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Research Article

Imposex in *Thaisella chocolata* (Duclos, 1832) (Gastropoda: Muricidae) Caldera Bay, Chile

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ABSTRACT. Imposex syndrome is the superimposition of a pseudopenis and vas deferens in the reproductive system of female gastropods. This anomalous virilization is attributed to a hormonal imbalance induced by tributyltin (TBT), an organotin compound, present in antifouling paints. This study analyzes the severity, extent, degree and indices of imposex in a population of *Thaisella chocolata* from Caldera Bay, Atacama Region, Chile. Sex was determined by biopsy of the gonad from 180 specimens and imposex by the concurrence of ovarian, capsule gland, vas deferens and pseudopenis in abnormal females. The proportion of females affected with imposex was estimated using the formula described by Gibbs *et al.* (1987). The degree of the syndrome progress was classified using five scales of imposex and Vas Deferens Sequence Index (VDSI), Relative Penis Index (RPSI) and Relative Penis Size Index (RPSI) were calculated. Results show that 100% of the females of *T. chocolata* from Caldera Bay suffer imposex. Macroscopic analysis revealed atrophy of the vagina and femenine glands. Some females lacked ingestive gland and seminal receptacle. Cytological disorders as modification of internal epithelium, occurrence of acellular areas, lack of basal lamina and presence of pyknotic nuclei, and overlapping of prostate tissue in the three females' glands were found. Anatomical and histological alterations associated with imposex determine the absolute sterility of female. The syndrome reached maximum degrees in all scales except for that of Barreiro *et al.* (2001). It is recommended the use of VDSI for *T. chocolata*, since provides a realistic measure of the virilization and reproductive potential of the females and would not be affected by the shape and size of the penis.

Keywords: *Thaisella chocolata*, anatomy, reproduction, tributyltin, TBT, imposex, histopathology.

Imposex en *Thaisella chocolata* (Duclos, 1832) (Gastropoda: Muricidae) de Bahía Caldera, Chile

RESUMEN. El imposex es la superimposición de pseudopene y vaso deferente en el sistema reproductor de las hembras de gasterópodos. Esta virilización se atribuye a un desbalance hormonal inducido por el tributilestaño (TBT), un compuesto organoestañoso, presente en las pinturas anti-incrustantes. Este estudio analiza la severidad, proporción, grados e índices de imposex en una población de *Thaisella chocolata* de Bahía Caldera, Chile. El sexo se determinó por biopsia de la gónada de 180 ejemplares y el imposex por la concurrencia de ovario, glándula de la cápsula, vaso deferente y pseudopene en hembras anormales. La proporción de hembras afectadas se estimó a través de la fórmula de Gibbs *et al.* (1987). Se clasificó el grado del síndrome aplicando cinco escalas y se calcularon los Índices del Estado del Vaso Deferente (VDSI), Longitud Relativa del Pene (RPLI) y Tamaño Relativo del Pene (RPSI). Los resultados muestran que el 100% de las hembras de *T. chocolata* provenientes de Bahía Caldera sufre de imposex. El análisis macroscópico reveló atrofia de la vagina y de las glándulas femeninas. Algunas hembras carecen de glándula ingestiva y receptáculo seminal. Se determinó alteraciones citológicas como la modificación del epitelio interno, ocurrencia de áreas acelulares, falta de lámina basal y presencia de núcleo picnótico, y superposición de tejido prostático en las tres glándulas femeninas. Las alteraciones anatómicas e histológicas asociadas al imposex determinan la esterilidad absoluta de las hembras. El síndrome alcanzó los grados máximos en todas las escalas aplicadas, excepto en la de Barreiro *et al.* (2001). Se recomienda el uso del VDSI para *T. chocolata,* ya que, es una medida realista de la virilización y potencial reproductivo de las hembras y no estaría afectado por la forma y tamaño del pene.

Palabras clave: *Thaisella chocolata*, anatomía, reproducción, tributilestaño, TBT, imposex, histopatología.

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INTRODUCTION

Anthropogenic pollution has severe effects on the biology of marine organisms (Collado *et al.*, 2010). One example is the so-called imposex syndrome, defined by Smith (1971) as the superimposition of non-functional male sex organs in the female genital system of gastropods. In extreme cases, imposex prevents the normal reproduction of specimens and can even cause death and extinction of affected female populations (Oehlmann *et al.*, 1991). This virilizing alteration is induced by the organotin compound called TBT (tributyltin), used as a biocide in antifouling paints and other products (Castro *et al.*, 2012).

