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Morphological expression and histological analysis of imposex in *Gemophos viverratus* (Kiener, 1834) (Gastropoda: Buccinidae): a new bioindicator of tributyltin pollution on the West African coast

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ABSTRACT

We describe the reproductive system and morphological expression of imposex in the marine gastropod *Gemophos viverratus* (Kiener, 1834) for the first time. This species can be used as a bioindicator of tributyltin (TBT) pollution on the west coast of Africa, a region for which there is a lack of information regarding levels and impacts of TBT pollution. Adult *G. viverratus* were collected between August 2012 and July 2013 in São Vicente Island (Republic of Cabo Verde), at pristine sites on the northeastern and eastern coasts and at Porto Grande Bay, the major harbour in the country. All the females ($n = 81$) caught outside the bay were normal, but 99% ($n = 108$) of those collected inside the bay exhibited degrees of abnormal virilization. Seven levels of imposex intensity, from stage 0 (normal) to stage 6 (sterilized females), are described following at least three different pathways of evolution (a, b and c). Female sterilization was caused mainly by abnormal growth of 'tumour-like' tissues in the oviduct. A peculiar characteristic of imposex in this species is the development of a distal prostate by females, resembling the same organ exhibited by males. In the laboratory, imposex could be artificially induced by injecting very small doses of TBT into females, demonstrating that TBT is a causative agent of this syndrome.

INTRODUCTION

Tributyltin (TBT) compounds began to be used as biocidal agents of antifouling systems (AFS) in the mid-1960s. Due to unmatched antifouling effectiveness and economic advantages, TBT-based AFS soon dominated the market, covering an estimated 70% of the world fleet in the early 2000s (De Mora & Pelletier, 1997; Evans, 1999; Yebra, Kiil & Dam-Johansen, 2004). However, the adverse effects caused by TBT compounds in nontarget organisms led to a global ban of TBT-based AFS since 2008, through the AFS Convention (IMO, 2001).

Unfortunately, a high amount of TBT was released into coastal waters for decades, accumulating in sediments. The high persistence of TBT in deeper and anoxic sediments makes this compartment a dangerous reservoir of the biocide, which slowly remobilizes to the water column or contaminates benthic organisms through particle ingestion for many years (Burton, Phillips & Hawker, 2006; Lee, Hsieh & Tien, 2006; Galante-Oliveira *et al.*, 2010). Moreover, there are still regions where the AFS Convention has not been implemented. According to the IMO Convention status of February 2014 (IMO, 2014), the majority of West African countries, including the Republic of Cabo Verde, have yet officially to ratify the AFS Convention. Hence, it is likely that TBT pollution may still cause serious impacts on marine and estuarine ecosystems around the world for many years to come.

One of the most notable effects of TBT in nontarget organisms is imposex, a condition in which female caenogastropods develop male sexual characters, such as a penis and a vas deferens (Blaber, 1970; Smith, 1971). The specificity and strong positive relationship between imposex intensity and the level of TBT pollution, as well as the fact that imposex is triggered by very low concentrations of TBT in the environment (a few nanograms per litre of seawater), has allowed the imposex phenomenon to be effectively used worldwide as a biomarker for TBT pollution (Gibbs *et al.*, 1987; Schulte-Oehlmann *et al.*, 1997; Matthiessen & Gibbs, 1998). The intensity of imposex is usually measured through a scoring system, where the sequence of vas deferens and penis formation is classified into progressive stages, generally ranked from 0 (normal female) to 6 (female that has become sterile due to high levels of imposex) (Gibbs *et al*., 1987). This classification system, usually called the vas deferens sequence (VDS) and commonly represented by a VDS scheme (Schulte-Oehlmann *et al*., 1997), is suitable for many species, but an extended version with multiple pathways was proposed by Fioroni, Oehlmann & Stroben (1991) to fit more species. This was thereafter refined by Stroben, Oehlmann & Fioroni (1992) to represent the morphological expression of imposex in a wide diversity of caenogastropods. Imposex is known to occur in more than 260 species of gastropods around the world (Titley-O'Neal, Munkittrick & MacDonald, 2011). Although this number suggests the global scale of the phenomenon, there remains a surprising asymmetry among the geographical regions for which imposex has been reported. The main information gap occurs in Africa (Titley-O'Neal *et al*., 2011) where, to date, imposex has been reported only for species collected in Tunisia (Abidli *et al*., 2013), the Mediterranean coast of Morocco (Lemghich & Benajiba, 2007), Ghana (Nyarko & Evans, 1997) and the eastern seaboard of South Africa (Marshall & Rajkumar, 2003). The total lack of information regarding the occurrence of imposex for the almost entire coast of West Africa constitutes a serious setback, not only for the countries in the region, where it hinders any TBT-biomonitoring studies and ecological risk assessments, but also for the global effort to implement regulatory policies and monitor their effectiveness. This is of great concern since the West African coast is a region with increasing importance for shipping trade routes connecting the continents (UNCTAD, 2013). In fact, according to a recent study, 3,000,000 containers of cargo passed through West Africa in 2011 (CATRAM, 2013).

