

## Integrative evidence for the first record of *Clavelina oblonga* (Ascidiacea: Clavelinidae) in a highly turbid Caribbean habitat

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**ABSTRACT.** Ascidians are key components of benthic marine communities worldwide. They contribute to biofouling and increase the structural complexity of benthic habitats by providing substrate for other organisms. Despite their ecological relevance, the ascidian fauna of the Colombian Caribbean remains poorly documented. We provide the first confirmed record of the colonial ascidian *Clavelina oblonga* Herdman, 1880 in Colombia, based on an integrative approach that combined morphological examination with DNA barcoding of the mitochondrial cytochrome oxidase subunit I (COI) gene. Specimens were collected from artificial substrates, including concrete structures, metallic pilings, and submerged ropes at depths of 0.5–2.0 m within a marina in the Atlántico Department on the Caribbean coast of Colombia. The presence of this species may represent either an overlooked native population or human-mediated dispersal associated with maritime traffic. These findings highlight the importance of sustained regional monitoring and the use of molecular tools to improve the documentation of ascidian diversity.

**Key words:** artificial substrates, biodiversity, biofouling, Colombian Caribbean, colonial ascidian, marina.

## INTRODUCTION

Ascidians are an important component of marine fouling fauna, and although numerous studies have addressed this group in the Caribbean (Van Name 1921, 1924, 1930; Millar 1962; Van der Sloot 1969; Millar and Goodbody 1974; Hernández-Zanuy 1990; Goodbody 2000; Rocha et al. 2005, 2010), substantial knowledge gaps persist. Ascidian distributions are often facilitated by maritime transport and the availability of hard substrates (Lambert 2001, Lins et al. 2018). Consequently, ports, marinas, and docks provide stable habitats for native and invasive species and favor their establishment and persistence (Lambert and Lambert 2003, Cohen et al. 2005, Lambert 2005, Koplovitz et al. 2016).

Along the central Caribbean coast of Colombia, environmental conditions are strongly shaped by the influence of the Magdalena River Delta, one of the largest fluvial systems in the region. The discharge of this system generates a pronounced plume characterized by strong sediment loads, high turbidity, and marked salinity gradients, which creates heterogeneous

coastal environments (Higgins et al. 2016, Gracia et al. 2021). These conditions are particularly relevant for filter-feeding organisms, such as ascidians, which are sensitive to variations in sediment loads. Increased sedimentation may lead to higher oxygen consumption and greater energetic demands associated with coping with sediment accumulation. In some cases, excessive sediment deposition can clog the filtering organs of ascidians, reduce aerobic metabolism, and ultimately cause population declines in the most sensitive species (Torre et al. 2012). Likewise, given that light availability can influence ascidian distributions, turbidity is also a determining factor of their distributions, as it can reduce light penetration and create conditions, such as those in shaded environments, which may favor the settlement of larvae that exhibit negative phototaxis (Young and Chia 1984, Houle 2015). Additionally, salinity fluctuations represent a key ecological factor that influences species distributions, settlement, and survival (Naranjo et al. 1996, Epelbaum et al. 2009, Loureiro et al. 2021).

Despite the ecological marine diversity of the Colombian Caribbean, studies on colonial ascidians remain scarce.

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Ascidians are subject to dispersal limitations and are frequently associated with artificial substrates (Lambert 2001, Koplovitz et al. 2016). These characteristics increase the potential for cryptogenic or non-native species to occur within this group, which supports the need for continuous monitoring (Lambert 2005). In this context, the present study aimed to identify colonies of Clavelinidae found in a marina located in a highly turbid environment using an integrative morphological and molecular approach and to evaluate their occurrence in relation to environmental variables such as light availability. This study contributes to understanding how local environmental conditions and artificial substrates may shape the distribution of ascidian species in the Colombian Caribbean.

## MATERIALS AND METHODS

### Study area

This study was conducted at Puerto Velero Marina (PVM; 10°56'37.89"N, 75°02'26.94"W), located in the Atlántico Department on the Caribbean coast of Colombia. The coastal zone of the Atlántico Department is strongly influenced by the discharge of the Magdalena River, with the annual suspended sediment load estimated at  $145 \pm 47 \times 10^6 \text{ t}\cdot\text{y}^{-1}$  (Higgins et al. 2016). However, this average excludes contributions from 26 additional micro-watersheds that flow into the coastline (Rangel-Buitrago et al. 2020). The climate in the area is characterized by 3 seasons: dry (December–April), rainy (August–November), and transitional (May–July) (Rangel-Buitrago et al. 2016). Tides in the region are mixed semidiurnal, with maximum amplitudes of 0.6 m (Torres and Tsimplis 2012).