The extent of anatomical malformations is dependent on the concentration of TBT in the environment (Gibbs & Bryan, 1986) and can range from mild, forming a pseudopenis primordium to very severe obstruction of the gonoduct (Oberdörster & McClellan-Green, 2002). Various scales and indices have been developed to standardize the stages of imposex that consider mainly the degree of development of the cephalic structures (pseudopenis and vas deferens) and occlusion of the vagina (Gibbs *et al.*, 1987; Fioroni *et al.*, 1991; Oehlmann *et al.*, 1991; Stroben *et al.*, 1992; Barreiro *et al.*, 2001; Fernandez *et al.*, 2002; Rodríguez *et al.*, 2008). A few scales use microscopic alterations associated with imposex (Oehlmann *et al.*, 1991; Schulte-Oehlmann *et al.*, 1997; Huaquín *et al.*, 2004; Horiguchi *et al.*, 2014).

Determining the occurrence and severity of the syndrome is particularly important in commercial species, since these are subjected regularly to fishing mortality. In addition, the edible species should be studied due to the potential effects of TBT on human health, including impaired immune system, altered response to physiological stimuli (IPCS, 1990), promoting of adipogenesis (Bernardes *et al.*, 2013) and even it is also suspected to act as a carcinogen (Yamabe *et al.*, 2000; Nakanishi, 2007).

Thaisella chocolata (Duclos, 1832) is a gastropod extracted for human consumption (Avendaño *et al.*, 1998) and represents a significant benthic resource species for the artisanal fisheries sector in northern Chile; however, due to high levels of exploitation since the beginning of its extraction in 1978, with a maximum of 8,244 ton in 1986 (Avendaño *et al.*, 1998), protective measures that included the establishment of a minimum capture size of 5.5 cm and a fishing ban for one or two years was decreed by region (Avendaño *et al.*, 1998). Currently, this species is extracted in the north and in the south of Chile; in 2015 total landings were 492 ton (SERNAPESCA, 2015).

Anatomical observations on females of *T. chocolata* from Caldera Bay, Atacama Region, led us to hypothesize the imposex occurrence in this species. In that locality a number of productive activities are performed, that includes the shipment of mineral concentrates, fuel discharge, artisanal and industrial fisheries, aquaculture and tourism (Castillo & Valdés, 2011).

In order to determine the occurrence and intensity of imposex in female of *T. chocolata* from Caldera Bay, this study characterizes the anatomical and histopathological abnormalities of the female reproductive system and estimates the proportion, grades and indices of imposex. To propose *T. chocolata* as a sentinel species, different scales of imposex were applied to determine the scale that best fits the alterations observed in this species, including the scales of Gibbs *et al.* (1987), Stroben *et al.* (1992), Barreiro *et al.* (2001), Fernandez *et al.* (2002) and Rodríguez *et al.* (2008).

MATERIALS AND METHODS

One hundred and eighty individuals of *T. chocolata* were collected by hookah diving in Caldera Bay (27°2'47"S, 70°49'29"W), Atacama Region, Chile, from down to 16 m depth, on February 2012. Live specimens were transferred to the wet laboratory of the North Catholic University, Coquimbo, where they were kept in 200 L aquaria with circulating seawater and continuous aeration for five days until analysis. For each specimen we recorded the maximum length of the shell with a vernier with 0.01 mm accuracy. The maximum length of the penis of males and the pseudopenis of females were measured from the base of the body to the distal end using the Image Pro-Plus program. Soft tissue was extracted by breaking the shell and sex was determined by biopsy of the gonad observed *in vivo* in a photonic microscope Olympus CX31 with magnification 40x to 1000x.

Females were classified with imposex observing the ovarian, albumen, ingestive and capsule glands, vagina, vas deferens, pseudopenis and penis duct of males and females. Photograph of these organs were taken with a camera Nikon FX 35A. Transverse and longitudinal sections of the organs previously named were extracted. The sections were observed and described under a stereoscopic microscope Nikon SMZ-10 in order to clarify their delimitation with other structures and location of the gonoduct.

