

# A first record of biphallia in imposexed female of *Leucozonia nassa* (Caenogastropoda: Fasciolaridae)

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Imposex is characterized by the development of male characteristics, such as the penis and vas deferens, in female prosobranch gastropods, caused by organotin compounds contamination. In Brazil, imposex was observed in the muricids *Stramonita haemastoma* and *Stramonita rustica* and in the olivid *Olivancillaria vesica*. During the low tide, 54 adult individuals of *Leucozonia nassa* were manually collected in February 2004 at Ilha do Japonês tidal flat, Cabo Frio County, Rio de Janeiro State, south-east Brazil. About 98.15% of females presented imposex (N=30). Their penises were smaller than male ones. Only 1 imposexed female presented a biphallia problem, showing 2 flattened penises with a small scourge in the edge. This is the first record of biphallia in an imposexed female of fasciolarid *Leucozonia nassa*.

Imposex is characterized by the development of male characteristics, such as the penis and vas deferens, in female prosobranch gastropods (Smith, 1971). This abnormality is caused by the exposure of those animals to organotin compounds (OTs) in antifouling paints (Gibbs & Bryan, 1987; Matthiessen & Gibbs, 1998), used world-wide as covering of boats and other metallic structures in order to prevent incrustations and corrosion, which are processes that drastically reduce the income of boats and the durability of these structures.

The study of imposex has been used at several places around the world as a tool to monitor contamination by organotin compounds, since the methods of chemical analysis are extremely onerous (Oehlmann et al., 1996). In Brazil, imposex was observed in the muricids *Stramonita haemastoma* (Linnaeus, 1767) (Castro et al., 2000; Fernandez et al., 2002; Fernandez et al., 2005; Castro et al., 2007a,b) and *Stramonita rustica* (Lamarck, 1822) (Camillo et al., 2004; Castro et al., 2004, 2007) and in the olivid *Olivancillaria vesica* (Gmelin, 1791) (Caetano & Absalão, 2003).

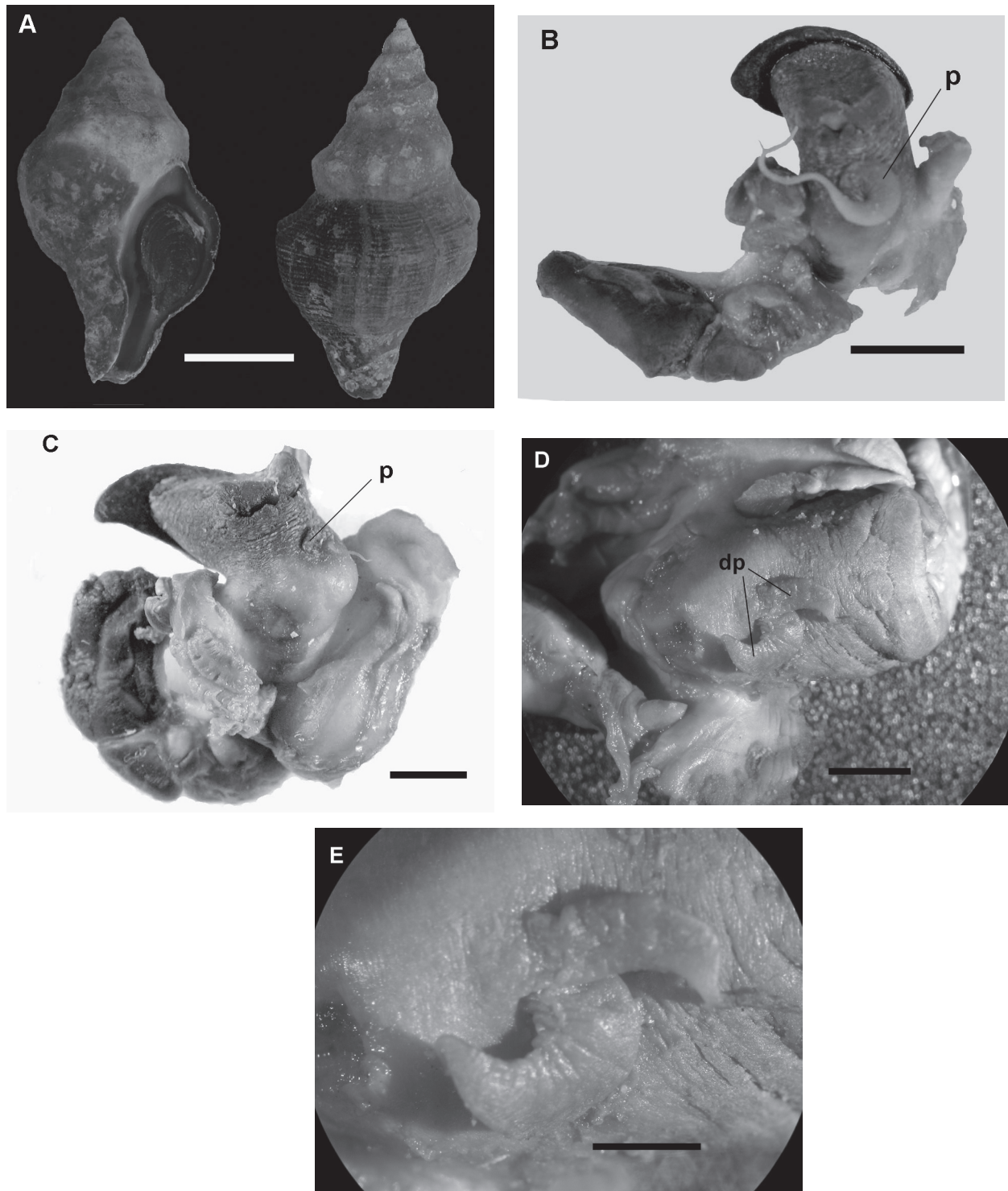
During the low tide, 54 adult individuals of *Leucozonia nassa* (Figure 1A) were manually collected in February 2004 at Ilha do Japonês tidal flat, Cabo Frio County, Rio de Janeiro State, south-east Brazil. The animals were kept in plastic boxes containing seawater from the respective sites and were taken to the laboratory. The molluscs were preliminarily narcotized with magnesium chloride solution 3.5% (Huet et al., 1995). The soft parts were examined using a binocular microscope. Individuals of *Leucozonia nassa* with oviducts and penis were considered imposexed females.