Puerto Velero Marina is a small to medium-sized marina located in a semi-enclosed and sheltered coastal embayment (Fig. 1). The structures present at PVM include concrete slabs, metal pilings, plastic buoys, and submerged ropes, all of which provide suitable settlement substrates for marine organisms. The arrangement of the slabs and the space under the walkways create well-lit areas near the outer sides and shaded areas beneath these pathways. In the PVM area, Gracia et al. (2021) reported an average sedimentation rate of  $39.5 \pm 38.6 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$  (for an 8-month period), which suggests low water visibility during most of the year, and an average rate of  $10.5 \pm 7.6 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$  during the rainy season. Additionally, Gracia et al. (2021) registered an average sea surface temperature of 28.1 °C at a depth of 0.5 m, which ranged from 23.9 °C in the dry season to 33.3 °C at the end of the rainy season.

### Sampling

Sampling and observations of well-lit and shaded areas in PVM were conducted between August 2024 to March 2026 using snorkeling gear. It is important to note that PVM substrates have been continuously monitored through random sampling since 2017; however, ascidians of this family have

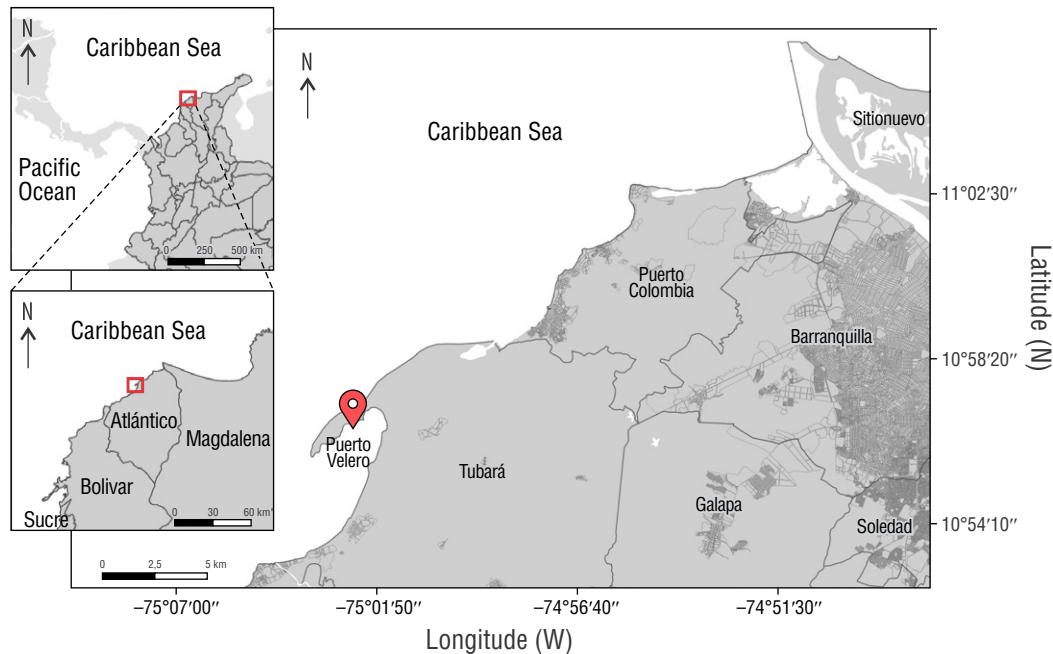
only been observed from 2024 onwards. Additionally, the presence of Clavelinidae colonies was assessed on natural substrates in areas adjacent to PVM in the Atlántico Department, namely Caño Dulce (November 2024), Mallorquín Coastal Lagoon (January and July 2025), and Puerto Caimán (March 2026), as well as within the larger Puerto Velero area outside PVM (continuously monitored through random sampling since 2017).

