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# First Record of Amphioxus *Branchiostoma californiense* (Amphioxiformes: Branchiostomatidae) Adjacent to a Shallow Submarine Hydrothermal System at Banderas Bay (Mexico)

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**Abstract:** Shallow submarine hydrothermal vent systems assume differentiated environmental conditions. Some specimens of *Branchiostoma californiense* were found in the sediments of the influence area of the shallow hydrothermal venting in Punta Mita. This is the only lancelet species registered for the Mexican Pacific. The meristic and morphometric characteristics of the organisms first collected in unconsolidated sediments of this shallow system were reviewed, in order to determine the species. We confirm that it is the same species. This represents the first record of it for both the Banderas bay and in the influence area of a shallow hydrothermal system.

**Keywords:** Cephalochordata; lancelet; hydrothermal system; record; shallow vent; Banderas Bay

## 1. Introduction

Amphioxus, also known as sea lancelets, are cephalochordates that can be best recognized by their notochord, which is visible through the body wall [1]. In addition, they have distinguishable metamerization, marked by body muscle bundles (myotomes), which are grouped and separated by connective tissue (myosepts) [2]. According to Del Moral-Flores, et al. [1], there are 35 valid species of cephalochordates, all included in the family Branchiostomatidae, and divided into three genera: *Asymmetron*, *Branchiostoma*, and *Epigonichthys* [3–6]. The *Branchiostoma* genus is the most diverse, with 23 valid species [7].

The cephalochordates are cosmopolitan and usually inhabit the shallow marine waters near the coasts [1,7], although some species can inhabit depths greater than 200 m. The work of Kon, et al. [8] established that they can spend most of their lives forming part of the plankton [4,9,10]. They are mainly associated with thick sediments, such as sand, gravel, or shells' remains [9,11]. These animals are dioecious and without apparent sexual dimorphism [1], and their larvae can be benthic or planktonic [3,6,12]. The adults are occasional swimmers, since they prefer the benthic substrate, where they spend most of their life filtering their food in small particles towards plankton [13].

Along the Mexican Pacific coasts, only the amphioxus *Branchiostoma californiense* (Andrews, 1893) has been reported [1,7].

According to Canet and Prol-Ledesma [14], in Mexico there are three shallow submarine hydrothermal ventings, one of which is at Punta Mita, Nayarit, where anomalously high heat flow values have been calculated [15]. The study area is part of a shallow submarine hydrothermal system at Punta Mita (SSHSPM), in the Las Coronas fissure [16,17]. The hydrothermal discharges reach temperatures up to 89 °C. The emanated gas is composed mainly of $N_2$ (88%) and $CH_4$ (12%), and the fluids are less saline than seawater and significantly enriched in Si, Ca, Li, B, Ba, Rb, Fe, Mn, and As [18].

The aim of this research was to determine and describe the species of cephalochordates found in the sediments of the SSHSPM.

## 2. Materials and Methods

On 23 November 2017, 400 m away from the coast, in front of the beaches of Punta Pantoque in Banderas Bay, Nayarit, Mexico, dive three of the SisVoc–CUC1 expedition took place at active hydrothermal venting, coordinates: 20°44′54.9″ N, 105°28′38.4″ W, at a water depth of 10 m, within the SSHSPM (Figure 1). The SSHSPM covers an area of 300 $m^2$ at a depth of 10 m [18], and have an influence area of approximately 1 $km^2$ [14].