The male penis has a robust base and a long scourge in the edge (penis length=9.4 ± 1.0 mm) (Figure 1B). Sometimes its thread-like white tip extended far into the palial cavity; sometimes it was completely retracted into the red base of the copulatory organ.

About 98.15% of females presented imposex (N=30). Their penises were smaller than male ones (5.96 ± 3.15 mm in length) (Figure 1C) and, in some cases, a well developed vas deferens was observed. Only 1 imposexed female presented a biphallia problem, i.e. it had a double penis. These flattened penises were similar in shape and length (4.35 and 4.75 mm, respectively), with a small scourge in the edge (Figure 1D&E). This is the first record of biphallia in an imposexed female of fasciolarid *Leucozonia nassa*.

The mechanism leading to imposex in female prosobranch is not well understood, but the inhibition of the cytochrome P450-mediated aromatase activity (CYP19) (responsible for the conversion of androgens to estrogens) by organotin compounds has been suggested as a possible mechanism for imposex, resulting in increased tissue androgen levels (Bettin et al., 1996).

This tissue proliferation, in advanced levels, blocks the females' genital system (Gibbs & Bryan, 1987; Gibbs et al., 1987). This situation usually causes sterilization and death due to the presence of aborted capsules in the capsule gland (Gibbs & Bryan, 1986; Gibson & Wilson, 2003). Reproductive failure was observed in populations of muricid *Nucella lapillus* (Linnaeus, 1758) which severely declined during the 1980s in southern England (Gibbs & Bryan, 1986) due to imposex effects and their intracapsular development (no larval exchange from healthy populations) (Spence et al., 1990). This is an important concern from a conservationist point of view, since high levels of imposex could lead some gastropod populations with intracapsular development to extinction (Gibbs & Bryan, 1986).



**Figure 1.** *Leucozonia nassa*. (A) Adult shell; (B) soft parts of an adult male; (C) soft parts of an imposed female; (D) soft parts of an imposed female with biphallia; (E) detail of the double penis. Abbreviations: dp, double penis; p, penis. Scale bars: A, 15 mm; B, 5 mm; C, 6 mm; D, 4.5 mm; E, 2.4 mm.

Some countries, such as England and Ireland, have introduced a law that prohibits the use of organotin on vessels under 25 m and other structures (Evans et al., 1991; Michin et al., 1995). This law has been successful in reducing levels of OTs and imposex levels in dogwhelks over a 6 year period (Michin et al., 1995). Other studies have shown interesting results against imposex effects, such as the co-exposure to 3-MC (3-methylcholanthrene) (McClellan-Green & Robbins, 2000). It appeared to mediate the endocrine-disrupting effects of OT exposure by reducing the penis size in imposed females of *Ilyanassa obsoleta* (Say, 1822) and restoring their capacity to lay eggs (McClellan-Green & Robbins, 2000).

The main problem of organotin contamination monitoring is to know the effects of each organotin contamination level on the bioindicators. In another way, the relative organotin sensitivity among species was not tested (Camillo et al., 2004). Since it is probably different between species (Stroben et al., 1995), more studies are required to clarify this question.

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