Two colonies used for morphological analysis were relaxed in seawater with added menthol crystals before being fixed and preserved in 10% formalin (Rocha et al. 2005). An additional colony was preserved in absolute ethanol for morphological analysis and molecular barcoding. For zooid analysis, 10 zooids per colony were isolated from the tunic matrix, and their tissues were examined using hematoxylin staining. In addition, larvae were extracted from the atrial cavity and mounted on permanent slides. Identification was conducted following the guidelines of Van Name (1921), Rocha et al. (2012), and Ordoñez et al. (2016), in accordance with WoRMS (2026) taxonomy. The biological specimens were collected in compliance with the permit granted to *Universidad del Atlántico* by *Agencia Nacional de Licencias Ambientales* based on Resolution 1214 of 29 September 2017 and Ethics Resolution 00594 of 26 April 2018. The voucher material was deposited in *Museo de Historia Natural Marina de Colombia-Makuriwa* (Santa Marta) under the acronym INV TUN.

Salinity was measured during each field survey using a PAL-SALT refractometer (ATAGO Co. LTD., Tokyo, Japan). Field surveys were conducted monthly during a total of 13 months within the period of August 2024 to April 2026. Temperature was continuously recorded at 2-h intervals using HOBO Pendant MX2201 data loggers (Onset Corporation, Bourne, USA) during the data collection and monitoring period of August 2025 to April 2026. These variables, together with those reported by Gracia et al. (2021), constitute the environmental dataset currently available for the PVM area.

### Molecular analysis

Two zooids of one colony were selected for molecular analysis (INV TUN0364). Genomic DNA was isolated using a DNA2000 kit (CorpoGen, Bogotá, Colombia), and the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the 5X Hot FirePol Blend Master Mix to Load RTL and the universal COI primers LCO1490 (5'-GGT-CAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). A COI fragment of ~610 bp was obtained. Annealing conditions were set at 50 °C for COI. The thermal cycling protocol consisted of an initial denaturation step at 95 °C for 10 min, followed by 35 cycles for 30 s at 94 °C, annealing at 50 °C for 30 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 5 min. Amplified products were sequenced by CorpoGen using the Sanger method,



**Figure 1.** Study area in the Colombian Caribbean where *Clavelina oblonga* was recorded.

with both strands sequenced. Forward and reverse sequences were assembled and edited using Geneious Prime v. 2023.0.4 (Kearse et al. 2012). Edited sequences were assembled and compared with the GenBank database to confirm marker identity. The resulting sequences were deposited in GenBank under the accession numbers PZ324367 and PZ324368.

## RESULTS

Morphological information and DNA barcoding confirmed the identity of *Clavelina oblonga* Herdman, 1880 in the Colombian Caribbean, where it was recorded exclusively on hard substrates in Pvm. The present study constitutes the first record of the family Clavelinidae Forbes & Hanley, 1848 and *C. oblonga* in the Colombian Caribbean. The taxonomy of *C. oblonga* is given below.

Class Ascidiacea Blainville, 1824  
 Order Aplousobranchia Lahille, 1886  
 Family Clavelinidae Forbes & Hanley, 1848  
 Genus *Clavelina* Savigny, 1816  
*Clavelina oblonga* Herdman, 1880 (Fig. 2a–g)

### Examined material

INV TUN0362 (01/8/2024) and INV TUN0364 (16/11/2024) were found attached to concrete slabs at a depth

of ~0.3 m in the shaded areas of the main walkway in Pvm. INV TUN0365 (10/5/2025) was collected from a submerged plastic rope at a depth of 2 m in a well-lit area. A total of 10 zooids from each colony were morphologically analyzed. However, INV TUN0364 consisted of a small colony, and all its zooids were used for molecular analysis following morphological examination, which left only the tunic as a tissue voucher. Additionally, observational records were obtained between May 2025 and April 2026. Most records were supported by photographic evidence.

### Diagnosis

Colonial ascidian. Tunic smooth, mostly transparent, with scattered whitish flecks along the endostyle and peripharyngeal groove (Fig. 2a–c); firmer and more consistent toward base. Basal region light beige due to adhered sediment. Zooids partially embedded; abdomens integrated into a common basal mass; thoracic portions free within thick anastomosed digitations fused basally. Zooids 2.6 cm long when removed from the tunic; however, measurements varied, with less developed zooids measuring ~0.5–1.0 cm; body divided into thorax and elongated posterior abdomen (Fig. 2d). Oral and atrial siphons with smooth margins; circular musculature more evident in the atrial siphon. Oral tentacles (16–19), alternating in size. Pharynx with 15 rows of stigmata (Fig. 2e), consistent with the description of Van Name (1945); dorsal tubercle

simple and horizontally elongated. Esophagus long; stomach circular with 4 longitudinal folds. Gonads within the intestinal loop. Basal region with stoloniferous vessel network (Fig. 2f). Incubation pouch present in atrial cavity, containing brooded larvae. Larva (tadpole type) with 3 adhesive papillae arranged triangularly; ocellus and statocyst present (Fig. 2g).

