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Genitalia Malformations in *Stramonita haemastoma* (Gastropoda: Muricidae) from Atlantic and Mediterranean Coast

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Abstract

Stramonita haemastoma is a gonochoric marine gastropod. The examination of the genital tract has revealed the occurrence of malformations affecting the penis of males *S. haemastoma*, as for the females only aphallia was recorded. Investigations of the genital tract malformations were made in snails sampled monthly from Bizerta Channel (Northern Tunisia), over one-year period of sampling (June 2009 to May 2010). *S. haemastoma* was sampled in 2013 from sixteen sites located in the Mediterranean and Atlantic coast. The development of such type of malformations was associated to the use of antifouling paints. However, this assumption should be supported by further bioassays.

Keywords: *Stramonita haemastoma*; Penis malformations; Aphallia; Mediterranean coast; Atlantic coast

Introduction

Malformations in marine gastropods, often associated with pollution, may have severe consequences on the marine system [1,2]. For instance, imposex had caused reproductive failure and in consequence population decline of several gastropod species [1-5]. Undeniably, imposex is the most common malformation reported in marine gastropods; it affects the genital tract of normal female by imposing the development of male sexual characters onto female [6]. According to the literature, imposex is induced following an exposure to tributyltin (TBT) used in antifouling paints [7]. Several marine gastropod species were found affected by this abnormality, in 2005 Shi et al. [8] published a list with 260 species affected by imposex [8,9]. This list has considerably increased since this date with the addition of new affected species as *Heleobia australis* [10], *Nassarius mutabilis* [11] and *Plicopurpura pansa* [12].

TBT may induce another genital tract malformation called Dumpton Syndrome (DS), corresponding to the under-development of male sexual characters on both sexes (male and imposex affected female) [13]. The Dumpton syndrome is also known as aphallia in marine gastropods, this deficiency was firstly observed in the snail *Nucella lapillus* sampled from Dumpton Gap (England) [13] and then in four other localities: Brest (France) [14], Brittany (France) [14], Spain [15] and Portugal [16]. DS was also recorded in other gastropods as *Ocenebrina erinacea* [17]. Lima [17] noted a high rate of aphallic females *S. haemastoma* (42.6%) during an experimental induction of imposex. After that, [18] Queiroz et al. noted a high occurrence of aphallia in females *S. haemastoma* sampled from the Northeast of Brazil and suggested the possibility of the presence of the allele responsible for the Dumpton Syndrome at high frequencies in *S. haemastoma* population. Male affected by DS may have an undersized penis, an under-developed vas deferens, in the extreme cases they may present a split prostate, and a completely absence of the penis [13]. Female affected by DS developed or not an excrescence instead of a penis, which is considered an advantage in severely TBT-polluted site [13].

Besides to those genital abnormalities, morphological malformations were reported to affect the penis [7,14,19-24], vas deferens [25] or both penis and vas deferens [26-28]. Penis malformations were reported in *Hexaplex trunculus* [22,24,26,28], *Bolinus brandaris* [26,29], *Hinia reticulata* [27], *Nucella lapillus* [14], *Cyclope neritea* [30,31], *Leucozonia nassa* and *Leucozonia ocellata* [21].

There has been some report on imposex in *Stramonita haemastoma* in any areas of the world but not much was known in this part of the country. Contrary to its congeneric species *H. trunculus* [7,22,24] and *B. brandaris* [26,29]. In the present study, we reported malformations affecting the genital tract of males and females imposex-affected *S. haemastoma* collected from both Mediterranean and Atlantic coast.

Materials and Methods

Adult gastropod, *S. haemastoma,* were sampled monthly during 12 months from June 2009 to May 2010 in a rocky site located in Bizerta Channel. In totality, a sample of 1035 gastropod/month with shell length 28 m to 80 m is collected. Sampling was performed by SCUBA and some time by hand (snorkelling) at a station located in the artificial channel linking the Bizerta lagoon to the Mediterranean Sea (Figure 1). The length of the channel of Bizerta is about 1500 m, 240 m broad and 12 m depth. It is characterized by strong currents, an intense maritime traffic and chemical and urban pollution [32-34]. The direct and indirect discharges of urban and industrial wastes have contaminated the lagoon by various toxic compounds such as organo-chlorinated pesticides [35,36], halogenated aromatic compounds like polychlorinated biphenyls (PCBs) [2], polycyclic aromatic hydrocarbons (PAHs) [37], heavy metals and organotins compounds [38,39].

30 to 45 individuals were collected in 2013 from 5 and 11 North-Eastern Atlantic and Western Mediterranean coasts, respectively (Figure 1 and Table 1). In the laboratory, specimens were anaesthetized in a 1:1 mixture of 7.5% $MgCl_2$ and seawater. Shell and penis length were measured using a vernier calliper to the nearest 0.1 mm. The genital tract was examined under a binocular microscope.

