First *in situ* observation of an aphyonid fish (Teleostei, Ophidiiformes, Bythitidae)

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Abstract

Aphyonids are poorly-known, live-bearing brotulas (Ophidiiformes, Bythitidae) that until recently were considered to be in a distinct family, Aphyonidae. A single, ca. 9.3 cm total length aphyonid observed during a remotely-operated vehicle survey in the Mariana Archipelago at 2504.2 m on Explorer Ridge (20.68152°N, 145.08750°E) is the first seen alive in its natural habitat. Collection to verify its identification was not possible, but based on observations it was a species of either *Barathronus* or *Nybelinella.* The fish swam 1-10 cm over sediment between rocks and small boulders on a 45° talus slope. Swimming speeds were consistently slow, 0.33 ± 0.15 body lengths per second, and the fish appeared to be neutrally buoyant. Although there are few other records of aphyonid-clade fishes in the Pacific away from continental margins, this observation suggests that they will be found elsewhere in the basin when appropriate methods are used to detect these small fishes in the high-relief, rugose habitats of central Pacific oceanic islands and seamounts.

1. Introduction

Aphyonid-clade brotulas are little-known, viviparous deep-sea fishes found worldwide at bathyal and abyssal depths of 230 to 5600 meters (Nielsen *et al.*, 1999). They are paedomorphic brotulas, with gelatinous, elongate, and translucent bodies, loose skin, reduced musculature, poorly ossified bones (although *Barathronus* Goode and Bean, 1886 has more solidly-ossified bones than other genera), no swim bladder, and reduced eyes (Günther, 1887; Nielsen, 1969). There is almost no information about their life history, ecology, or behavior (Nielsen *et al.*, 1968;

Okiyama and Kato, 1997; Nielsen *et al*., 1999). They were placed in a distinct family within the Ophidiiformes, Aphyonidae, until the phylogenetic review of Møller *et al*. (2016), who determined that the aphyonid clade is nested within the Bythitidae as a derived group. Those authors did not give the clade a formal, named taxonomic rank, but referred to it as the aphyonids. In this paper, we refer to them as aphyonid-clade fishes or aphyonids to recognize their status as a species-group within the Bythitidae without implying that the clade has a formal taxonomic name. The clade contains 28 species in seven genera (Nielsen, 2016; Nielsen, 2017). Many species are known only from one or a few specimens (Nielsen, 1969, 1984a,b, 2015, 2016; Nielsen and Eagle, 1974; Shcherbachev, 1976; Nielsen and Machida, 1985; Okiyama and Kato, 1997; Nielsen *et al*., 1999; Nielsen and Møller, 2008; Ohashi *et al*., 2013; Nielsen *et al*., 2015).

Aphyonids are thought to be demersal (living near the bottom) or benthic (living on the bottom) (Nielsen *et al*., 1999), although it was suggested that some species might be pelagic or benthopelagic (Nybelin, 1957; Nielsen, 1969; Cohen and Nielsen, 1978). There were no observations of live aphyonids in their natural habitat until 2016, when a single, live aphyonid was observed during a remotely-operated vehicle (ROV) dive in the Mariana Archipelago (Fig. 1). The purpose of this paper is to describe that observation.

2. Methods and Materials

The ROV dive was conducted from the NOAA ship *Okeanos Explorer* during surveys of seamounts in the region of the Mariana Archipelago (Fig. 2(a,b)) from 20 April to 9 July 2016 (Amon *et al*., 2017). The *Okeanos Explorer*, operated by the NOAA Office of Ocean Exploration and Research (OER), is a 68.3 m vessel dedicated to deep ocean exploration. The ship is equipped with various types of sonars to map both the seafloor and water column, CTDs, a dual-body ROV for in situ videography to 6,000 m, and a telepresence mode of scientific participation by internet-accessible satellite links to shore (Bell *et al*., 2016, 2017) (http://oceanexplorer.noaa.gov/okeanos/about.html). Most data and other products from *Okeanos Explorer* expeditions are publically available from OER (Mesick *et al*., 2016) (http://oceanexplorer.noaa.gov/okeanos/data.html). The vessel typically carries only two onboard scientists. Other scientists participate by telepresence at Exploration Command Centers (ECCs) or from their offices and homes (Kennedy *et al*., 2016). During the Mariana expedition, one of the authors (PF) was aboard the ship and most of the others (BCM, MEG, AL) participated by telepresence at ECCs located at the NOAA Inouye Regional Center and the University of Hawai'i at Mānoa on O'ahu.

