Record of a bypass on the oral siphon of *Ascidia sydneiensis* (Tunicata: Asciidiacea) in a tropical bay from south-eastern Brazil

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*Ascidia sydneiensis* Stimpson, 1855 is a solitary ascidian (Order Phlebobranchia) first described in the China Sea and Japan. Recently, it has been recorded in subtropical and tropical regions and is abundant in the Caribbean (Rocha et al., 2005; Worms, 2020). In Brazil, it was first recorded in the São Paulo State (Bjornberg, 1956) and ranges from Ceará to Santa Catarina (Rodrigues, 1962; Rocha and Nasser, 1998; Rocha and Costa, 2005; Rocha and Kremmer, 2005; Rocha et al., 2012). On the coast of Rio de Janeiro State, it was recorded at Ilha Grande Bay, Sepetiba Bay, Arraial do Cabo, and Búzios (Marins et al., 2010; Granthon-Costa et al., 2016).

*Ascidia sydneiensis* occurs on both natural and artificial hard substrates (Rodrigues, 1962; Rocha and Kremmer, 2005). *Ascidia sydneiensis* has a cartilaginous and soft tunic and, in vivo, is colourless or grayish and usually covered by epibionts. The atrial siphon, which is lobed and has strong circular muscles, is distant from one to two-thirds of its body length from the terminal oral siphon (Kott, 1985). This note reports the occurrence of an individual of *A. sydneiensis* with a filtering anatomical anomaly.

An individual with an additional siphon opening (Figure 1) was collected in January/2018 from a natural substrate at Ilha da Amarração, next to Ilha Grande, Rio de Janeiro state, Brazil (23°10'58.0"S, 44°10'47.7" W). This atypical individual had a small projection with rims and other morphological features, on the tunic next to a larger oral siphon. After collection, the animal was anesthetized with menthol and fixed in 10% formaldehyde. The presence of the third opening was not noticed during the removal of the animal from the substrate but was noted in the laboratory during dissection. Part of the larger oral siphon on the mantle projects into a secondary siphon (bypass) that connects to the tunic and then to the exterior. Both siphons show evident lobes, seven on the larger and five on smaller. The latter also shows projections on its margins, similar to those reported by Kott (1985) and Bonnet and Rocha (2011). Siphon musculature has longitudinal and circular muscle bundles, with the former being more developed. Our specimen showed marked longitudinal muscles along the siphon lobes. Seven bands were observed on the larger siphon, and four on the smaller. There was a gap between the two oral siphons, with an almost absence of muscular fibers and some of the muscle bundles showing changes in direction (Fig. 2).

Both siphons lead to a shared channel with a single tentacular region that projects into the branchial sac. No other structural modifications were observed as compared to the normal anatomy of *A. sydneiensis*. Although filtration activity through the additional siphon was not observed, we assume that...
Figure 1. *Ascidia sydneiensis* with anomaly in the filtering system, with two oral and one atrial siphons: a) external appearance of the specimen inside the tunic; b) detail of the oral siphon with the tunic, the red arrow indicates the second siphon; c) appearance of the specimen outside the tunic; and d) detail of the anomaly in the oral siphon with the red arrow indicating the second oral siphon.

Figure 2. *Ascidia sydneiensis* individual modification in the filtering structure: A) distribution of the muscle bundles present in the siphons; and B) Siphon muscle bundles shown indicating their distribution between the siphons. Muscles bundles colored red are shared by both siphons, while those colored blue are exclusive to the major siphon.
it was functional as the syphon opened when relaxed with menthol and because of the presence of shared muscle bundles on both siphons.

In contrast to the bypass recorded here, the presence of additional siphonal openings in ascidians in the wild has only been reported for atrial siphons, never on oral siphon. Such duplications and bypasses can occur naturally or be experimentally induced (Geoge, 1937; Berril, 1951). *Styela clava* and *Polycarpa mytiligera* (Order Stolidobranchia) have been reported with an additional atrial siphon resulting from predation (Epelbaum *et al.*, 2007; Gordon & Shenkar, 2018). The experimental obstruction of the atrial siphon of *Styela plicata* resulted in a bypass on that organ, highlighting the plasticity of the ascidian body growth (Geoge, 1937). Some species, such as *Ascidella aspersa* and *Ciona intestinalis* (Order Phlebobranchia), have shown true duplication of their atrial siphons including internal structures (Kerkenberg and Hofmann, 1989; Hamanda, 2015). Individuals of the genera *Phallusia*, *Ascidia*, *Ciona*, and *Ascidella* had two atrial siphons on the third day after settlement, although those doubled siphons merged at the end of metamorphosis, resulting in a single structure (Kerkenberg and Hormann, 1989). Thus, if any abnormality occurs during this developmental stage, an anomalous adult could result, such as one with a duplication of the atrial siphon (Epelbaum *et al.*, 2007).

The dual opening of the oral siphon observed in *A. sydneiensis* is suggested to be either regeneration after predator damage to the siphon or obstruction. A new siphon, or new opening, can develop in just a few hours (Geoge, 1937), originated from epidermal or atrial epithelial tissues (Berril, 1951). As both siphonal openings share several structures, such as a ciliated groove and tentacles, it can be considered a bypass. However, even that possibility raises questions about regeneration, as the "regenerated" siphon has a similar rim structure and reflects the plasticity of the individual in adapting to water and food intakes. The evolutionary meaning of regeneration or the reorientation of body structures such as the neural complex, internal organs, the digestive system, and even peripheral structures such as siphons in ascidians, can reflect a recovery from predation or competitive pressure from overgrowth (Brow *et al.*, 2009; Dahlberg *et al.*, 2009; Gordon & Shenkar, 2015). Sublethal predation may be responsible for maintaining evolutionary regenerative capacity of ascidians (Lindsay, 2010; Gordon and Shenkar, 2018).

Between 2009 and 2020, 245 individuals of the genus *Ascidia* were collected during predator exclusion experiments in Rio de Janeiro State and just a single case of a siphonal bypass was recorded among 126 individuals of *A. sydneiensis*. Although ascidians with some degree of duplication of the atrial siphon and associated structures are rare in nature (Epelbaum, 2007), this phenomenon could be quite significant for future studies in areas such as adaptative ecology, evolutionary biology, embryology, and morphology that use ascidians as a model.

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**AUTHOR CONTRIBUTIONS**

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