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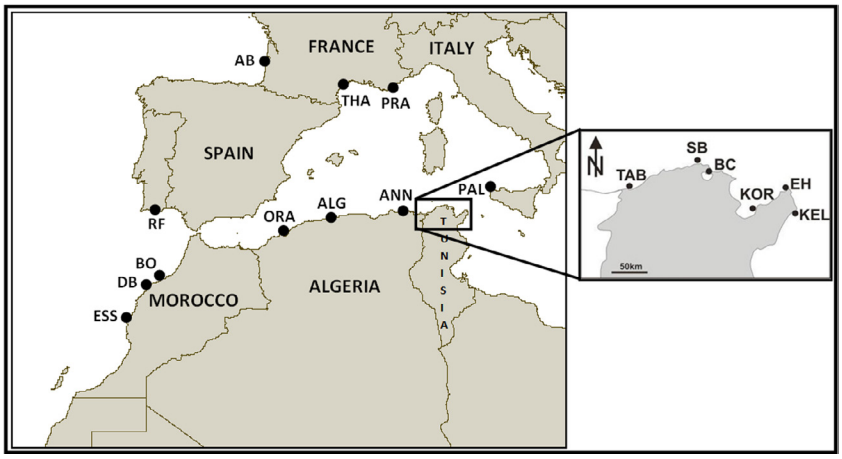

Figure 1: Sampling localities of *Stramonita haemastoma*.

				GPS coordinate	
Areas	**Sample code**	**Sample name**	**Year of collection**	**Latitude**	**Longitude**
North-Eastern Atlantic	AB	Arcachon Bay	2013	44°592602 N	-1°214601 E
	RF	Ria Formosa	2013	36°988717 N	-7°983696 E
	BO	Bouznilca	2013	33°828000 N	-7.145958 E
Western Mediterranean basin	DB	Dar Bouaaza	2013	33°534499 N	-7°827555E
	ES S	Essaouira	2013	31°692465 N	-9°815664 E
	THA	Etang de Than	2013	43°393343 N	3°702986 E
	ORA	Can	2013	35°901409 N	-0°331933 E
	ALG	Algiers	2013	36°45873 N	2°50838 E
	ANN	Annaba	2013	36°898241 N	7°771432 E
	TAB	Tabarca	2013	37°514243 N	9°869430 E
	BC	Bizerta channel	2010 to 2013	36136030 N	11°101338 E
	SB	Sidi Bechir	2013	37°345694 N	9°735649 E
	KOR	Korbous	2013	36°816849 N	10°566951 E
	KEL	Kelibia	2013	38°126280 N	12°793723 E
	EH	El Houaria	2013	37.033306 N	11°059370 E
	PAL	Palermo	2013	43°332742 N	3°612416 E

Table 1: Sample location of *Stramonita haemastoma*.

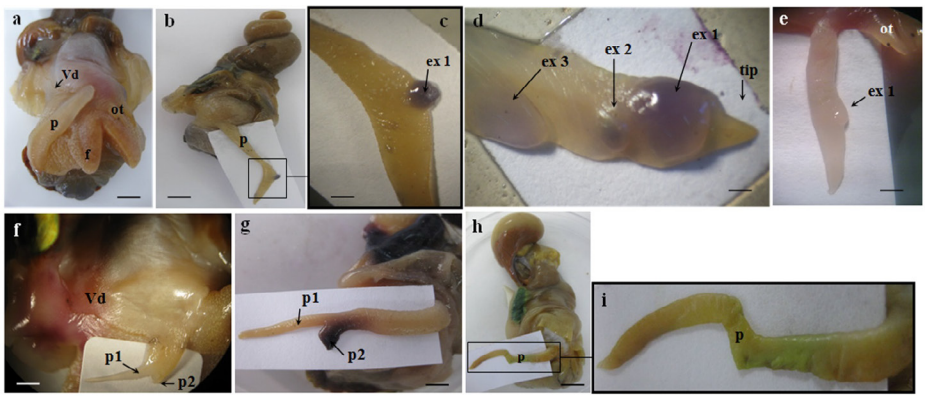

Figure 2: Penis malformations in males *Stramonita haemastoma*. a: normal penis; b–g: abnormal penises observed in Bizerta Channel (Tunisia); h-i: abnormal penises observed in Bouznikha (Morocco). ex 1: excrescence 1; ex 2: excrescence 2; ex 3: excrescence 3; f: foot; ot: ocular tentacle; p: penis; p1: penis 1; p2: penis 2; vd: vas deferens. Scale bars=1 mm.

