of light, which may be indicative of luminescence-based reproductive interactions (McFall-Ngai and Dunlap, 1983; Sasaki et al., 2003), were not observed in *S. versicolor*. Behavioral studies of *S. versicolor* in the wild and additional studies of aquarium-held fish are needed to examine the possible involvement of luminescence in reproduction and other activities, such as predator avoidance.

The ability of the fish to turn on and off the ventral emission of light and to adjust its intensity in response to changing levels of down-welling light suggests that the fish can detect self produced luminescence. We note in this regard the clearing of ventromedial portions of the argenteum of the eyes of *Siphamia versicolor*. The ocular patch and stripe on each eye might allow light from the upturned flanges of the ventral diffuser to enter the eye. According to this scenario, the eye would receive light from two sources, the ambient environment, via the lens of the eye, and the light organ, via the upturned flange of ventral diffuser and the ocular

patch and stripe. Comparison of the levels of light from these two sources and integration of this information would then permit the fish to adjust the extent to which it opens and closes the light organ shutter, thereby adjusting the intensity of its ventral luminescence in response to changes in the intensity of down-welling light. The ocular patch and stripe of the eyes of *S. versicolor* may be novel for fish and other vertebrates. Apparently the only instance of a possibly similar structural modification is that of the cyprinodontid fish, *Poecilia reticulata*, a nonluminous species. The eyes of *P. reticulata* bear a black patch on the dorsal–ventral meridian passing through the optic nerve; the patch, however, is opaque, and its function is unknown (Kunz and Wise, 1977). An intriguing possible functional parallel to the situation in *Siphamia*, however, was described recently for the bacterially luminous sepiolid squid *Euprymna scolopes*; the light organ of the squid has photoreceptor capability (Tong et al., 2009), giving the squid the ability to detect the light produced by its symbiotic bacteria. Much additional work will be necessary with *S. versicolor* to determine if the ocular patch and stripe actually function to allow the fish to detect and adjust its ventral luminescence.

The more complete understanding of the functional morphology of the luminescence system of *Siphamia versicolor* presented here provides a foundation for examining the behavioral ecology of the fish in the natural reef habitat from the perspectives of light emission, feeding, reproduction, and predator avoidance. Detailed knowledge of the morphological components of the luminescence system also provides the necessary foundation for examining the ontogenetic formation of the luminescence system during development of the fish (Dunlap et al., 2009).

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LITERATURE CITED

Ahrens G. 1965. Untersuchungen am Leuchtorgan von *Leiognathus klunzingeri* (Steindachner). Z Wiss Zool 173:90–113.

Allen GR. 1972. Observations on a commensal relationship between *Siphamia fucolineata* (Apogonidae) and the crown-of-thorns starfish, *Acanthaster planci*. Copeia 1972:595–597.

Bassot JM. 1968. Les organs lumineux à bacteries symbiotiques du Téléostéen *Anomalops*. Données histologique. Bull Soc Zool France 93:569–579.

Bassot JM. 1975. Les organs lumineux à bacteries symbiotiques de quelques Téléostéens Leiognathides. Arch Zoo Exp Gén 116:359–373.

Breder CM Jr, Rosen DE. 1966. Modes of Reproduction in Fishes, Garden City, Natural History Press,941 p.

Dunlap PV, McFall-Ngai MJ. 1987. Initiation and control of the bioluminescent symbiosis between *Photobacterium leiognathi* and leiognathid fish. Ann New York Acad Sci 503:269–283.

Dunlap PV, Davis KM, Tomiyama S, Fujino M, Fukui A. 2008. Devlopmental and microbiological analysis of the inception of bioluminescent symbiosis in the marine fish *Nuchequula nuchalis* (Perciformes: Leiognathidae). Appl Environ Microbiol 74:7471–7481.