Additionally, a linear regression was performed to establish the effect of the Average Pseudopenis/Penis Length (APL), using as covariate Average Shell Length (ASL) between males (n = 90) and females (n = 90) of the same locality. Subsequently, by an analysis of co-

variance (ANCOVA) the effect of these variables was established. Normality of the data was determined using the Kolmogorov-Smirnov and Lilliefors tests and homogeneity by the Levene test. Significant differences between treatments (male and females) were detected by ANDEVA.

The histopathological alterations of the female system affected by imposex were analyzed in 20 females. From each female we obtained cross sections of 0.5 cm^3 of ovary, glands, oviduct and vagina. The tissue pieces were fixed in Davidson's fixative for 24 h and processed according to the standard technique described by Martoja & Martoja-Pierson (1970). Sections 5 µm thick were stained with Harris hematoxylin and eosin, and then mounted with Flotexx® resin. The preparations were observed with a magnification 40x to 1000x and photographed in a light microscope equipped with an Olympus CX31 U-CMAD3 camera. Measurements of organs and histological structures were performed using the Image Pro-Plus program.

The proportion of females affected was estimated in 90 specimens through the following formula: I% = (number of females with imposex/total number of females)x100 (Gibbs *et al.*, 1987). The progress of imposex was determined by applying the scale of Gibbs *et al.* (1987) and indices of imposex of Gibbs were estimated as follows: Vas Deferens Stages Index (VDSI) = (Σ of degrees of VDS/total number of females analyzed), Relative Penis Length Index (RPLI) = (average pseudopenis length in females/average penis length in males)x100 and Relative Penis Size Index (RPSI) = (average pseudopenis length in females /average penis length in males)^{3}x100 (Gibbs *et al.*, 1987). We also applied the scales of Stroben *et al.* (1992); Barreiro *et al.* (2001); Fernandez *et al.* (2002) and Rodríguez *et al.* (2008), who consider the development of vas deferens, pseudopenis, the convolution of the oviduct and the occurrence of aborted ovicapsules.

RESULTS

Anatomical and histopathological alterations

The 90 females of *T. chocolata*, sex corroborated by gonadal histological analysis, had prostate gland tissue in the mantle cavity, a pseudopenis and vas deferens at different levels of development. The female reproductive system of *T. chocolata* from Caldera Bay fits the characterization of imposex, which consist, from anterior to posterior, of a pseudopenis, penile duct, vas deferens, vagina, capsule, ingestive and albumen glands, seminal receptacle, oviduct and an ovary.

The pseudopenis is located on the right tentacle of the specimens; it is of sigmoidal shape and has a filiform extension at its end corresponding to a filament (Fig. 1a). Other abnormalities were detected in the head region of two specimens. In one, two pseudopenises are originated from the same base on the right tentacle (Fig. 1b); one measured 0.8 cm and the other 0.5 cm in length. In a second specimen there was a dense round structure located on the vas deferens. It was pigmented pinkish brown with white spots and measured 1 cm length (Fig. 1c). Moreover, in this individual the anterior and posterior ends of pseudopenis were fused by their epidermis (Fig. 1c).

The epidermis of the pseudopenis is similar to that of the head and foot of the specimen and is formed by ciliated columnar cells interspersed with mucous cells. Underlying the epidermis are muscle cells of circular, longitudinal and diagonal arrangement. The pseudopenis contains a closed, narrow and pleat lumen penis duct. The conduit consists of ciliated columnar epithelium and is closely surrounded by a thick and compact layer of circular muscle and connective tissue (Fig. 2a). The male conduit has a large lumen surrounded by a layer of circular muscle (Fig. 2b).

For females there was a positive correlation between the ASL (x axis) and the APL (y axis) (ANCOVA, n = 90; r = 0.7; $P < 0.05$) (Fig. 3). Besides, it was determined that the length of the pseudopenis of females (n = 90; ASL: 83.9 ± 11.1 mm and APL: 10.6 ± 2.0 mm; $P < 0.05$) was significantly less than the penis of males (n = 90; ASL: 82.8 ± 9.5 mm and APL: 17.8 ± 2.4 mm; $P < 0.05$).