High definition video of the aphyonid was collected with dual-body ROV. The upper body of the system was the camera platform *Seirios,* equipped with lighting to 72,000 lumens, two high-definition cameras, and a Sea Bird 9/11+ CTD for recording ambient environmental data (Gregory *et al*., 2016). *Seirios* was primarily used to view the lower, main ROV body for system control and navigation, but was also used to obtain wide-field video records of habitats. The lower body of the system was the *Deep Discoverer* (D2), also equipped with 2 Insite Pacific Inc. Mini Zeus HD video cameras that were capable of obtaining macro-photography images in addition to video from a distance. The D2 also had a Sea Bird 9/11+ CTD for obtaining ambient environmental data during the surveys. The D2 lights were LED systems capable of 96000 lumens, on four swing arms that allowed the angle and positioning of the lights to be adjusted for optimal videography at different focal lengths. The ROV system had high-resolution navigation, using Doppler (DVL) bottom lock and PHINS inertial navigation system heading reference. The D2 was equipped with a mechanical arm and collection boxes that allowed samples of rocks, corals, and sponges to be collected, but there was no capability to collect mobile organisms like fishes (Gregory *et al*., 2016). Parallel lasers, 10 cm apart, gave size scales in the videos. ROV dives were conducted during daylight hours, with surveys beginning at the deepest end of the planned transects and moving upslope until the end of the dive.

Videos from the surveys were viewed by the authors onboard the ship or in telepresence in real time. Preliminary identifications of fishes were done during the dives, assisted by internet chatroom and teleconference communications. After the dives, frame-grabs from the videos and video segments made available by OER were examined more carefully to verify or alter identifications in consultation with taxonomists who were knowledgeable about the organisms recorded. Video segments used for this paper are available at the OER video portal (https://www.nodc.noaa.gov/oer/video/) using the search parameters of the date (2016-06-30) and depth range (2490 m and 2520 m). The aphyonid was initially identified from information in Nielsen *et al*. (1999), and the identification was later verified by consultation with the third author (Nielsen), Peter Rask Møller, and Werner Schwarzhans (Natural History Museum of Denmark, University of Copenhagen). The length and body proportions of the fish were estimated from the parallel lasers of the ROV using close-up images when the fish was only a few millimeters off bottom, adjacent to the laser lights visible on the substrate, with its body in a straight position parallel to the plane of the camera view.

Swimming kinematics were characterized using ImageJ (Schneider *et al.*, 2012). Tail beat frequency, swimming speed, and tail beat amplitude were determined from straight swims with complete undulatory cycles. Tail beat amplitude was measured from the fish's midline. To account for frequent camera movements and zooms, each video frame was calibrated to the size of the fish, and all measurements were taken in terms of fish body length. Travel distance was determined in relation to recognizable points in the sediment and rock formations. Swimming events when the fish used only active pectoral fin strokes, or when there was clear interference from ROV-generated current flow, were excluded from the analysis. Results are presented as mean ± standard deviation.

3. Results

The aphyonid was first encountered on Explorer Ridge (20.68152°N, 145.08750°E) at 23:29:10 on 30 June 2016 GMT (09:29:10, 1 July 2016 local time in the Mariana Islands) (Fig. 2(b,c)). From the parallel laser measurement of the ROV, it was about 9.3 cm total length and 8.5 cm standard length. Environmental measurements recorded at first sighting were: D2 depth = 2504.2 m, temperature = 1.78°C, salinity = 34.64, and dissolved oxygen = 3.70 mg/L. The observations continued for 8 minutes 48 seconds, after which the fish took shelter in a crevice under a boulder. By the end of the observations, the D2 depth varied upwards to 2492 m, temperature increased to 1.80°C, and dissolved oxygen varied between 3.57 and 3.71 mg/L (salinity did not vary). No other megafauna were observed in the immediate area where the fish was seen except a small, pale organism drifting in the water about 0.5-1 m above the bottom 16 seconds after the aphyonid was first sighted (Fig. 3). It was not possible to obtain a view of that organism that was sufficient to identify it even to phylum or to accurately measure its size. Because it appeared to be about the same size, shape, and color as the aphyonid, it is possible that this was another individual of the same species.