Dunlap PV, Kojima Y, Nakamura S, Nakamura M. 2009. Inception of formation and early morphogenesis of the bacterial light organ of the sea urchin cardinalfish, *Siphamia versicolor* (Perciformes: Apogonidae). Mar Biol 156:2011–2020.

Eibl-Eibesfeldt I. 1961. Eine Symbiose zwischen Fischen (*Siphamia versicolor*) und Seeigeln. Z Tierpsychol 18:56–59.

Fishelson L, Gon O, Goren M, Ben-David-Zaslow R. 2005. The oral cavity and bioluminescent organs of the cardinal fish species *Siphamia permutata* and *S. cephalotes* (Perciformes, Apogonidae). Mar Biol 147:603–609.

Froese R, Pauly D, editors. 2010. FishBase. World Wide Web electronic publication. Available at: www.fishbase.org, version, March, 2010.

Gon O. 1996. Revision of the cardinalfish subgenus *Jaydia* (Perciformes, Apogonidae, *Apogon*). Trans R Soc S Afr 51:147–194.

Gon O, Gouws G, Allen GR, Dunlap P. 2009. Preliminary phylogenetic analysis of the cardinalfish genus *Siphamia* (Perciformes, Apogonidae). Abstract, 8th Indo-Pacific Fish Conference, Fremantle, Western Australia.

Haneda Y. 1965. Observations on a luminous apogonid fish, *Siphamia versicolor*, and on others of the same genus. Sci Rep Yokosuka City Mus 11:1–12.

Harvey EN. 1922. The production of light by the fishes Photoblepharon and Anomalops. Carnegie Inst Publ 312:45–60.

Hastings JW. 1971. Light to hide by: Ventral luminescence to camouflage the silhouette. Science 173:1016–1017.

Herring PJ, Morin JG. 1978. Bioluminescence in fishes. In: Herring PJ, editor. Bioluminescence in Action. London: Academic Press. pp 273–329.

Iwai T. 1958. A study of the luminous organ of the apogonid fish *Siphamia versicolor* (Smith and Radcliffe). J Wash Acad Sci 48:267–270.

Iwai T. 1959. Notes on the luminous organ of the apogonid fish, *Siphamia majimai*. Am Mus Nat Hist 13:545–550.

Iwai T. 1971. Structure of luminescent organ of apogonid fish, *Siphamia versicolor*. Jap J Ichthyol 18:125–127.

Johnson GD, Rosenblatt RH. 1988. Mechanisms of light organ occlusion in flashlight fishes, family Anomalopidae (Teleostei: Beryciformes), and the evolution of the group. Zool J Linn Soc London 94:65–96.

Kaeding AJ, Ast JC, Pearce MM, Urbanczyk H, Kimura S, Endo H, Nakamura M, Dunlap PV. 2007. Phylogenetic diversity and co-symbiosis in the bioluminescent symbioses of *Photobacterium mandapamensis*. Appl Environ Microbiol 73: 3173–3182.

Kessel M. 1977. The ultrastructure of the relationship between the luminous organ of the teleost fish *Photoblepharon palpebratus* and its symbiotic bacteria. Cytobiology 15:145–158.

Kunz Y, Wise C. 1977. Regional differences of the argentea and sclera in the eye of *Poecilia reticulata* P. (Teleostei: Cyprinidontidae). Zoomorphologie 87:203–215.

Lee KH, Ruby EG. 1994. Effect of the squid host on the abundance and distribution of symbiotic *Vibrio fischeri* in nature. Appl Environ Microbiol 94:1565–1571.

Leis JM, Bullock S. 1986. The luminous cardinalfish *Siphamia* (Pisces, Apogonidae): Development of larvae and the luminous organ. In: Uyeno T, Arai R, Taniuchi T, Matsuura K, editors. Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fish. Tokyo, Japan: Japan Ichthyological Society. pp 703–714.

McFall-Ngai MJ, Dunlap PV. 1983. Three new modes of luminescence in the leiognathid fish *Gazza minuta* (Perciformes: Leiognathidae): Discrete projected luminescence, ventral body flash and buccal luminescence. Mar Biol 73:227–237.