The present work analyses, for the first time, the morphological expression of imposex in the caenogastropod *Gemophos viverratus* (Kiener, 1834) (previously included in genus *Cantharus*, see WoRMS, 2014). This species occurs from the Canary and Cabo Verde Archipelagos to Angola, living on sandy and rocky bottoms to 10 m depth (Rolán, 2005). No previous study has addressed the occurrence of imposex in this species, but this phenomenon was fully described in a related species, *Cantharus cecillei*, from the coast of China by Shi *et al*. (2005).

The main objectives of this work are (1) to find an adequate VDS scheme to classify the levels of imposex intensity in *G. viverratus*, (2) to understand how imposex causes female sterility and (3) to test under controlled conditions if TBT can induce imposex in this species. These steps constitute a first approach before *G. viverratus* can be effectively tested as a bioindicator in field surveys and proposed as a TBT-pollution bioindicator for West Africa.

MATERIAL AND METHODS

Gemophos viverratus *sampling and imposex analysis*

A total of 371 adult *Gemophos viverratus* were collected between August 2012 and July 2013 on the coast of São Vicente Island, Republic of Cabo Verde. These were collected by hand in the intertidal rocky shore of pristine sites on the northeastern and eastern coast of the island and also at Porto Grande Bay, the major harbour in the country (Fig. 1B).

In the laboratory, the specimens were narcotized for 60 min using 7% $MgCl_2$ in distilled water. The shells were cracked open with a bench vice and the reproductive systems of males and females examined under a stereomicroscope.

The observed morphological expression of imposex in *G. viverratus* was compared with the general VDS scheme available for caenogastropods (Stroben *et al*., 1992) and also with the VDS scheme available for the Asian indicator species *C. cecillei* (Shi *et al*., 2005). A more complete assessment of the spatial gradient of imposex in *G. viverratus* and its organotin body burdens in São Vicente Island was also performed, but the complete dataset will be presented elsewhere (Lopes-dos-Santos *et al*., submitted); the present work aims mainly to describe imposex expression in this species. A detailed inspection of the anatomical changes caused by imposex was performed by examining the animals under a stereomicroscope and by analysing histological sections of target organs, both in normal individuals of both sexes and in imposex-affected females. After narcotization and examination under a stereomicroscope, the animals were fixed in Bouin's solution for 24 h and then preserved in 70% ethanol. Complete series of histological sections of 18 specimens embedded in paraffin were made (at 5–7 μm) using a Leica microtome, then stained with haematoxylin-eosin and mounted in DPX resin for observation by light microscopy.

TBT injection experiment

In order to test if TBT is able to induce imposex in *G. viverratus*, adult specimens were collected in July 2013 at the pristine site of Praia do Calhau, on the eastern coast of São Vicente Island (Fig. 1B). In the laboratory, specimens were acclimatized for 1 week in 15-l aquaria filled with artificial seawater (Prodac International® Sea Salt in dechlorinated tap water) at a salinity of 36, continuously aerated, biologically filtered and maintained at 20 ± 1°C with a photoperiod of 16 h light and 8 h dark, the same conditions as the exposure period. After acclimatization, animals were narcotized in 7% $MgCl_2$ in distilled water for

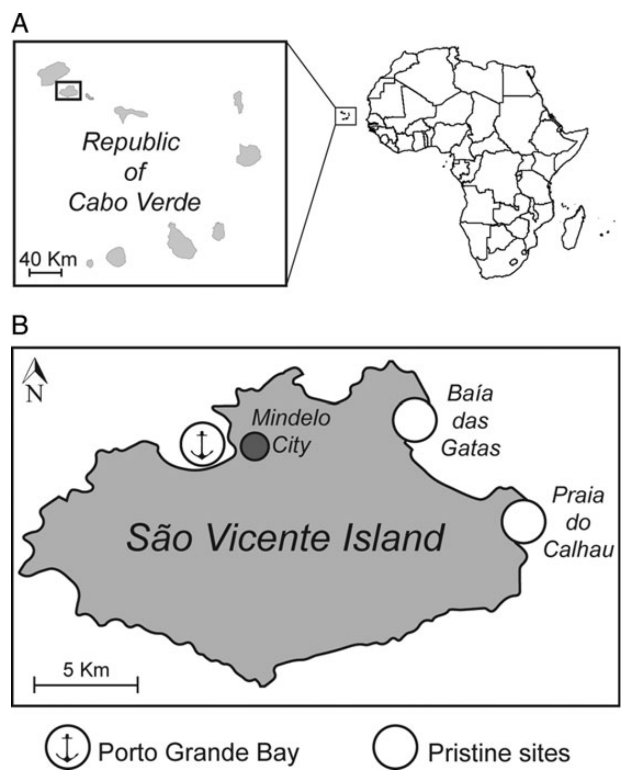

Figure 1. A. Location of São Vicente Island in the Republic of Cabo Verde. **B.** Map of São Vicente Island showing location of sampling stations for *Gemophos viverratus*.