### Molecular analysis

BLAST searches against GenBank identified *C. oblonga* as the closest match for both sequences. Sequence identities were 100% with specimens from the Azores (AY603106.1) and Puerto Rico (MT637981.1), with query coverages of 96% and 94%, respectively. The next closest matches were also *C. oblonga* sequences from Puerto Rico, with 100% sequence identity (MT637963.1 and MT637945.1) and query coverages of 95% and 92%, respectively. Similarly high sequence identity values (>99.8 %) were also detected in specimens from Brazil (MK397826.1), Spain (KF309648.1), Greece (PQ397366.1), and the United States (MK397825.1).

### Ecology

In PVM, colonies were found attached to concrete slabs, metallic pilings, and ropes in both shaded and well-lit areas. Observations of non-collected colonies suggest that the species is abundant in the area, particularly on submerged ropes. No evidence of colonization was found on the surrounding natural substrates. The average salinity in PVM was  $27.9 \pm 1.4$  psu, with a minimum value of 25.6 psu in October 2025 and a maximum value of 30.8 psu in March 2026. Continuous temperature data recorded every 2 h from August 2025 to April 2026 ranged from  $26.3$  °C to  $32.3$  °C, with average day and night temperatures of  $28.9 \pm 1.5$  °C and  $29.8 \pm 0.6$  °C, respectively. Non-quantitative field observations indicated persistently low water visibility (<2 m) during nearly all field trips that prevented direct observations of the seafloor. Water conditions were only clear enough to allow visual observation of the seafloor in October 2025.

Colonies were observed growing in association with macroalgae and various invertebrates, including sponges, bryozoans, and other ascidians (Fig. 2a–c). Additionally, 3 polychaete individuals were found among the stolons of the zooids in specimen INV TUN0362. Most examined colonies showed a heavy accumulation of organic material and sediments at the stolon base (Fig. 2b–c).

## DISCUSSION

### Biogeography and distribution

*Clavelina* is a globally distributed genus that currently comprises 49 recognized species (WoRMS 2026). In the tropical Atlantic, 6 species are known to occur: *Clavelina oblonga*; *Clavelina picta* (Verrill, 1900); *Clavelina puertosecensis*