Results

Penis malformations in males *S. haemastoma*

In male *S. haemastoma*, the normal penis has a bent shape, a large triangular tip, tapering gradually toward the snail head and without flagellum (Figure 2a). At the center of the penis there is a penis duct which is a curved closed tube beginning from the vas deference and reaching the tip of the penis. This tube ensures the passage of the sperm from the prostate to the female during the period of reproduction. The length of the penis increases considerably during the period of

reproduction extending from May to October, increases in penis length may exceed 5 mm to 6 mm during this period. Malformations affecting the male penis were observed in 2 out of 16 stations. The first station is located in the Western Mediterranean basin (Bizerta Channel, Tunisia), the second one is in the North-Eastern Atlantic Ocean (Bouznika, Morocco). Penis malformations were mainly observed in Tunisian population, 9 males were found with malformed penis in Bizerta Channel, against 1 male in Bouznika station. Malformations observed in Bizerta Channel consist in the development of a bud tissue (excrescence) close to the tip (Figures 2b-2d), or at the half of the total penis length always at the posterior side (Figure 2e). The number of excrescences may reach three at the level of the tip of the main penis (Figure 2d). Another malformation consists in the differentiation of a supplementary small penis measuring 2.3 mm in length and without a penis duct (biphallic penis) (Figure 2f). In addition to the main penis which appear undersized (3.5 mm in length), filamentous and with an incomplete penis duct (Figure 2f). Another specimen was found with two penises, the main penis has a normal shape and length (17.6 mm), the second penis is more developed compared to the last case and with a complete penis-duct (3.7 mm in length) (Figure 2g). Four Aphallic males were found in Bizerta Channel. The malformation observed in Bouznika station corresponds to the development of a long penis (23 mm) in the shape of the Z letter (Figures 2h and 2i). The rates of malformed males were 0.86% and 2.38% in Tunisian population and Moroccan population, respectively.

Aphallia in imposex-affected females *S. haemastoma*

Aphallia is reported for the first time in females *S. haemastoma*, aphallic females were observed only in the Western Mediterranean basin (Figure 3). Aphallic females were found in 7 out of 11 imposex-affected populations. Females without penis and with only a vas deferens were found in three Tunisian sites (Tabarca, 15.38% and El Houaria, 6.66%) and in one Algerian site (Oran, 12.5%) (Figure 3). While, females *S. haemastoma* with a vas deferens and a papilla were found in four stations, length of the papilla varied between 0.11 mm in Algiers (Algeria) and 0.33 mm in Korbous (Tunisia). Rates of females showing only a papilla varied between 5.88% and 50% in Korbous and Sidi Bechir, respectively.

Discussion

The present work highlights the occurrence of morphological malformations affecting the genital tract of males and imposex-affected females *S. haemastoma*. Malformations described in the literature are essentially related to the differentiation or the under-development of a part or a whole male genital tract in both sexes [22,26,40]. In Tunisia, penis malformations were first reported by Lahbib et al. [22] in males and imposex affected females *H. trunculus*, while Abidli et al. [26]

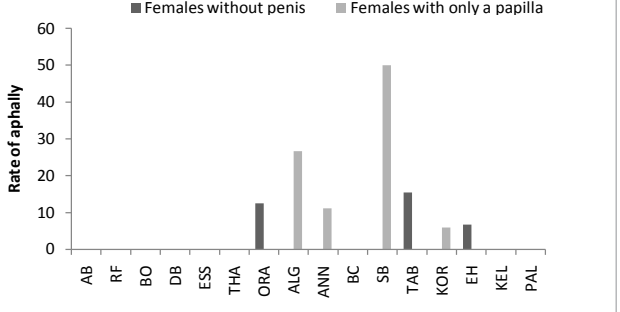

Figure 3: Rates of aphally in females *Stramonita haemastoma*.

detected malformations affecting the penis and/or the vas deferens of two muricids (*H. trunculus* and *B. brandaris*) sampled from Bizerta Channel (Table 2). In *S. haemastoma* only imposex was signalled in specimens sampled from Bizerta Channel [11,38]. Penis excrescences observed in 3 males *S. haemastoma* were previously observed in 1 male *H. trunculus* collected from Bizerta Channel [22] and in three other gastropod species (*Hexaplex trunculus*, *Bolinus brandaris* and *Hinia reticulata*) collected from the Atlantic coast [24,27,29]. Aphallia and biphallia were the most common malformations recorded in the literature (Table 2). Whereas, the observation of male with abnormal shaped penis (Z-shape) is newly reported in *S. haemastoma* collected from an Atlantic shore (Morocco). Abnormal shaped penis was only reported by Lahbib et al. [30] in 1 Male *Cyclope neritea*. In contrast, aphallia was widely reported in previous studies (Table 2), in the present study aphallia was detected in *S. haemastoma* in both sexes. Aphallic females were more frequent than males, similar finding was recorded in *Nucella lapillus* [16]. Usually, aphallia is reported as cases of DS, in other cases it was considered a path of imposex development [18]. However, there is a doubt about the occurrence of Dumpton Syndrome in this population, since the rate of aphallic males was low. Indeed, Queiroz et al. [18] did not found any aphallic male, while they noted a very high rate of aphallic imposexed females (46.2%). Same finding was reported by Santos et al. in *Nucella lapillus* 0.1% of aphallic males against 23.8% of aphallic females. Quintela et al. [18] noted 7% of aphallic males and 31% imposexed females. That's why those authors recommended thus the use of the diameter of the vas deferens instead of the size of the penis in studying the Dumpton Syndrome. As for imposex development, the aphallic path observed here was not observed presviously in *S. haemastoma* sampled from Tunisian coast [30,38]. This path was only observed in the Braszilian population [18].