60 min. Once narcotized, specimens were sexed and females were then examined under a stereomicroscope to screen any signs of imposex. All females observed were normal (i.e. not affected by imposex). They were then subjected through injection to the following experimental treatments: (1) water control (noninjected females); (2) TBT chloride (TBTCl, Sigma-Aldrich) at 1 μg Sn/g w/w soft body that was injected using dimethyl sulfoxide (DMSO, Sigma-Aldrich) as a vehicle and (3) DMSO control. Fifteen females were assigned to each treatment. DMSO and TBT females were first injected in the foot on day 0 with a volume proportional to the animal's soft body (3 μl/g w/w), using 5-μl microsyringes (Hamilton); a treatment boost by a second injection was performed on day 30 (1 month). The experiment ended 4 months after the first injection on day 0, during which period the animals were fed weekly with fresh pilchards and water was changed immediately after each feeding session. Females were then sacrificed and imposex assessed using the parameters proposed here (see below). The nonparametric Kruskal-Wallis test (K), implemented using the Statistica software package, was used to assess significant differences in VDS between treatment groups at the end of the experiment.

RESULTS

Spatial occurrence of imposex in São Vicente Island

A total of 371 *Gemophos viverratus* were analysed in the current work: 181 were obtained from the two pristine sites on the north-eastern and eastern coasts of the island, and 190 were collected from Porto Grande Bay. Among the 81 females collected at the pristine sites, none showed signs of imposex. On the contrary, of the 108 females collected inside Porto Grande Bay, only one female was normal (Fig. 2).

Anatomy of the reproductive system

There is a clear distinction between the male and female reproductive systems of *G. viverratus*. Sexes can easily be determined if animals are pulled slightly out of the shell, when a conspicuous penis can be seen behind the right ocular tentacle in males. Further differences are observed when the shell is removed and the mantle cavity is exposed. Females show a conspicuous capsule gland on the right side of the mantle cavity, beneath the rectum, leading forward to a vaginal opening (vulva) located in the right anterior region of the mantle cavity, in a ventral and anterior position in relation to the anus (Fig. 3A).

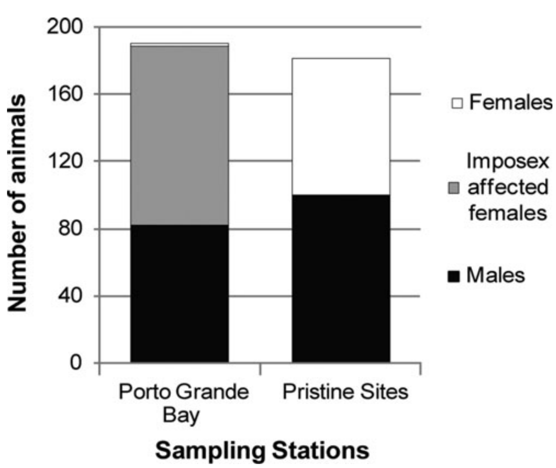

Figure 2. *Gemophos viverratus*. Imposex incidence in the harbour of Porto Grande Bay and in two pristine sites in the eastern part of São Vicente Island: Baía das Gatas and Praia do Calhau.

Histological sections reveal that the capsule gland leads to a muscular vestibule and then to the vagina and vulva. Between the vulva and the capsule gland (towards and just below the anus) the gonoduct gives rise ventrally to a long oval pouch—the bursa copulatrix—that extends beneath the capsule gland for about half of its length (Fig. 3B). Histological sections frequently showed the bursa filled with spermatozoa, which shows that, during copulation, the male inserts the penis into the vulva and discharges sperm and prostatic fluid into the bursa (Fig. 3B_5). From here, the sperm is conducted posteriorly through the ventral channel for fertilization somewhere close to the posterior end of the capsule gland, in the receptaculum seminis. The albumen gland lies in the posterior end of the duct behind the capsule gland. Between the albumen and the capsule glands a shallow darker ingesting gland is visible. The oviduct continues posteriorly towards the ovary, which lies over the digestive gland in the visceral mass.