The vagina is in the anteroventral end of the mantle cavity (Fig. 4a). The vulva is a shallow oval slot due to melting of the muscular walls of the vagina. In three of ten specimens, the vulva was occluded and instead shows a thin red line corresponding to the vas deferens, which concludes in the base of the pseudopenis and extends posteriorly to the muscular region of the vagina (Fig. 4a). On the outskirts of the vagina are located prostate gland acini (Fig. 4b).

The capsule gland is tubular and thinned at its anterior end. It is cream color and often has a nodular appearance on its surface (Fig. 5a). 16% of females contained compacted ovicapsules inside the gland, forming a brown solid sphere (Fig. 5b), which includes mucus mass, yolk granules and irregular fragments of fibrous sheets similar to layers of ovicapsules.The walls of the gland tend to be laminar, organized into two lateral lobes and the posterior lobe not distinguished. The gland measured on average 2.1 ± 0.5 cm in length (n = 10). The epithelium of the gonoduct has sections of different thickness and metaplastic appearance ranging from cylindrical to cuboidal cells (Fig. 5c). The organization of the glandular tissue is interrupted by

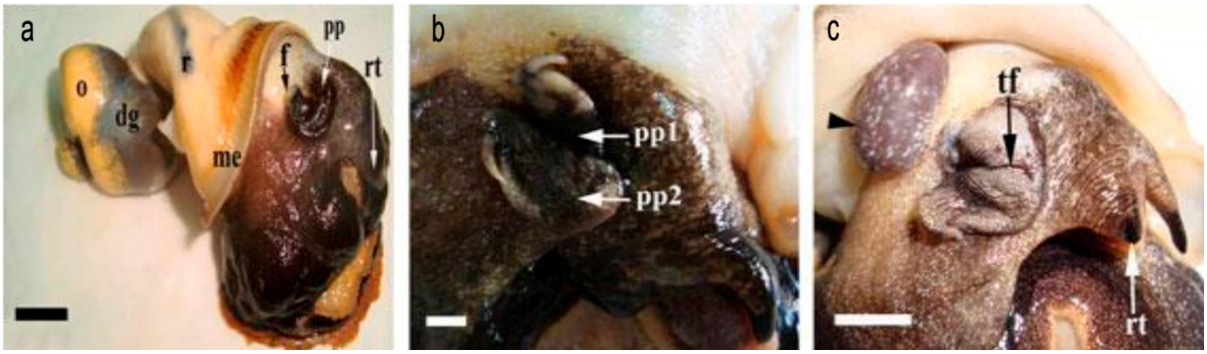

Figure 1. *Thaisella chocolata*. Pseudopenis and penis: a-c) Macroscopic view of the pseudopenis. a) Location of the pseudopenis, behind the right tentacle. In the anterior part of the pseudopenis is located a filiform extension, b) occurrence of two pseudopenis, c) dense structure (arrowhead) and pseudopenis tissue fusion. Scale bar: 1 cm, dg: digestive gland, f: filiform, me: mantle edge, o: ovary, pp: pseudopenis, r: rectum, rt: right tentacle, tf: tissue fusion.

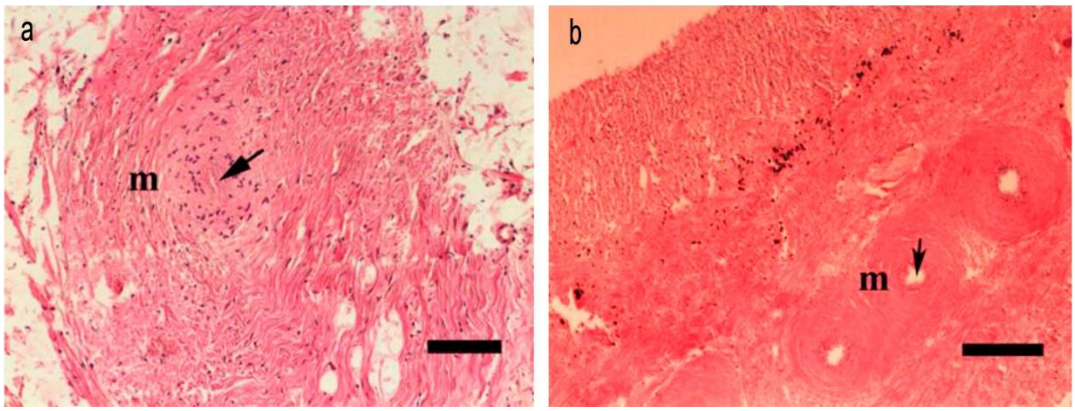

Figure 2. Trasnverse histological sections of the pseudopenis duct and penis duct of *Thaisella chocolata*. a) Narrow lumen (arrow) of the pseudopenis duct and surrounded by a thick circular musculature in female (scale bar = 400 µm), b) Lumen (arrow) of the penis duct surrounded by a circular muscle in male. (scale bar = 300 µm). m: muscle.