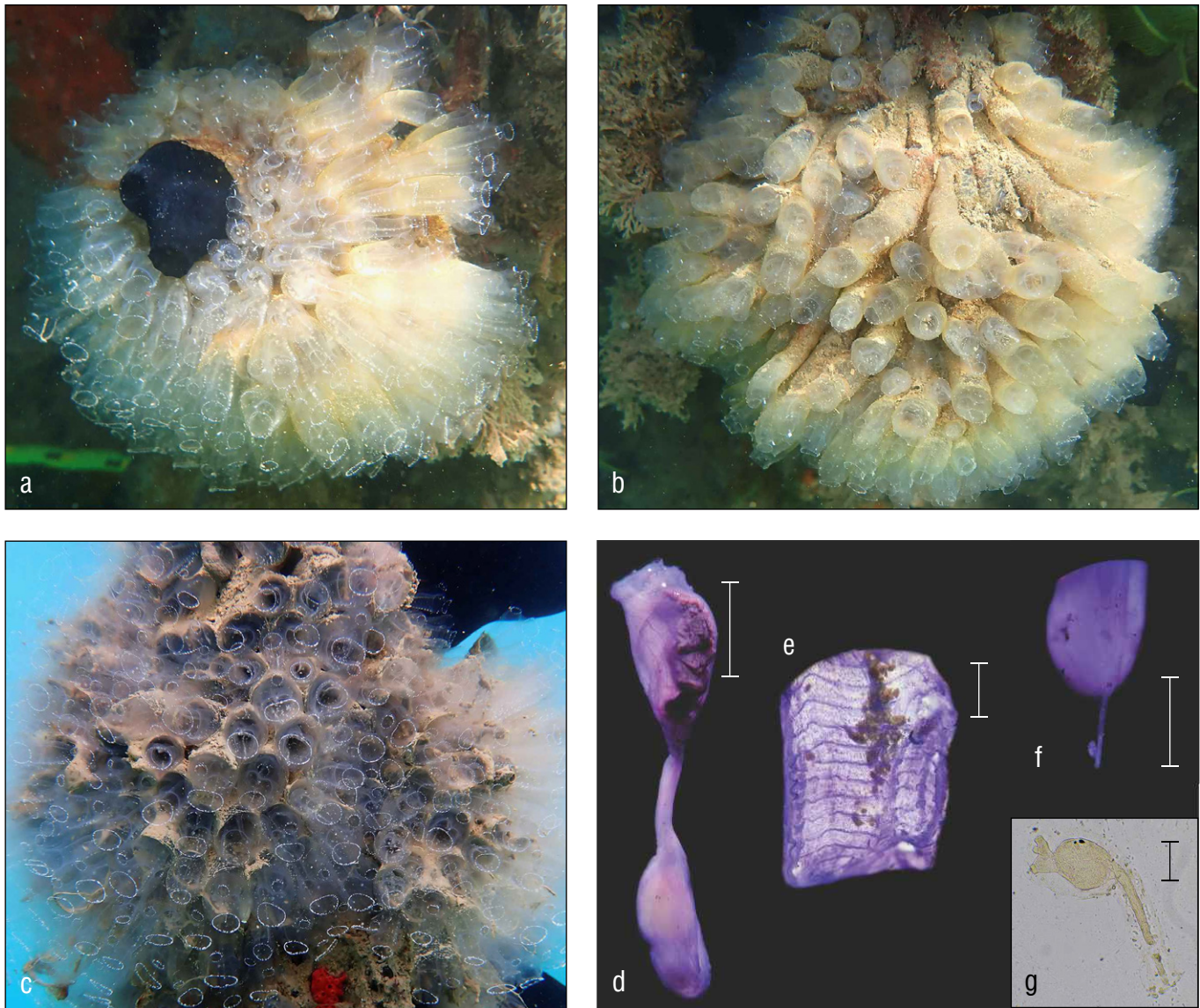
Millar & Goodbody, 1974; *Clavelina rochae* Turon & López-Legentil, 2024; *Clavelina pawliki* Turon & López-Legentil, 2024; and *Clavelina erwinorum* Turon & López-Legentil, 2024. Among these, *C. oblonga* is the most widely distributed species, as it occurs throughout the tropical Gulf of Mexico and western Atlantic, including Florida, Mexico, Jamaica, Belize, St. Thomas, Bermuda, Trinidad, Puerto Rico, Panama, Venezuela, Aruba, and Guadeloupe (Goodbody 2000, 2003; Rocha and Faria 2005; Palomino-Álvarez et al. 2019), as well as in Colombia (present study). This species has also been introduced in several regions outside its native range, such as the southern and southeastern coasts of Brazil, the Azores, Cape Verde, Senegal, Spain, Italy, and Croatia (Rocha et al. 2012, Ordóñez et al. 2016, Popovic et al. 2025).

*Clavelina picta* has been reported in the Bahamas, Bermuda, Belize, and Cuba, whereas *C. puertosecensis* is known to occur in Belize, Guadeloupe, and Jamaica (Goodbody 2000, Rocha and Faria 2005, Turon and López-Legentil 2024). Thus far, the most recently described species (*C. rochae*, *C. pawliki*, and *C. erwinorum*) are restricted to the Bahamas. In addition, unidentified *Clavelina* species have been recorded in Panama (Rocha et al. 2005) and Mexico (Palomino-Álvarez et al. 2019). Together, these records indicate the growing distribution of *Clavelina* in the tropical Atlantic and highlight the limited knowledge of this genus and its hidden diversity in the region.

### Molecular analysis

DNA barcoding using the COI gene is a reliable tool to identify metazoan species (Hebert et al. 2003), particularly colonial ascidians, which exhibit phenotypic plasticity and overlapping morphological traits that can make species-level identification challenging (Newlon et al. 2003, Selva-Prabhu et al. 2012). In this context, our study provides the first COI-based molecular identification of ascidians from the central Colombian Caribbean. Although molecular analyses were conducted on only one colony, the morphological uniformity among the 3 examined colonies confirmed the identification of the specimens as *C. oblonga*.