In turn, males show a large, conspicuous penis behind the right ocular tentacle that may reach, at rest, as much as one-third of the length of the animal (Fig. 3C). The penis is composed of an extensible basal portion and a tip that is enclosed in a sheath. Inside the organ runs a channel—the penis duct—through which sperm are discharged into the female (Fig. 3D_7). Leaving the penis, this duct continues posteriorly as a vas deferens, at first straight, but then highly convoluted to form a 'distal prostate' (Fig. 3D_8). This prostate, located behind the penis, is enlarged and elevated smoothly above the mantle floor when fully developed. From here the vas deferens resumes its linear configuration and runs backwards along the right side of the mantle floor towards the rectum. When the vas deferens passes ventrally close to the rectum it again becomes convoluted and, covered by an epithelium, produces a voluminous and conspicuous second prostate (the 'proximal prostate') that accompanies ventrally the intestine along the pallial cavity; the convoluted coils of the vas deferens can be seen by transparency within the epithelium (Fig. 3D_9). Thereafter, the duct runs posteriorly towards the testis that lies close to digestive gland in the visceral mass. In the reproductive season, below the surface of the visceral mass, the vas deferens is greatly coiled and distended by a white sperm mass, forming a seminal vesicle.

Imposex development

The female reproductive system described above is valid for animals collected other than from Porto Grande Bay and is referred as the 'normal condition'. However, 107 out of the 108 females caught inside this bay exhibited various degrees of abnormal virilization or imposex.

The most affected females, representing about 61% ($n = 66$) of all the specimens obtained inside the bay, showed a complete penis with a sheath and a tip inside it (a character that can therefore not be used to distinguish sexes at these locations) and a vas deferens running through the mantle floor up to the vulva (15% of cases) or passing laterally close to the vulva (85% of cases) (Fig. 3E). Some of these females ($n = 22$) developed distal and proximal prostates, resembling the males (Fig. 3E). In the most extreme cases, females also showed an enlarged capsule gland ($n = 5$), tissue excrescencies near the vulva ($n = 3$) and one female showed aborted egg capsules inside the capsule gland. Histological sections revealed that seven of these females were sterilized due to malformations and constrictions of the vulva, vestibule, vagina and bursa copulatrix, resulting from the growth of tumour-like masses of cells in the region corresponding to the proximal prostate of males (Fig. 4A, B). However, histological sections of the ovary in females with advanced stages of imposex did not reveal any signs of spermatogenesis.

We found that the morphological expression of imposex in *G. viverratus* was similar to that described for *C. cecillei* by Shi *et al.*