Limited characteristics for identification of the fish could be clearly seen from the video. The two most obvious features were the apparent lack of body pigmentation and the large size and color of the eyes, which were pale-yellow discs, seemingly concave, without pupils or lenses (Figs. 1, 4). The fish was not one of the slender-bodied aphyonid species (maximum body depth > 16.5% SL), although precise measurement of the body depth was not possible because of the angle at which the fish was observed. The head length (HL) was about 24.5% SL, head width 23.6% SL, head depth > 50% HL, interorbital width 18.2% HL, eye diameter about 22.2% HL, and the pectoral-fin length about 18.6% SL. The origin of the dorsal fin could not be seen to determine the pre-dorsal distance, although it appeared to be at about 33% SL. Accurate measurement of the pre-anal length was not possible, but it appeared to have been about 70% SL. Some fin rays were visible as opaque, narrow, elongate areas in the dorsal and pectoral fins, but complete counts of fin rays were not possible from the video. The lower jaw protruded slightly anterior from the tip of the upper jaw, but it was not possible to determine if the mouth was near horizontal or oblique. Several papillae or small crenulations were apparent in the snout area, and small papillae that may have been neuromasts or skin flaps of the cephalic and lateral-line sensory canals were apparent along the posterior edge of the preopercle and sides of the body. The fish was pale or translucent white except for the pale yellow eyes, yellow to yellow-cream inside the viscera, and pale pink inside the branchial cavity. The liver was visible as a yellowish mass at the anterior end of the gut. There were thick layers of translucent tissue under the skin in much of the head and body. The musculature was visible in the body as more opaque white tissue, as was the brain in the translucent skull. There were also more opaque areas in the snout and anterior part of the upper jaw that were probably muscles and bones of the dentigerous elements of the jaws and roof of the mouth. With their prominent opaque appearance, these jaw structures were likely among the more ossified structures in the fish.

The aphyonid was seen in the Mariana Trench Marine National Monuments Islands Unit (Fig. 2(a)), part of the Mariana forearc, the region between the Mariana Trench axis and the active volcanic island arc. The entire forearc has undergone north-south extension at the latitude of the sighting, resulting in steep fault scarps trending generally east-northeast across the entire forearc area and creating a fault-controlled basin from the island arc to the inner slope of the trench. The part of the fault scarp at which the fish was found had sediment-covered talus (Fig. 3). The sediment was pale-brown with numerous, tiny foraminiferal tests. The general slope in the area was 45°, the normal maximum angle of repose assumed by piles of debris. The talus pile had occasional chutes (large or small furrows), likely caused by debris moving down-slope and thus eroding the surface of the slope. Some of these chutes contained rocks, varying in size from pebbles to boulders. The fish was observed in one of these chutes, hovering about 2-10 cm above the bottom among rocks and boulders. The topography above the talus chute where the fish was seen was steeper, eventually reaching ~ 55°. A rock sample from the area above the location of the fish was a well-indurated siltstone. It had adherent sediment containing many fragments of foraminiferal tests, one olivine crystal, and many black volcanic glass fragments, all enclosed in fine clay-sized particles. The general color of the sediment was medium-brown as was the rock where it was not coated with a manganese oxide crust.