Previous studies have shown that *C. oblonga* exhibits very low genetic variation in the COI marker across its distribution. Stach and Turbeville (2002) and Rocha et al. (2012) reported only 4 haplotypes among colonies collected along the Atlantic coasts of the United States, Panama, and Brazil, which were also shared with introduced populations in the Mediterranean Sea (Ordóñez et al. 2016). The high sequence identity values (>99 %) obtained in the present study with specimens from Puerto Rico, Azores, Brazil, Spain, Greece, and the United States support the idea that *C. oblonga* maintains a highly conserved COI haplotype throughout its distribution range. Rocha et al. (2012) suggested that this low genetic variation may be associated with human-mediated dispersal and the frequent occurrence of the species in artificial environments such as marinas (Selva-Prabhu et al. 2012).



**Figure 2.** Field and laboratory observations of *Clavelina oblonga*. A colony growing on an artificial substrate (metallic piling); a specimen of *Phallusia nigra* is visible inside the colony (16 August 2025; depth: 0.6 m) (a). Colony showing a high accumulation of sediments (16 August 2025; depth: 0.7 m) (b). Colony growing on an artificial substrate (i.e., plastic rope) (25 April 2026; depth: 1 m) (c). Zooid removed from the tunic (d). Detail of the pharynx (e). Basal portion with a network of stoloniferous vessels (f). Tadpole-type larva showing 3 adhesive papillae (g). Scale bars: 2.5 mm (d), 1 cm (e), and 1 mm (f, g).

The fact that our specimens were collected from a marina is consistent with this ecological pattern and further supports the role of such habitats in facilitating both the spread and genetic homogenization of *C. oblonga*.

**Ecology: habitat associations and environmental influences**

*Clavelina oblonga* has been recorded on both natural and artificial substrates within its native distribution range (Goodbody 2000). The species was among the most abundant ascidians recorded in the mangroves of La Restinga National

Park, Venezuela (Rocha et al. 2010). A study conducted in harbors and marinas in Puerto Rico (Streit et al. 2021) reported the presence of *C. oblonga* on artificial substrata in 4 of the 10 marinas surveyed at depths of <2 m. Streit et al. (2021) reported an average salinity of 34.9 psu across the 10 sampled areas, although no ascidian species were recorded in one site that exhibited markedly lower salinity (25 psu). The mean salinity estimated in the present study was 27.9 psu, which suggests the species is tolerant to broader salinity range.

In its invasive range, *C. oblonga* has been found on a variety of substrates, including natural rocks, pier columns, mussel

socks, oyster lantern nets, and mussel and oyster farming gear (Rocha et al. 2012, Ordoñez et al. 2016, Popovic et al. 2025). In addition, the species has been reported in shallow waters at depths <2 m (Goodbody 2000) and at depths of 7 m and 11 m (Palomino-Álvarez et al. 2019).

According to Palomino-Álvarez et al. (2019), specimens from Campeche (Mexico) were found associated with the hydroid *Macrorhynchia philippina* Kirchenpauer, 1872, which may provide protection against predation. In PVM, *C. oblonga* was observed in association with multiple invertebrate taxa; however, given the high levels of biofouling on the artificial structures in this marina (Gracia et al. 2021), such associations should be interpreted with caution. Space availability in these environments is often a limiting factor that strongly influences settlement patterns and forces close spatial associations among diverse sessile organisms (Osman and Whitlatch 1995, Lambert 2007).

Observations of *C. oblonga* suggest that it is a shade-tolerant species that typically thrives in areas where overhanging mangroves provide protection from direct sunlight (Goodbody 2003). However, in the Colombian Caribbean, *C. oblonga* has also been recorded in well-lit environments, although the high-water turbidity at PVM likely acted as a natural light filter, creating conditions functionally similar to those of shaded habitats.

From the observations conducted in 2025, 3 main patterns were identified in the present study. First, small colonies occurring at depths of less than 1 m were observed in August 2025 and September 2025. These remained in as polyps with low development during October 2025 and were no longer visible in 2026. Their disappearance in 2026 may have been related to salinity fluctuations associated with the onset of the rainy season (Lambert and Lambert 2003, Rocha et al. 2017) or to the continuous input of suspended sediments. However, the observations were recorded incidentally and not from continuous monitoring of specific individuals. Thus, the influence of other localized factors typical of marina environments cannot be ruled out. Furthermore, this explanation remains uncertain, as the species was observed in well-developed colonies in other areas of the marina, particularly on submerged ropes where numerous colonies were recorded in November 2025. This heterogeneity in distribution, which was characterized by colonies thriving in one spot while disappearing in another nearby, is consistent with patterns observed in other anthropogenically modified embayments, where environmental variables and community structure have been observed to vary notably over very short distances (Tracy and Reynolds 2014).