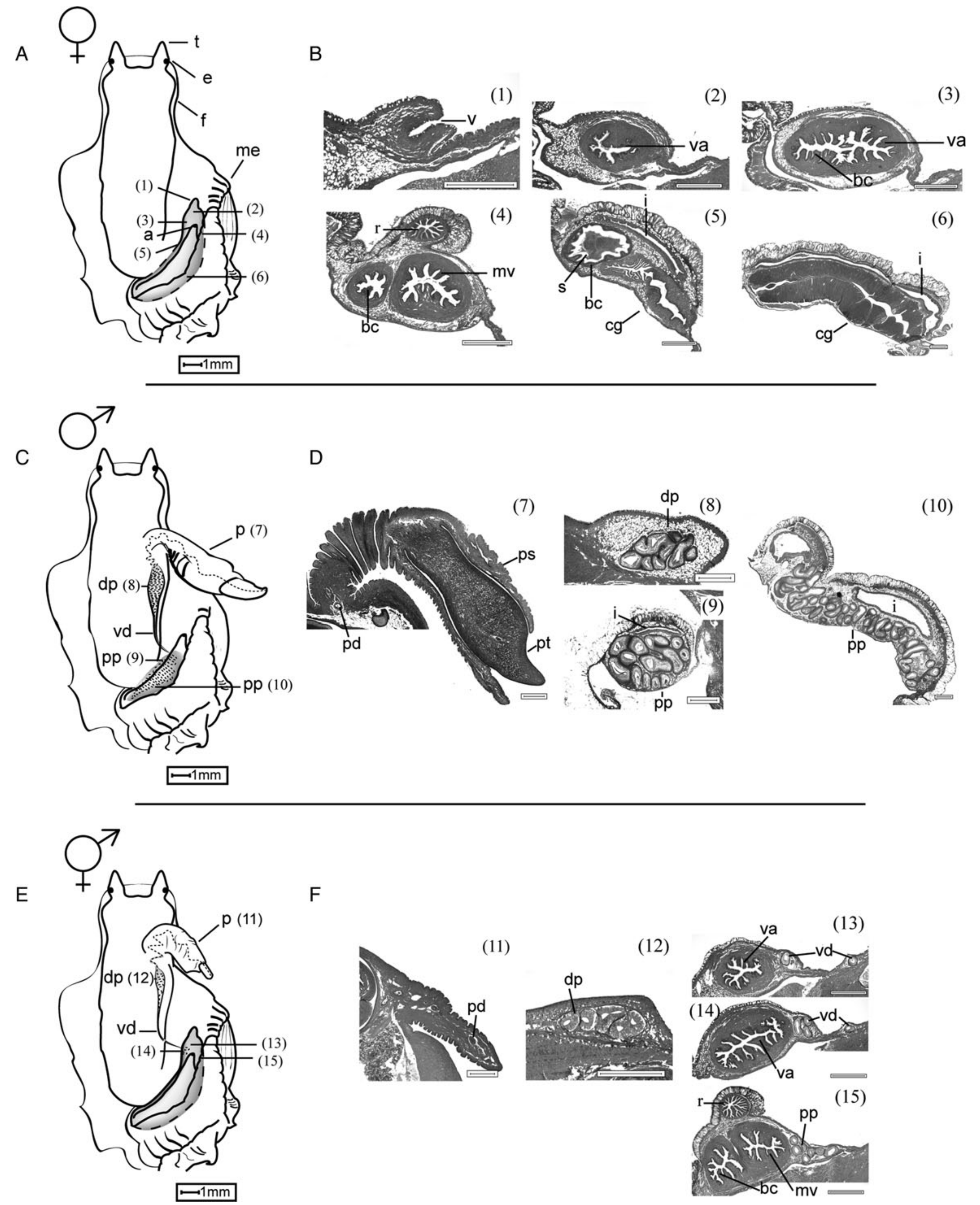

Figure 3. *Gemophos viverratus*. **A.** Female removed from shell and mantle cavity opened mid-dorsally to display anterior genital tract. **B.** Histological sections of organs indicated in **A**. **C.** Male removed from shell and mantle cavity opened mid-dorsally to display anterior genital tract. **D.** Histological sections of organs indicated in (**C**). **E.** Female with imposex and exhibiting VDS stage 4*. **F.** Histological sections of organs indicated in **E**. Abbreviations: a, anus; bc, bursa copulatrix; cg, capsule gland; dp, distal prostate; e, eye; f, foot; i, intestine; me, mantle edge; mv, muscular vestibulum; p, penis; pd, penial duct; pp, proximal prostate; ps, penial sheath; pt, penis tip; r, rectum; s, sperm; t, tentacle; v, vulva; va, vagina; vd, vas deferens. Scale bars **B, D, F** = 500 μm.

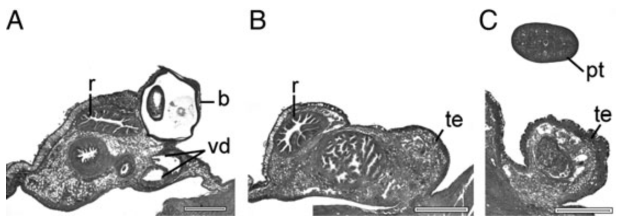

Figure 4. *Gemophos viverratus*. Abnormal growth of tumour-like excrescences and/or blisters in sterile females with advanced stages of imposex; sections from region of vulva and vagina, which are deformed and can no longer be recognized. **A.** Anterior genital tract showing blister and initial growth of proximal prostate. **B.** Tumour-like excrescence growing in anterior genital tract blocking vagina. **C.** Tumour-like excrescence growing in zone where distal prostate would develop in imposex-affected female. Abbreviations: b, blister; te, tissue excrescences; other abbreviations as in Figure 1. Scale bar = 500 μm.

(2005), but as this VDS classification scheme was originally derived from the more general one proposed by Stroben *et al.* (1992), both are intrinsically related and will be discussed here in parallel. These two VDS classification schemes have been used to describe *G. viverratus* imposex stages that were found (written in *italic*) or not found in this study (although some specific differences and adaptations of the VDS classification for *G. viverratus* will be noted in due course; Fig. 5).

Stage 1: type a: small penis, without a penial duct or penial sheath, behind the right ocular tentacle; type b (not observed): a short distal vas deferens section behind the right tentacle; *types c, c*:* a short proximal vas deferens tract beginning at (type c) or passing by (type c*) the vaginal opening.

Stage 2: type a: penis without a penial sheath but with a closing or closed penial duct, behind the right ocular tentacle; type b (not observed): vas deferens with a short distal and also a short proximal tract; types c, c* (not observed): small penis (without penial duct), often as a small ridge and a short proximal vas deferens tract.