The aphyonid was first seen at a small sedimented area between groups of rocks, which in turn were amid groups of small boulders. The fish's body was nearly straight except for slow undulations of the end of the tail. It drifted very slowly and laterally a few centimeters upslope. As the ROV approached, it twisted into a C shape about 90° downslope, straightened its body, made a quick flexure, and straightened its body again (Fig. 4(a)). Slight body flexures continued, producing a truncated undulatory motion that was combined with occasional sculls of the pectoral fins. The fish remained a few millimeters above the bottom. The body motion increased after about a minute, perhaps in response to the currents produced by the ROV thrusters, and shortly thereafter the fish engaged in rapid anguilliform swimming, moving several centimeters downslope and laterally to its left toward rocks. It then bumped into the rocks, presumably resulting from disorientation caused by the lights and other sensory stimuli from the ROV. The rocks were not undercut enough for the fish to shelter beneath them. The fish continued around the rock group with anguilliform movement and pectoral-fin sculling, bumping into the rocks several more times, usually maintaining a height above bottom of one to a few millimeters, but touching bottom occasionally (Fig. 4(b)). It moved back over the sediment after about two minutes. At that time, the ROV thrusters disturbed the sediment, sending a cloud of it over the fish. The cloud dispersed after several seconds, indicating that a moderate current was present. The fish did not show a startle response to this disturbance and it continued its slow, undulating swimming, bumping into more rocks as it turned back upslope. A few sediment particles settled on the top of the fish's head and nape, but the fish did not make active attempts to dislodge them. It turned, began to circle back, occasionally rising to a centimeter or two above bottom, and sometimes descending to touch the sediment. At 3 ½ minutes, it angled its body slightly head-down and touched the sediment with its snout. It then returned to its position parallel to bottom, a few millimeters above the sediment. Similar behavior, with several instances of bumping snout-first into rocks, continued for the rest of the observations as the fish circled in the same small area (Fig. 4(c)). It always moved slowly, sometimes away from the ROV and sometimes toward it, without an apparent overall direction. After about four minutes, its swimming slowed even more and it drifted above the bottom with fewer tail flexures and pectoral-fin sculls. After 6 minutes (Figs. 1, 4(d)), it had moved laterally to the left toward a group of larger (25-30 cm) rocks that had overhanging or undercut areas at the sea floor. It drifted slowly into one of the small overhangs, propelled slightly by flexures of the tail end, and sheltered in the shadow of the undercut. Observations stopped at that time, and the ROV continued on its course upslope.

Measurements of swimming kinematics were possible at seven occasions in the video. Tail beat frequency ranged from 0.35 to 0.68 beats per second (0.49 ± 0.13). Swimming speeds were consistently slow, 0.33 ± 0.15 body lengths per second. Maximum tail beat amplitude in each stroke averaged 25 ± 3% of body length (range 20-28%).

4. Discussion

The fish described here is in the aphyonid clade within the Bythitidae (order Ophidiiformes), judging from the combination of joined vertical fins, gelatinous body, transparent, scale-less skin, gut length, and buried, degenerate eyes. Among the seven known aphyonid genera (Nielsen, 2016), it could belong to either *Barathronus* Goode & Bean, 1886 or *Nybelinella* Nielsen, 1969. The species of these genera have an unpigmented or pigmented, compressed body, with head higher and broader than body, an oblique mouth cleft and light or dark-blue peritoneum (Nielsen, 1969, 2017; Nielsen et al., 1999; Nielsen et al. 2015). *Barathronus* is found in all oceans and contains 10 species, of which four are slightly pigmented or unpigmented: three from the Atlantic (*B. linsi* Nielsen et al., 2015; *B. multidens* Nielsen, 1984; and *B. unicolor* Nielsen, 1984) and one from the Indo-Pacific (*B. affinis* Brauer, 1906). *Nybelinella* has three species from the Atlantic and Indian Oceans; of which two are unpigmented (*N. erikssoni* [Nybelin, 1957] and *N. brevianalis* Nielsen, 2017) and one is lightly pigmented (*N. brevidorsalis* Shcherbachev, 1976). The two genera are easily separated, but the diagnostic characters (dentition, form of the gill rakers and filaments on the anterior gill arch, and the form of the vertebral centra) cannot be seen in the video. Among aphyonids, *Barathronus* has the most strongly developed bones of the jaws and roof of the mouth (Nielsen, 1969). Meristic characters are necessary to reach a specific identification within both genera. However, these characters also are not visible in the video. Consequently, we conclude that the fish seems to belong to one of the two genera (*Barathronus* and *Nybelinella*) and to one of the six unpigmented species within those genera, or it may be an undescribed species as suggested by the apparent anterior position of the dorsal-fin origin.