The mechanism implicated in the development of penis malformations was widely discussed but still unknown; some authors supported the hypothesis linking genitalia malformations to the presence of oragnotin compounds in the aquatic environment. In this context, [41] observed penis malformations in juveniles *Buccinum undatum* exposed to a very high concentration of TBT (500 ng/L). Later, Lahbib et al. [22] supported this idea based on the fact that penis malformations were only found in highly TBT-polluted site. However, the low rates of penis malformations recorded in previous studies: (0.32%) in Portuguese *H. trunculus* [24], (1.34%) in Tunisian *H. trunculus* [26], (0.56%) in Tunisian *B. brandaris* [26], (1.69%) in Spanish *B. brandaris* [29], (0.2%) and (2.44%) in Brazilian *Leucozonia nassa* and *Leucozonia ocellata*, respectively [21]. Penis malformations reported in the present study were very low (0.86%) in Tunisian *S. haemastoma* against (2.38%) in Moroccan population. Factors other than tributyltin were probably implication in the development of those malformations. This assumption is supported by the observation of biphallic males among museum specimens of *H. trunculus* collected before the use of the biocide TBT (tributyltin) [7]. Generally, malformations are evidence of environmental problems generating negative impacts on live organisms [42,43], but they may also appear naturally. Indeed, further investigations are needed in order to elucidate the causal factor generating such malformations in marine gastropods.

Conclusion and Recommendations

The present work elucidates the occurrence of malformations affecting the genital tract of males and imposex-affected females *S. haemastoma*. The mechanism implicated in the development of penis malformations is supported by the hypothesis linking genitalia malformations to the presence of oragnotin compounds in the aquatic

Studies	Species	Locations	Penis malformation		
			N	Nmm	Malformation
Present study	*Stramonita haemastoma*	Bizerta Channel	1035	3	Penis excrescences
		--	--	1	Biphallic penis
		--	--	1	Biphalic penis/undersized
		--	--	4	Aphallic
		Bouznika	42	1	Penis Z-shape
Sanchez-Marin et al. (2015)	*Nucella lapillus*	Portuguese coast	1433	2	Aphallic
		--	--	-	Undersized penis
Abdili et al. (2009)	*Hexaplex trunculus*	Gulf of Tunis	522	6	Biphallic penis
	--	--	--	1	Triphallic penis
	Bolinus brandaris	Gulf of Tunis	531	3	Biphallic penis
Lahbib et al. (2008)	*Hexaplex trunculus*	Steg Tunis North Lake	14	1	Bifurcated tip
	Hexaplex trunculus	Small Gulf of Tunis	48	1	Biphallic penis
	Hexaplex trunculus	Bizerta Channel	42	2	Penis excrescences
Terlizzi et al. (1998)	*Hexaplex trunculus*	Italian coast	600	--	Bifurcated penis
		--	--	1	Aphallic
Terlizzi et al. (1999)	*Hexaplex trunculus*	Italian coast	3000	4	Aphallic
Barreiro et al. (1999)	*Nucella lapillus*	Spanish coast	1602	39	Aphallic
Vasconcelos et al. (2006)	*Hexaplex trunculus*	Ria Formosa	621	2	Penis excrescences
Ramon and Amor (2001)	*Bolinus brandaris*	Spanish coast	59	1	Penis excrescences
Stroben et al. (1992)	*Hinia reticulata*	Brittany and Normandy Coast	2760	4	Penis excrescences
Huet et al. (2008)	*Nucella lapillus*	French coast	1847	19	Aphallic
Costa et al. (2014)	*Leucozonia nassa*	Southeast Brazil	493	1	Biphallic penis
	Leucozonia ocellata	--	58	2	Biphallic penis
Gibbs (1993)	*Nucella lapillus*	UK	--	--	Aphallic
		--	--	--	Undersized penis

Table 2: Penis malformations in *Stramonita haemastoma* with comparison to data reported in the literature. N: number of specimens; Nmm: number of malformed males.

environment generating negative impacts on live organisms. Indeed, further investigations are needed in order to clarify the causal factor generating this malformation in marine gastropods.

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