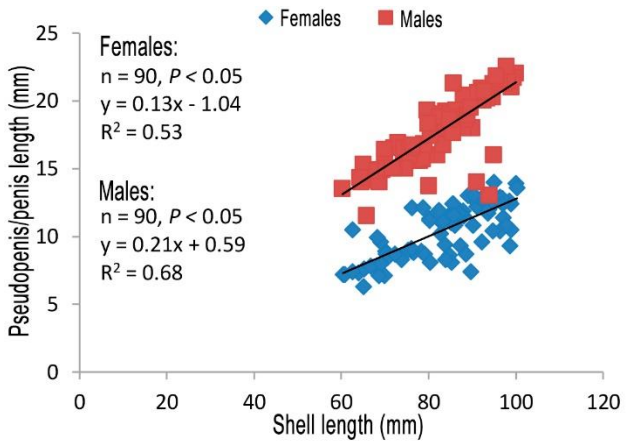

Figure 3. Lineal correlation of the shell length (mm) and pseudopenis/penis length (mm) of females and males of *Thaisella chocolata* from Caldera Bay.

acellular areas with homogeneous appearance and low dye affinity (Fig. 5d). Cell clusters were detected in scattered regions of the gland with pyknotic nuclei (Fig. 5e) and in the periphery of the gland are acini of prostate gland (Fig. 5f).

The ingestive gland is formed by numerous dark brown color saccules composed of cylindrical epithelium cells and granular cytoplasm. On the outskirts of this gland are located acini of the prostate gland (Fig. 6). Only six of ten specimens had an ingestive gland.

The albumen gland is located on the ventral right side, prior to the kidney. It is tubular in shape, curved in its dorsal end and cream color. It measures on average 0.5 ± 0.3 cm in length (n = 10). It consists of two side lobes, delimited by a ciliated columnar epithelium with oval and basal nuclei. In some sections the epithelium is modified like a pseudostratified epithelium (Fig. 7a) and lacks basal lamina (Fig. 7b). The demarcation between albumen and capsule glands is diffuse (Fig. 7c) and the acini of the prostate gland

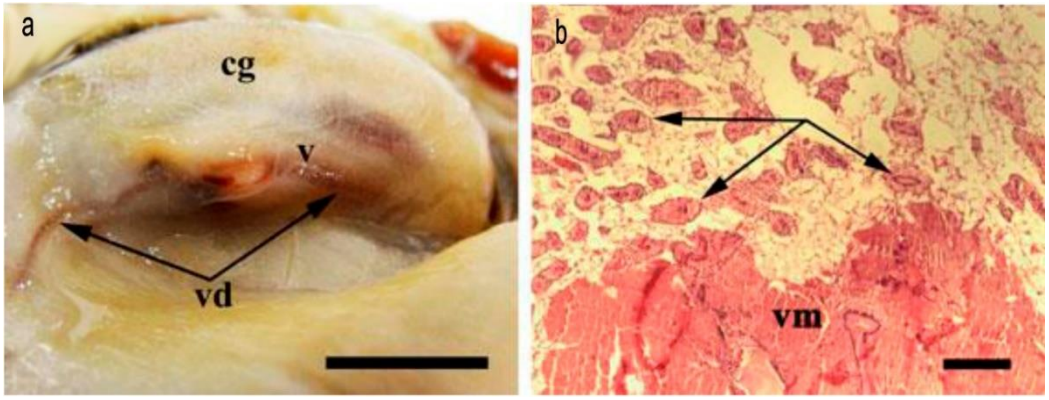

Figure 4. Vulva of *Thaisella chocolata* female. a) Macroscopic view of vulva occluded by the vas deferens (scale bar = 1 cm), b) transverse histological section of prostatic glands (arrows) on the periphery of the vulva musculature (scale bar = 200 µm). cg: capsule gland, v: vulva, vd: vas deferens, vm: vulva musculature.