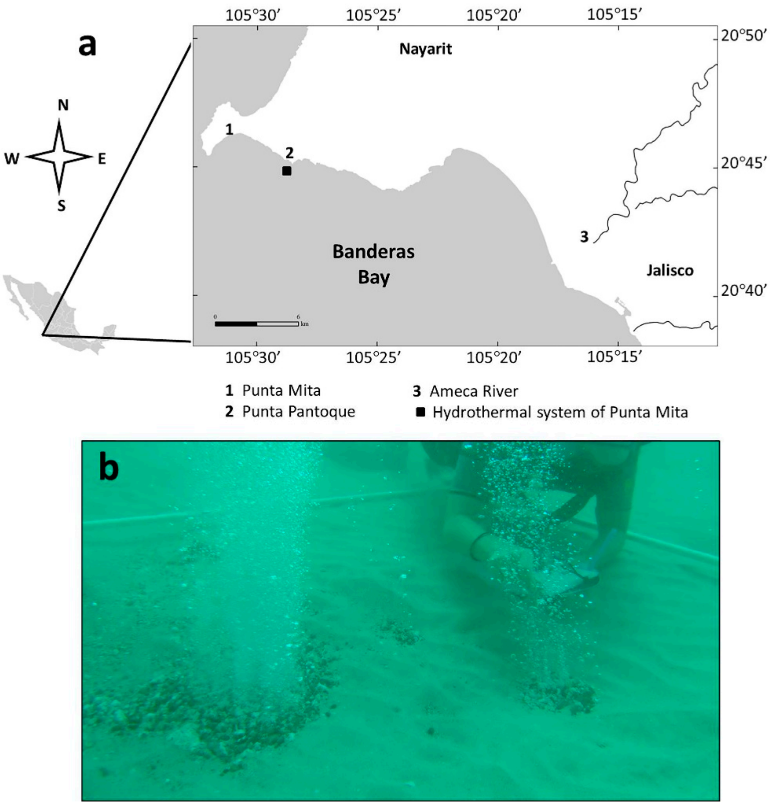


**Figure 1.** Study area: (**a**) location of the shallow submarine hydrothermal system of Punta Mita—the little black square indicates the study area; (**b**) photograph of three active hydrothermal vents.

Based on temperature and the hydrothermal vent position (considered the center), the study area was divided into three zones for sampling: vent zone (VZ) (0–0.4 m); middle zone (MZ) (0.4–3 m); and external zone (EZ) (3–6 m) (Table 1). Three cores of marine sediments were collected in each zone ($n$ = 9) by scuba diving, using PVC plastic cores of 10 cm long × 11 cm diameter. The cores were inserted at 5 cm of depth into sediment at random points in each zone. Subsequently, the sediment cores were stored in a cooler and taken to the laboratory, where they were then filtered using a 1 mm mesh sieve. Specimens were preserved in 96° alcohol, and then deposited in the first benthic invertebrate collection associated with SSHSPM, at the Centro de Sismología y Volcanología de Occidente of Centro Universitario de la Costa, Universidad de Guadalajara, in Puerto Vallarta, Mexico.

**Table 1.** Number of cephalochordates in each study zone. VZ = vent zone, MZ = middle zone, and EZ = external zone.

| | Zone | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | VZ | | | MZ | | | EZ | | | |
| Sample | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | |
| Sampled cephalochordates | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 4 | 8 |

The sediment temperature was recorded at 0, 3, and 5 cm depth in each of the three zones (see Figure 2) using a Taylor™ analog soil thermometer, 6099N model, 1″ diameter hood, 6″ stem and temperature ranging from −10 to 110 °C. Meanwhile, a YSI™ Professional 1030 multiparameter probe (Pro1030) with a 20 m cable, was used to determine the pH, salinity, and water temperature at the sea bottom. Sediment temperature and three parameters with three replicas in each zone (see Figure 2, Table 2). For the identification of collected cephalochordates, we used the descriptions of Kirkaldy [2], Hubbs [19], Poss and Boschung [3], Del Moral-Flores, et al. [1] and Galván-Villa, et al. [7]. The species were observed using an Optika® 50 × stereoscopic microscope (Via Rigla, Bergamo, Italy). The specimens were stained with methylene, to highlight their physical features in the photographs, and they were measured with a STEREN® digital Vernier caliper her-411.