The behavior exhibited by the aphyonid that we observed was certainly altered by the presence of the ROV. The highly reduced, lens-less eyes of aphyonids, like those of many fishes in the sunless bathyal and abyssal ocean, are not capable of forming images (Munk, 1966a,b). Although the tiny eyes of some aphyonids, such as *Sciadonus,* may be entirely functionless (Munk, 1966a,b), the larger eyes of *Barathronus* and *Nybellina* are probably able to sense the weak light produced by bioluminescent organisms as discussed in general for deep-sea organisms by Warrant and Locket (2004). The powerful lights of the ROV were likely a strong sensory overload for the fish, altering its behavior during our observations. These behavioral changes may have biased the swimming kinematic characters from normal.

The fish appeared to be neutrally buoyant, likely achieved through poorly ossified bones, the liver (visible in the video), and through the accumulation of gelatinous tissues below the skin (e.g., Yancey *et al.*, 1989). In addition to serving a buoyancy function, this watery layer of gelatinous tissue may also improve swimming efficiency at low growth cost (Gerringer, Univ. Hawai'i, unpublished results). Swimming speeds were relatively slow, as might be expected in a cold environment with limited interaction distances of predator and prey (Childress, 1995). Because observations of the fish were always less than about 10 cm above bottom, these observations provide evidence that this aphyonid is demersal or benthic rather than benthopelagic as has been previously suggested for some genera (Nybelin, 1957; Nielsen, 1969; Cohen and Nielsen, 1978).

To our knowledge, this record is the first of an aphyonid from the region of the Mariana Archipelago and Mariana Trench at the convergence of the eastern edge of the Philippine tectonic plate with the western edge of the Pacific Plate. It is also the first record of an aphyonid from the vicinity of an oceanic island group in the western North or central Pacific. The absence of records of aphyonids in the central Pacific is probably an artifact of low sampling effort, of the difficulty in sampling many of the habitats there, and of the aphyonids' small size and behavior that makes them unlikely to be captured by conventional trapping methods. Aphyonid specimens have been collected by trawling, a technique usually limited to low-relief, soft-substrate habitats.

Bathyal and abyssal trawling surveys have been conducted primarily along continental margins or islands near locations of major oceanographic institutions, for logistical and financial reasons. There have been few such surveys in the vast area of the central Pacific oceanic islands and seamounts. We also note that our observation of an aphyonid was at a high-relief, rocky seamount habitat with a 45° slope, a habitat that would be difficult to sample by trawling. Aside from trawling surveys, sampling for bathyal and abyssal fishes in the central Pacific has been conducted with baited traps and camera systems, or by submersible and ROV surveys. Aphyonids have not been recorded at baited cameras or captured by baited traps, thus they do not appear to be scavengers. Instead, they are thought to feed on small crustaceans including copepods or polychaetes (Nielsen, 1969, 1984b). Although aphyonids are paedomorphic with highly reduced body tissue, the relatively opaque structures in the snout and anterior head seen in the live specimen suggests the presence of well-developed feeding structures for predation on small crustaceans.

The small size of aphyonids makes them less likely to be noticed during submersible and ROV surveys than larger fish. We have searched carefully and hopefully for aphyonids in the *Okeanos Explorer* ROV surveys and expect that other ichthyologists have done the same during other surveys, but no other observations of live aphyonids are known. Although there are few records of aphyonids at Pacific islands or seamounts, we expect them to be found throughout tropical, subtropical, and temperate areas of the central Pacific when adequate sampling and observations are done that can detect them.

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Figure captions.

Fig. 1. The first aphyonid-clade brotula (Ophidiiformes, Bythitidae) observed alive in its natural habitat, six minutes after the fish was first observed at 2504.2 m on Explorer Ridge (20.68152°N, 145.08750°E) in the Mariana Archipelago.