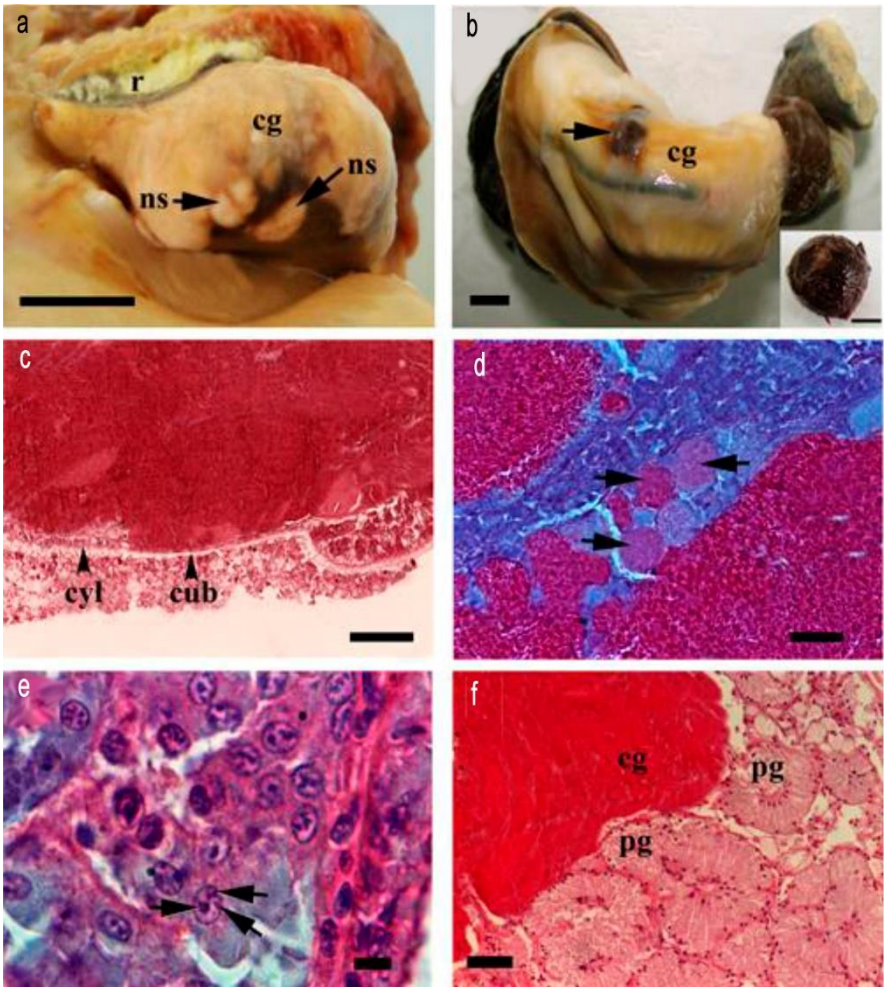

Figure 5. Capsule gland from female *Thaisella chocolata.* a) External view of the gland. Note the nodular structures, b) gland with aborted ovicapsule mass (arrow). Inset: external view of aborted ovicapsule mass, a-b (scale bar = 1 cm). c-f) transverse histological sections of capsule gland, c) modified cylindrical epithelium of the gonoduct (scale bar = 80 µm), d) glandular tissue with acellular areas and low dye affinity (arrows) (scale bar = 20 µm), e) detail of pyknotic nuclei (arrows) (scale bar = 5 µm), f) prostatic glands on the periphery of capsule gland (scale bar = 70 µm). cg: capsule gland, cyl: cylindrical, cub: cubic, n: nodular, r: rectum, pg: prostatic gland.