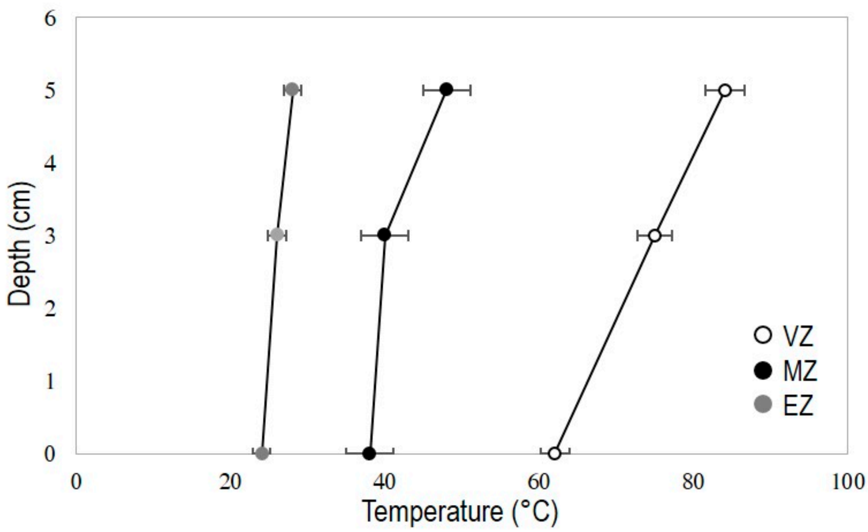


**Figure 2.** Sediment temperature at 0, 3, and 5 cm depth. VZ = vent zone, MZ = middle zone, and EZ = external zone.

**Table 2.** Physical and chemical measurements in each zone of study (average ± standard error). VZ = vent zone, MZ = middle zone, and EZ = external zone.

| Zone | pH | Salinity (ppt) | Water Temperature (°C) |
|---|---|---|---|
| VZ | 7.67 ± 0.01 | 17.2 ± 0.07 | 89 ± 0.47 |
| MZ | 8.04 ± 0.00 | 34.9 ± 0.04 | 26.8 ± 0.09 |
| EZ | 8.05 ± 0.00 | 35.8 ± 0.02 | 25.6 ± 0.21 |

## 3. Results

In the marine sediments of the SSHSPM, eight cephalochordates were found. None was found in VZ, but three were found in MZ and five in EZ (Table 1).

### 3.1. Pore-Water and Marine Sediments Measurements

The sediment temperature at 0, 3, and 5 cm depth of each zone is shown in Figure 2. The pH, salinity, and water temperature of each zone varied, as shown in Table 2.

### 3.2. Diagnosis

Collected specimens for this study presented similar diagnoses and these diagnoses coincided with those published previously [1–3,7,19]. The total number of myotomes was 65–71, of which 43–45 were preatriopore myotomes, 15–20 were between atriopore and anal, 8–9 were postanal myotomes, with 320–345 dorsal storage chambers, and 14–16 buccal cirri.

### 3.3. External Morphology

The bodies of collected specimens are elongated, translucent, compressed, and sharp at both ends. Under the microscope, the pharynx, the myotomes, notochord, intestine, and storage chambers of the dorsal and caudal fin are highlighted. The myotomes, some internal organs, the central part of the trunk, the area over the intestine and the caudal fin, are a solid color with a whitish hue, and the intestine is black. Specimens have a reduced rostrum. Their sizes ranged between 1.2 and 1.55 cm and there was no presence of well-differentiated gonads, which infers that they were in the juvenile stadium. This can be reinforced by the fact that the length of the prebranchial region is proportionally bigger in juveniles. According to the diagnosis and their external morphology, the specimens collected in SSHSPM belong to the species *Branchiostoma californiense*, Andrews, 1893 (Figure 3).

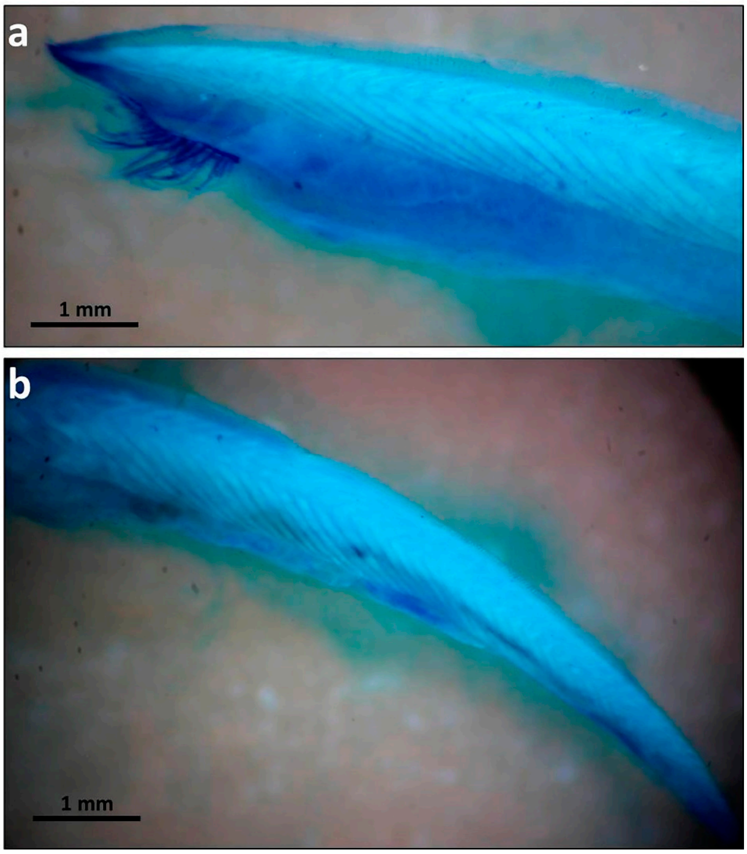


**Figure 3.** Side view of *Branchiostoma californiense* collected specimen: (**a**) anterior region; (**b**) posterior region. Its blue color is due to staining with methylene.