Figure 2. Location of the observation of a live aphyonid: (a) Mariana Archipelago (black rectangle) within Pacific Ocean; the United States Exclusive Economic Zones are indicated in white (from Pacific Islands Fisheries Science Center, 2010); (b) the Mariana Trench (white dashed line) to the east of the Mariana Islands and Explorer Ridge (black cross) where the aphyonid-clade bythitid was observed; (c) the planned ROV dive transect at the Explorer Ridge where the aphyonid was observed; the approximate location of the observation is indicated by the white arrow. Map from the NOAA Office of Ocean Exploration and Research.

Fig. 3. The sea floor characteristics of the site and initial position of the aphyonid (indicated by the arrow on the right) 16 seconds after the first sighting of the fish (30 June 2016, 23:29:26 GMT), showing the ca. 45° talus slope on the fault scarp and cobbles to boulders draped with pelagic sediment. The only other megafaunal organism seen at this area was the small, pale one at the top left corner of the photograph (arrow on the left). The two light dots on the sediment in the center of the photograph, above the short scale bar, are parallel laser beams 10 cm apart, for a size reference.

Fig. 4. The aphyonid in different aspects of its behavior during the ROV observations: (a) about 1 minute (30 June 2016, 23:29:41 GMT) after it was first sighted; (b) about 1 ½ minutes after the first sighting; (c) at 4 minutes 7 seconds after first sighting (30 June 2016, 23:33:17 GMT) when

it flexed to a circle and turned almost 180°; (d) about 6 minutes after the first sighting (30 June 2016, 23:34:52 GMT).

Figures.

Fig. 1. The first aphyonid-clade brotula (Ophidiiformes, Bythitidae) observed alive in its natural habitat, 6 minutes after the fish was first observed at 2504.2 m on Explorer Ridge (20.68152°N, 145.08750°E) in the Mariana Archipelago.