Stage 3: type a: penis without penial sheath but with a duct continuing in a short distal part of the vas deferens; *types b, b*:* vas deferens running continuously from the right ocular tentacle over the floor of the mantle cavity up to (type b) or passing by (type b*) the vaginal opening; *types c, c*:* penis without penial sheath but with a penial duct continuing in a distal portion of the vas deferens; additionally, a short proximal part of the vas deferens arises from (type c) or near (type c*) the vaginal opening.

Stage 4: types 4, 4:* penis frequently with a penial sheath and a continuous vas deferens extending from the penis up to (stage 4) or passing by (stage 4*) the vaginal opening; the distal prostate starts to develop and becomes noticeable behind the penis; the proximal prostate also starts to develop beneath the vulva and capsule gland but is difficult to see, although easily detected in histological sections.

Stage 5: type a (not observed): the vagina is reduced and the vulva is absent; a more-or-less extended prostate gland can be found at the vulva; type a* (transferred to stage 4 because the prostate development may not cause sterility in this species): a complete vagina and an open vulva with a more-or-less extended prostate gland near the vaginal opening; *type b:* the vaginal opening is occluded by proliferating vas deferens tissue, often forming nodules and blisters; *type b*:* an open vulva but a massive proliferation of cells in the region of the vagina creates nodules and blisters that cause blockage or contortion of the oviduct; types c, c* (not observed): the ontogenic closure of the pallial oviduct is incomplete, and a continuous vas deferens can be found up to (type c) or near (type c*) the vaginal opening.

Stage 6: types a, a*, b (not observed) and *b** (only one female of this type was found in the present study) differ from the corresponding types of stage 5 by the presence of aborted egg capsules in the oviduct.

The percentage of females collected inside Porto Grande Bay showing each of the VDS stages described above is given in Figure 6.

TBT injection experiment

The results obtained from the TBT injection experiment are shown in Table 1. There was no mortality and females in both controls (water and DMSO) did not show any signs of imposex at the end of the experiment. In contrast, 67% of the TBT-injected females presented different degrees of vas deferens development, although none developed a penis. About 3% of the females showed a complete vas deferens tract running through the mantle floor from a position near the base of the right ocular tentacle to the vulva. The Kruskal-Wallis test revealed a significant difference in the VDSI between the three groups of females ($H = 24.7$; $P < 0.001$), indicating that TBT has the ability to induce imposex in *G. viverratus*.

DISCUSSION

Imposex-affected females of *Gemophos viverratus* were only found inside Porto Grande Bay, an area where typical TBT-pollution sources (i.e. port terminals, marina and shipyard) are located (ENAPOR, 2013). Females collected in Porto Grande Bay exhibited different degrees of imposex, which could be well described by the general classification scheme proposed by Stroben *et al.* (1992), after some adaptations. In this scheme the VDS is ranked in seven levels, from stage 0 to stage 6, and all these stages were observed in *G. viverratus* females. In this classification, the first appearance of the penis *vs* the vas deferens categorizes different substages or pathways denoted a, b and c. Substage a indicates that the penis is the first organ to appear during the virilization process, while b and c describe the condition where the vas deferens is the first to emerge; the difference between b and c concerns the position where the vas deferens starts to develop, near the right ocular tentacle (distal tract) or near the vaginal opening (proximal tract), respectively. The expression of imposex in *G. viverratus* may follow a, b and/or c depending on the VDS stage, but we admit that perhaps more paths could be found if more animals had been analysed.

We believe that the precise path a, b or c through which imposex develops is not a random process, because imposex artificially induced in the laboratory by TBT administration led to the development of vas deferens without penis formation, contrasting with experiments using other species in which penis formation was concomitant with vas deferens development (e.g. Barroso *et al.*, 2002; Mensink *et al.*, 2002). This fact may suggest that, in the particular case of *G. viverratus,* the initial eruption of the vas deferens or the penis may be dependent on the levels and body distribution of TBT in the tissues, or on environmental factors, which differ under field and laboratory conditions. Further studies are necessary to clarify this matter.

One major discrepancy detected between the imposex expression observed in *G. viverratus* and the VDS classification scheme cited above is that the vas deferens frequently does not meet the vulva but, instead, deviates and passes to the side. The general typical VDS classification scheme (Stroben *et al.*, 1992) recognizes this possible deviation at stage 4 for some species and assigns an asterisk to this pathway. However, our results show that this condition is very common in *G. viverratus* and could be observed in virtually any VDS stage that embraces the proximal vas deferens tract. For stage 4 the majority of the *G. viverratus* females showed a vas deferens passing laterally to the vulva (i.e.