### 3.4. Distribution

*B. californiense* is the only species of lancelet reported in the Mexican Pacific, from the western coasts of peninsula of Baja California, including the Gulf of California, to the coasts of Oaxaca [1,7]. The presence of this species has already been reported on the coasts of the state of Nayarit, Mexico, whose specimens have been deposited in the scientific collection of the Natural History Museum of Los Angeles County, Los Angeles, California, USA (LACM-22314, LACM-22418) [1]. This work represents the second record of this species for the state of Nayarit, Mexico, and the first record within Banderas Bay. This species is reported for the first time in sediments located in the influence area of a shallow submarine hydrothermal system.

## 4. Discussion

The lancelets collected and described here are identified as immature juveniles, due to their reduced size and the non-identification of developed gonads, matching the findings of Galván-Villa, et al. [7]. They reported a minimum size of 2.41 cm for mature adults found in Melendres Island (Sinaloa, Mexico) and Chamela Bay (Jalisco, Mexico). Both places are located near Banderas Bay (Nayarit, Mexico), and the proximity of these places reinforces our findings.

Del Moral-Flores, et al. [1] reported similar myotome formulas to those reported by Galván-Villa, et al. [7] and by us. The specimens of Del Moral-Flores, et al. [1] were collected on the coasts of Baja California, Baja California Sur, Sinaloa, Nayarit, Jalisco, and Oaxaca, all of which belong to the Mexican Pacific. This is consistent with our findings and also with the reported wide distribution of the species along the eastern Pacific, from Monterrey Bay, USA to Chame Point, Panama [3,20]. It should be noted that the *B. elongatum*, found along the Humboldt current [21], can be clearly differentiated from *B. californiense* by the myotomes formula. It has 44 pre-atrioporal, 18 pre-anal, and nine post-anal myotomes, while *B. elongatum* has 49 pre-atrioporal, 18 pre-anal, and 12 post-anal myotomes [22].

Prior to this work, *B. californiense* had not been reported in or near a shallow submarine hydrothermal system (<200 m) [23,24]. The specimens of this study were found in a range of 0.4–6 m away from active hydrothermal venting, which reach temperatures of up to 89 °C, but were not found within the vent sediments. Melwani and Kim [25] reported benthic infauna related to two shallow submarine hydrothermal systems, one in Concepcion Bay (Baja California, Mexico), and the other in White Point (California, USA). They did not find lancelets in either of these two hydrothermal systems. They concluded that high temperatures are the main factor influencing the low abundances of benthic infauna in the sediments of these two hydrothermal systems. The benthic infauna communities reported by Melwani and Kim [25] are subsets of surrounding communities of these two hydrothermal systems.

The work of Kon, et al. [8] reported lancelet *Asymmetron inferum*, for the first time, in an anaerobic and sulfide-rich bottom (229 m) environment, caused by whale-falls. Our study area contains some geochemical properties typical of deep-sea hydrothermal systems [24] and whale-falls [8], since SSHSPM has a reducing environment [18].

According to Melwani and Kim [25], the benthic communities of shallow vents differ from outside sediments, being lower. The above coincides with our results, because we did not find *B. californiense* in VZ.

Carpizo-Ituarte, et al. [26] established that the water surface temperature at the north of Banderas Bay, was 26–30 °C from April to September, and 22 °C and lower from October to March. This is under the temperatures we registered in the MZ and EZ areas, so the hydrothermal system could be influencing these zones.

## 5. Conclusions

According to the diagnosis and their morphology, we conclude that the lancelets found in this research belong to the *B. californiense* species. This is the first record of this species being reported in the sediments in the influence area of a shallow submarine hydrothermal system (MZ and EZ). The conditions in VZ exclude the lancelets in this zone. Greater monitoring in SSHSPM, both in VZ and in its surroundings, is necessary to reaffirm this finding.

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**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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