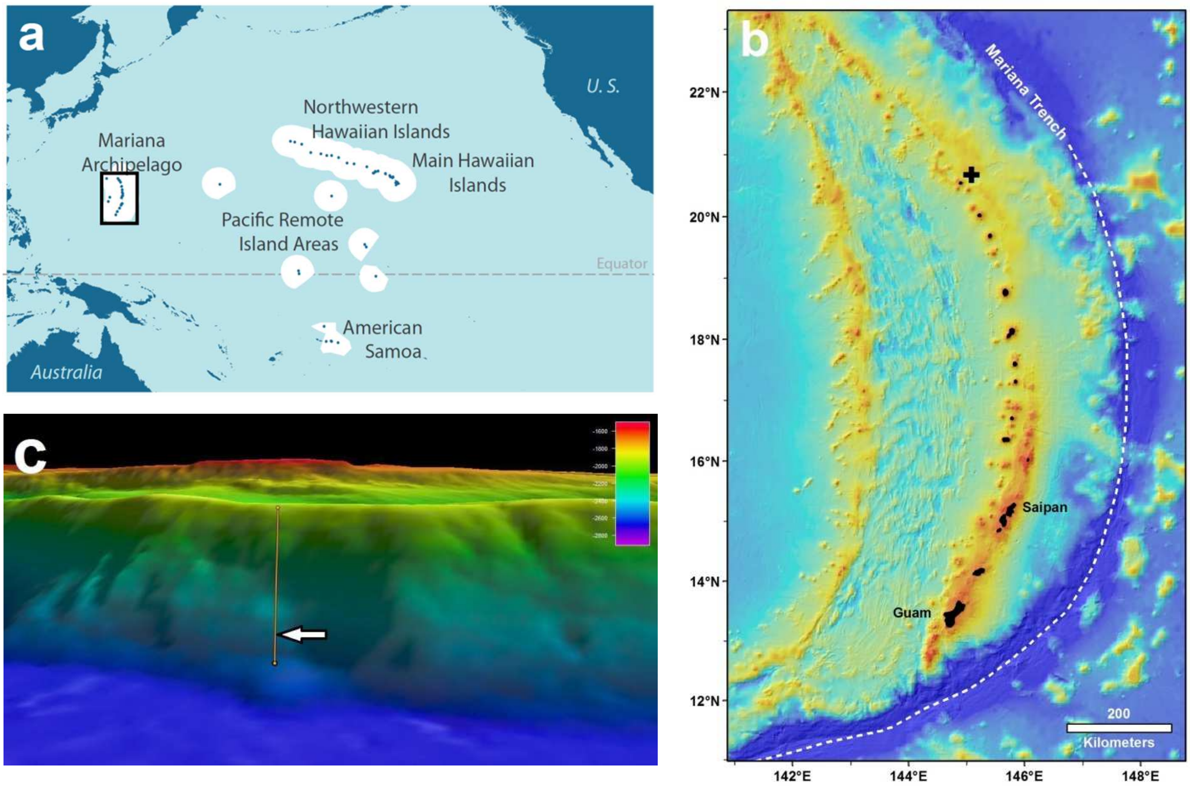

Figure 2. Location of the observation of a live aphyonid: (a) the location of the Mariana Archipelago (black rectangle) within Pacific Ocean; the United States Exclusive Economic Zones are indicated in white (from Pacific Islands Fisheries Science Center, 2010); (b) the location of the Mariana Trench (white dashed line) and Explorer Ridge (black cross) where the aphyonid-clade bythitid was observed; (c) the planned ROV dive transect at the Explorer Ridge and the approximate location of the aphyonid observation (white arrow). Map from the NOAA Office of Ocean Exploration and Research.