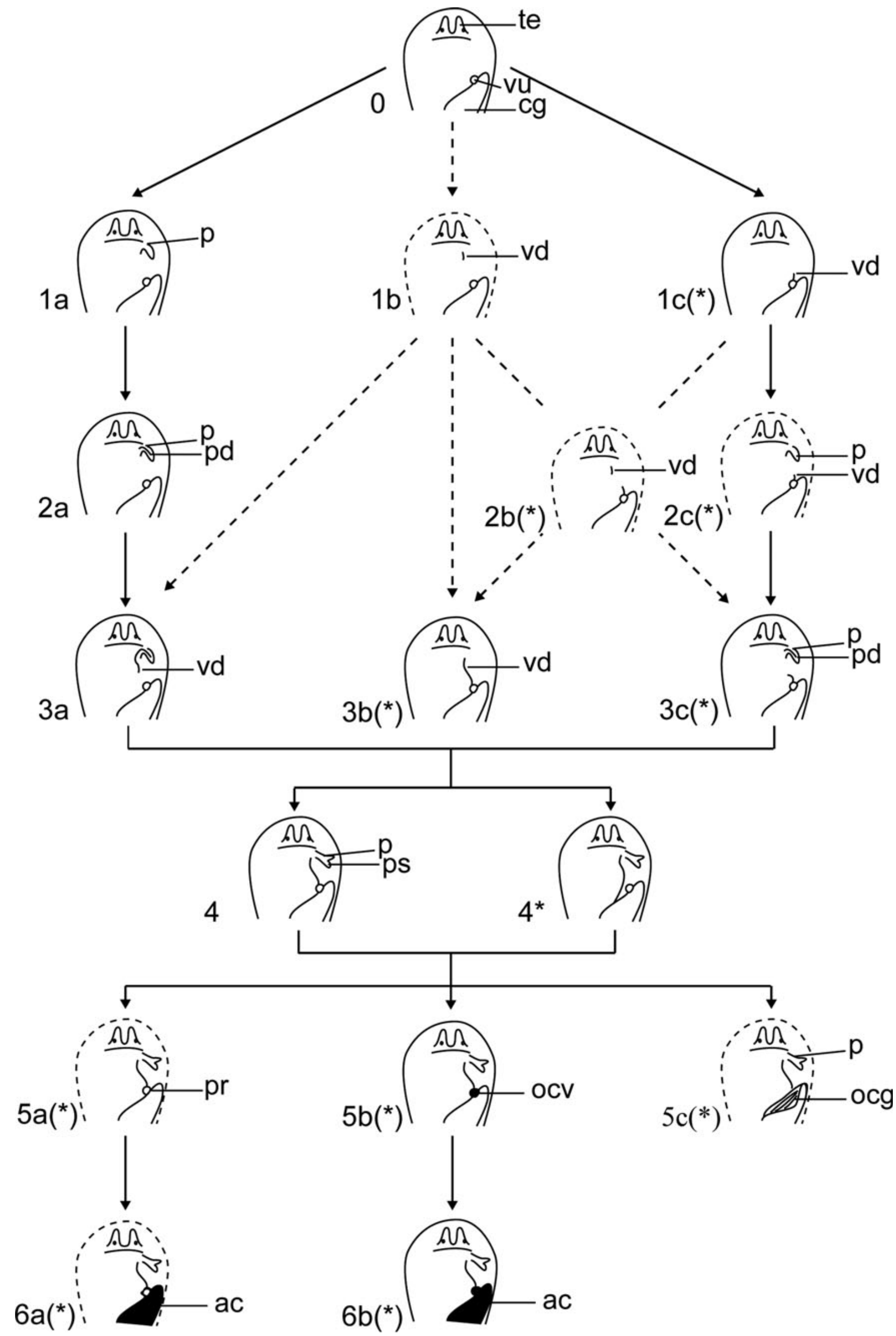

Figure 5. *Gemophos viverratus*. Imposex VDS classification scheme adapted from Shi *et al.* (2005) for *Cantharus cecillei*. The stages in dashed lines were not observed in the present study. Abbreviations: ac, aborted capsules; cg capsule gland; obc, open bursa copulatrix; ocg, open capsule gland; ocv, occlusion of the vulva; p, penis; pd, penial duct; pr, prostate; ps, penial sheath; te, tentacle; vd, vas deferens; vu, vulva.