The second pattern was characterized by colonies growing on submerged ropes at depths of  $\geq 1.5$  m that were generally well developed and, in some cases, dominant on the substrate. This pattern suggests that light exposure may play an important role in the distribution of *C. oblonga*. Under naturally high turbidity conditions and at greater depths, *C. oblonga* appeared to thrive, which is consistent

with previous reports describing the species as being shade tolerant (Goodbody 2000, Houle 2015, Turon and López-Legentil 2024).

The third pattern that should be considered is the observed seasonal biology of the species. *Clavelina* species are known to exhibit seasonal cycles in which colonies may undergo periods of regression, in which they drastically reduce their size or become visually undetectable by persisting only as dormant basal stolons or resistant buds before reappearing under more favorable environmental conditions, particularly during warmer periods (Berril 1951, De Caralt et al. 2002, Ordoñez et al. 2016). Reproductive dynamics may also be linked to this cycle. In the colonies collected from submerged ropes in May 2025 (INV TUN0365), most zooids contained a high proportion of embryos in various stages of development; only a few fully developed, free-swimming, tadpole larvae were observed at this time. Larvae in more advanced developmental stages were observed in specimens collected in August 2024 (INV TUN0362). Overall, these observations could indicate a seasonal pattern in the occurrence of the species and its early life-history stages in the study area.

In the Atlántico Department, a continuous input of suspended sediments promotes fine-particle accumulation on submerged structures, as observed on the stolons of the colonies in the present study that exhibited a thin layer of sediment that resulted in a “dirty” appearance of the tunic (Fig. 2b–c). Similar observations have been reported for ascidians inhabiting muddy or silty environments, where fine sediments often adhere to the tunic (Monniot et al. 1991).

### Dispersal pathways

The presence of *C. oblonga* in the region may be explained by 2 non-exclusive scenarios, given that the species is native to the Caribbean and its natural distribution includes parts of this area. One possibility is natural dispersal through oceanic currents or larval transport. Like most colonial ascidians, *C. oblonga* is a hermaphrodite that broods its offspring (Ordóñez et al. 2016), with eggs developing into lecithotrophic larvae that subsequently metamorphose into benthic adults (Young 2002). In *C. oblonga*, these larvae are short-lived in the water column and consequently have limited dispersal potential (Popović et al. 2025). Given this scenario, *C. oblonga* may have been historically present in the Colombian Caribbean but remained undetected due to the paucity of systematic inventories or very low population densities. Since 2017, species monitoring across multiple taxonomic groups has been conducted in the marina and in adjacent areas; however, *C. oblonga* has not been observed on other substrates. In contrast, other colonial and solitary ascidians have been recorded and are currently the subject of ongoing study. An alternative explanation is accidental human-mediated transport via biofouling on ship hulls, buoys, or ropes. In this case, the record would represent a translocation, secondary

spread, or human-assisted dispersal within the native range of *C. oblonga*, with eggs or larvae transported through anthropogenic vectors. The occurrence of *C. oblonga* in PVM supports this hypothesis, as recreational boats provide suitable substrates for fouling communities and may facilitate secondary spread when moving between ports and marinas (Ojaveer et al. 2018).

## CONCLUSIONS

This study provides the first morphological and molecular confirmation of *C. oblonga* in the Colombian Caribbean. Although previously described as a shade-associated species, in Puerto Velero, it was found in both shaded and well-lit areas with high turbidity, which suggests that the ecological plasticity of *C. oblonga* has allowed it to adapt to different environmental conditions and substrates. Its occurrence may reflect either a previously undetected native distribution or human-mediated dispersal and highlights the importance of monitoring fouling communities in Caribbean marinas.

## DECLARATIONS

### Supplementary Material

This work includes no supplementary material.

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### Conflict of interest

The authors declare they have no conflict of interest.

### Author contributions

Conceptualization: KBP, AG; Data curation: KBP, APR, SNC, AG; Formal analysis: KBP, APR, SNC, AG; Investigation: KBP, APR, SNC, AG; Methodology: KBP, APR, SNC, AG; Writing—original draft: KBP, APR, SNC, AG;

Writing—review & editing: KBP, APR, SNC, AG; Funding acquisition: AG.

### Data availability

The data for this study are available from the corresponding author by reasonable request.

### Use of AI tools

The authors did not employ any AI tools in this work.

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