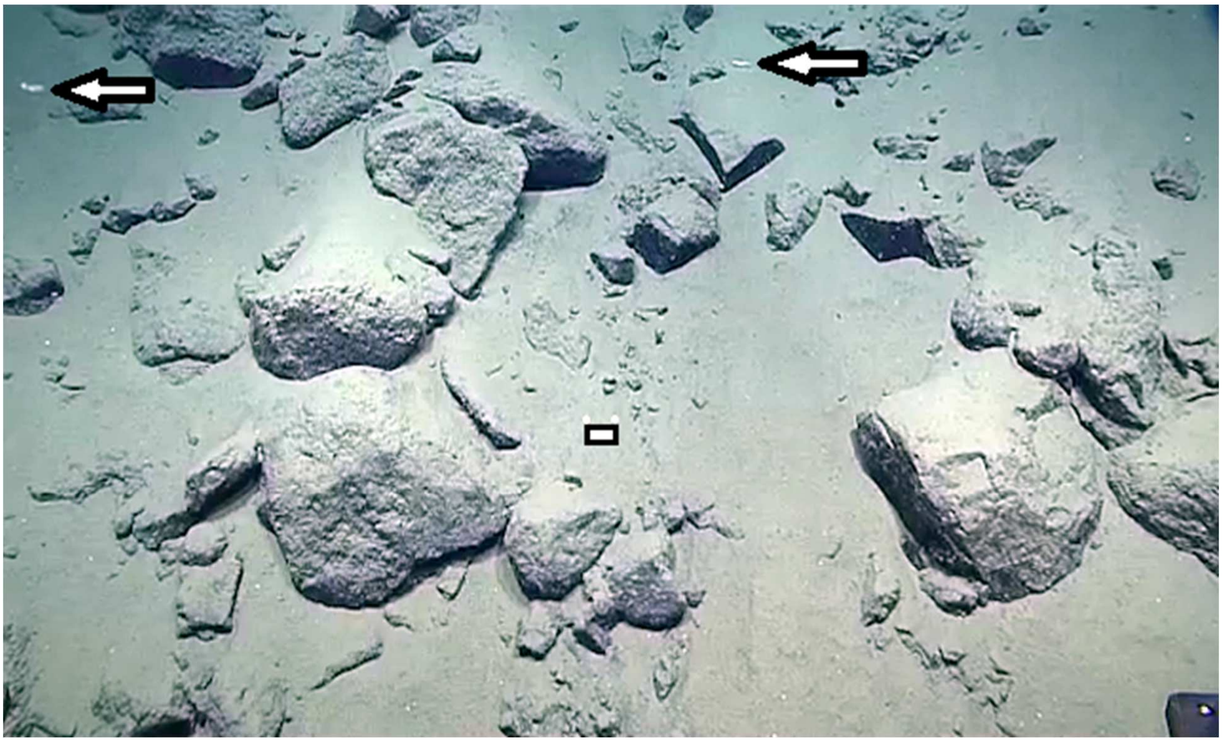

Fig. 3. Initial position of the aphyonid (indicated by the arrow on the right) 16 seconds after the first sighting of the fish at a ca. 45° talus slope on the fault scarp with cobbles to boulders draped with pelagic sediment. The only other megafaunal organism seen at this area was the small, pale one at the top left corner of the photograph (arrow on the left). The two light dots on the sediment in the center of the photograph, above the short scale bar, are parallel laser beams 10 cm apart, for a size reference.

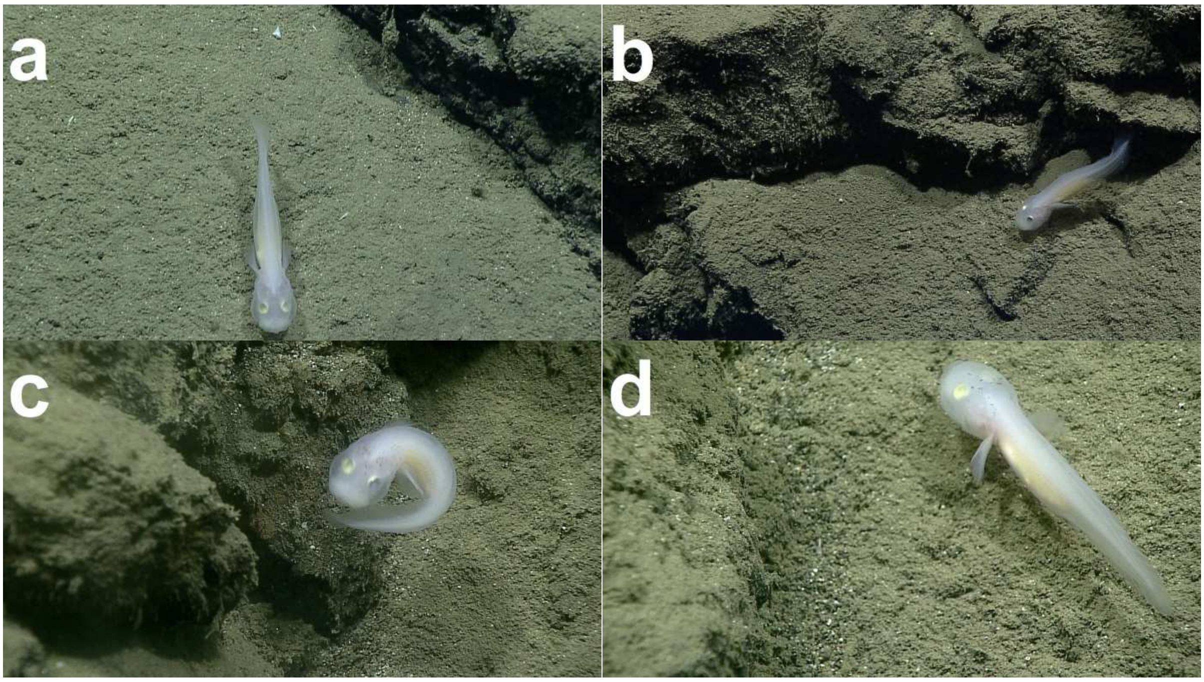

Fig. 4. The aphyonid in different aspects of its behavior during the ROV observations: (a) about 1 minute (30 June 2016, 23:29:41 GMT) after it was first sighted; (b) about 1 ½ minutes after the first sighting; (c) at 4 minutes 7 seconds after first sighting (30 June 2016, 23:33:17 GMT) when it flexed to a circle and turned almost 180°; (d) about 6 minutes after the first sighting, near the end of the observations (30 June 2016, 23:34:52 GMT).