VDS stage 4*). Another discrepancy between our results and the abovementioned VDS classification scheme is the fact that the proximal prostate development in females does not necessarily imply sterilization. In fact, histological sections of this region clearly demonstrate that the vas deferens at stage 4* does not run into the vulva, vagina or ventral channel. Instead, it passes by and becomes convoluted, initiating the primordium of an organ very similar to the male's proximal prostate (Fig. 3F_{15}). This prostate development does not replace the vagina and does not cause female sterilization by itself. We could observe the initial formation of this prostate in VDS stage 3b in some females, though it was predominant in stages 4, 5 and 6. Instead, sterilization of *G. viverratus* occurs by abnormal growth of tissues and blisters that block the vulva and constrict the vagina, vestibule and even the anterior region of the capsule gland (Fig. 4A, B). More rarely, the bursa copulatrix may become contorted and split,

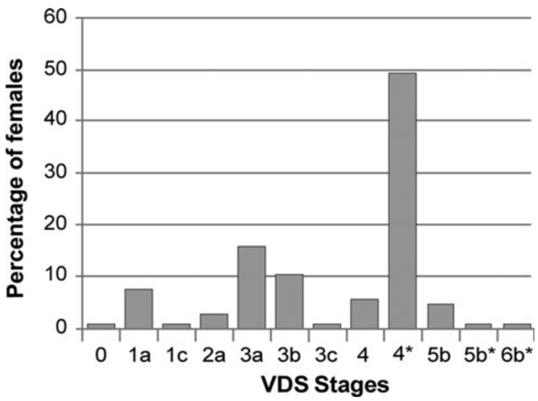

Figure 6. Percentage of female *Gemophos viverratus* ($n = 108$) collected at Porto Grande Bay exhibiting each stage of the VDS (compare Fig. 5).

Table 1. Imposex levels observed in the three groups of female *Gemophos viverratus* at the end of the 4-month experiment.

Treatment	*N*	SH (mm)	I %	VDSI
Water control	15	29.7 ± 2.0	0	0
DMSO control	15	28.6 ± 2.8	0	0
TBT	15	29.8 ± 3.2	66.7	1.4

Treatments: water control, noninjected females; DMSO control, females injected with DMSO; TBT, females injected with a solution of TBTCl using DMSO as solvent. *N*, number of females; SH, shell height mean ± standard deviation; I%, percentage of imposex-affected females; VDSI, vas deferens sequence index.

which may eventually impair fertilization. It seems that the endocrine disruption triggers tissue growth for the development of male sexual organs, a process that runs out of control and initiates tumour-like excrescences that push or substitute portions of the anterior tract of the gonoduct, causing blockage of the genital tract that impairs egg release and eventually copulation. Curiously these tissue excrescences were also observed behind the penis in females in an advanced stage of imposex, in the region corresponding to the distal prostate of males, indicating uncontrolled proliferation of cells in a similar way to that described above (Fig. 4C).

Some of these discrepancies from the typical VDS classification scheme were noted previously by Shi *et al*. (2005) regarding the expression of imposex in *C. cecillei* and, consequently, these authors proposed an updated scheme of imposex that incorporated, for instance, the deviation of the vas deferens near the vulva. The authors also proposed that stage 4* should no longer represent the end of imposex development, as it contributes to female sterility by a similar process described here. Our results corroborate the findings of Shi *et al*. (2005) and so their updated VDS classification scheme is suitable to describe the morphological expression of imposex in *G. viverratus*, except for some details: (1) the penial sheath in *G. viverratus* only appears in advanced stages of imposex and (2) some females may produce a conspicuous 'male-like' distal prostate behind the penis after VDS stage 4. Figure 5 shows the VDS classification scheme of Shi *et al*. (2005) after these modifications in order to describe better the expression of imposex in *G. viverratus*. The distal prostate is not represented in Figure 5, because it is not always conspicuous; nonetheless, the convolution of the vas deferens is always visible by histological analysis after VDS stage 4.

The factual evidence that TBT causes the development of imposex in *G. viverratus* comes from the successful induction of vas deferens formation by injecting very small doses of TBT into females. This causal relationship has been proved for many gastropod species by exposing females to very low concentrations of TBT in water and sediments, or by administrating TBT through food or injections at very low doses (Titley-O'Neal *et al*., 2011). This provides evidence that gastropods are indeed good bioindicators of TBT pollution, because this response is very specific to TBT. Nonetheless, this is not the only environmental contaminant able to induce imposex in gastropods. A related organotin—triphenyltin (TPT), used as biocide in AFS and as a pesticide in agriculture—is also able to induce imposex in a reduced number of species, namely in *Nassarius reticulatus* (Barroso *et al*., 2002) and *Thais clavigera* (Horiguchi *et al*., 1997). However, at least for Europe, this organotin occurs at lower concentrations than TBT (Sousa *et al*., 2009) and its action in inducing imposex in wild gastropod populations is probably negligible. TPT was also banned by the AFS IMO convention in 2008. Further research is needed to investigate if TPT is also capable of inducing imposex in *G. viverratus*.

The current work describes, for the first time, imposex expression in *G. viverratus*, proves that TBT is able to induce imposex in this species and provides a VDS classification scheme that may be used hereafter in field monitoring surveys. Further validation is required to test the usefulness of this gastropod as a TBT-pollution bioindicator, by testing how closely the intensity of imposex reflects the TBT environmental contamination in the field.

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