McFall-Ngai MJ, Dunlap PV. 1984. External and internal sexual dimorphism in leiognathid fishes: Morphological evidence for sex-specific signaling. J Morphol 182:71–83.

McFall-Ngai MJ, Morin JG. 1991. Camouflage by disruptive illumination in leiognathids, a family of shallow-water, bioluminescent fish. J Exp Biol 156:119–137.

Morin JG, Harrington A, Nealson K, Krieger N, Baldwin TO, Hastings JW. 1975. Light for all reasons: Versatility in the behavioral repertoire of the flashlight fish. Science 190:74–76.

Nelson JS. 2006. Fishes of the world, 4th ed. Hoboken: John Wiley & Sons.

Sasaki A, Ikejima K, Aoki S, Azuma N, Kashimura N, Wada M. 2003. Field evidence for bioluminescent signaling in the pony fish, *Leiognathus elongatus*. Environ Biol Fish 66:307–311.

Sparks JS, Dunlap PV, Smith WL. 2005. Evolution and diversification of a sexually dimorphic luminescent system in ponyfish (Teleostei: Leiognathidae), including diagnoses for two new genera. Cladistics 21:305–327.

Tamura R. 1982. Experimental observations on the association between the cardinalfish (*Siphamia versicolor*) and the sea urchin (*Diadema setosum*). Galaxea 1:1–10.

Tebo BM, Linthicum DS, Nealson KH. 1979. Luminous bacteria and light emitting fish: Ultrastructure of the symbiosis. BioSystems 11:269–280.

Thacker CE, Roje DM. 2009. Phylogeny of cardinalfishes (Teleostei: Gobiiformes: Apogonidae) and the evolution of visceral bioluminescence. Mol Phylogenet Evol 52:735–745.

Thresher RE. 1984. Reproduction in Reef Fishes. Neptune City, NJ: TFH Publications.399 p.

Tominaga Y. 1964. Notes on the fishes of the genus *Siphamia* (Apogonidae), with a record of *S. versicolor* from the Ryukyu Islands. Jpn J Ichthyol 12:10–17.

Tong D, Rozas NS, Oakley TH, Mitchell J, Colley NJ, McFall-Ngai MJ. 2009. Evidence for light perception in a bioluminescent organ. Proc Natl Acad Sci USA 106:9836–9841.

Tsuji FI, Haneda Y. 1966. Chemistry of luciferases of *Cypridina hilgendorfii* and *Apogon ellioti*. In: Johnson FH, Haneda Y, editors. Bioluminescence in Progress. Princeton: Princeton University Press. p 137–149.

Wada M, Kamiya A, Uchiyama N, Yoshizawa S, Kita-Tsukamoto, K, Ikejima K, Yu R, Imada C, Karatani H, Mizuno N, Suzuki Y, Nishida M, Kogure K. 2006. *Lux*A gene of light organ symbionts of the bioluminescent fish *Acropoma japonicum* (Acropomatidae) and *Siphamia versicolor* (Apogonidae) forms a lineage closely related to that of *Photobacterium leiognathi* spp. *mandapamensis*. FEMS Microbiol Lett 260:186–192.

Woodland DJ, Cabanban AS, Taylor VM, Taylor RJ. 2002. A synchronized rhythmic flashing light display by schooling *Leiognathus splendens* (Leiognathidae: Perciformes). Mar Freshwat Res 53:159–162.

Yoshiba S, Haneda Y. 1967. Bacteriological study on the symbiotic luminous bacteria cultivated from the luminous organ of the apogonid fish, *Siphamia versicolor* and the Australian pine cone fish, *Cleidopus gloria-maris*. Sci Rep Yokosuka City Mus 13:82–84.

Young JZ. 1933. The preparation of isotonic solutions for use in experiments with fish. Publ Staz Zool Nap 12:425–431.