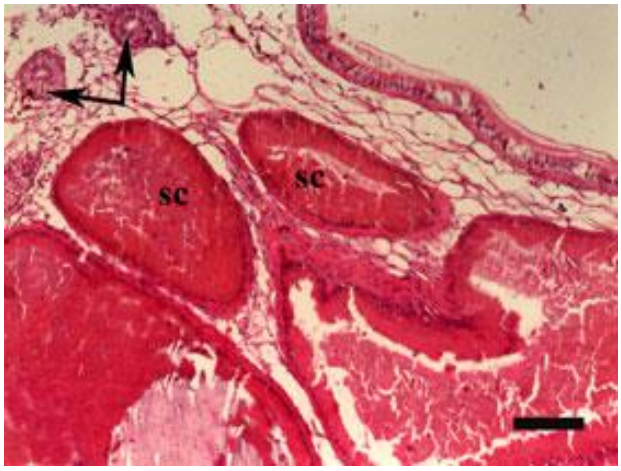

Figure 6. Ingestive gland of female *Thaisella chocolata* transverse histological section: Saccules of ingestive gland. It highlighted on the periphery of the gland, the occurrence of acini of prostatic gland (arrows). Scale bar = 200 µm, sc: saccules.

are located interspersed between the peripheral acini of the albumen gland (Fig. 7d).

The seminal receptacle corresponds to tubules located in a dorsal position between the albumen and ingestive glands. It consists of a cubic and simple epithelium surrounded by a layer of circular muscle.

The apical portion of the epithelial cells has spermatozoids adhered by their heads with their flagella directed towards the lumen. The seminal receptacle was present in three specimens of the ten analyzed.

The oviduct arises from the ovary and travels through the body as a straight tube of white color, internally lined by a cylindrical simple and ciliated epithelium, surrounded by a thick layer of circular muscle and connective tissue.The ovary is attached to the surface of the digestive gland. The color varies from cream to yellow-cream, according to the state of maturity. It is organized on elongated tubules composed of a germinal epithelium from which the previtellogenic and vitellogenic oocytes originate.

Proportion and degrees of imposex

All females of *T. chocolata* examined (n = 90) from Caldera Bay possessed a pseudopenis and vas deferens, which indicates that a high proportion suffers from imposex (I% = 100%). In the five scales applied, the largest proportion of females with imposex was classified in the higher grades, except for that of Barreiro *et al.* (2001) (Table 1).

In the scales of Gibbs *et al.* (1987) and Fernandez *et al.* (2002), on average 81% of females of *T. chocolata* examined had a vermiform structure called pseudo-penis and a continuous vas deferens, followed by 19% females with ovicapsules aborted in the lumen of the capsule gland, corresponding to the last grade in the scale of Rodríguez *et al.* (2008) (Table 1).

In addition to the superimposition of these two male organs and the occurrence of aborted ovicapsules in the female system of *T. chocolata* there were other anomalies, particularly in the vagina, considered by the scale of Stroben *et al.* (1992). In this scale, 48% of the analyzed females showed a reduction in the size of the vulva, nodules in the vaginal opening and/or the presence of prostatic tissue in the vaginal muscles, as well as the occurrence of a penis duct in the other 40% (Table 1). The VDSI index of imposex was 4.8 and the values of the other female indices were: RPLI = 59.6 and RPSI = 21.8

DISCUSSION

Anatomical and histopathological alterations

The most drastic anatomical and histological alterations identified in this study occur in the vagina and the reproductive glands. Occlusion of the vagina and its replacement by the vas deferens are the main cause of infertility in females. In addition, the capsule, ingestive and albumen glands are reduced in size and/or missing compared to females without imposex. This condition may prevent the normal production of secretory material used in packaging oocytes (Huaquín *et al.*, 2004) and in providing intracapsular material. Notable was the occurrence of ovicapsular materials and traces of yolk inside the capsule gland even when no presence of spermatozoids was recorded anywhere in the reproductive tract in these specimens. This suggests that females with imposex try to reproduce with still-unfertilized oocytes. The most revealing histological evidence of the extreme degree of virilization that females with imposex suffer is the presence of prostatic acini interposed in the vagina tissue and surrounding the reproductive glands.

The mechanisms underlying the masculinization and development of imposex have not been fully elucidated. One of the currently most accepted hypothesis ssuggests an activation induced by TBT on retinoid X receptors (RXR), normally present in the reproductive organs and the cephalic region (Urushitani *et al.*, 2013; Horiguchi *et al.*, 2014).

In Caldera Bay there are no measurements of TBT in the environment, however, this locality is characterized by the development of productive activities that involve the presence or building structures protected by antifouling paints (boats, dock

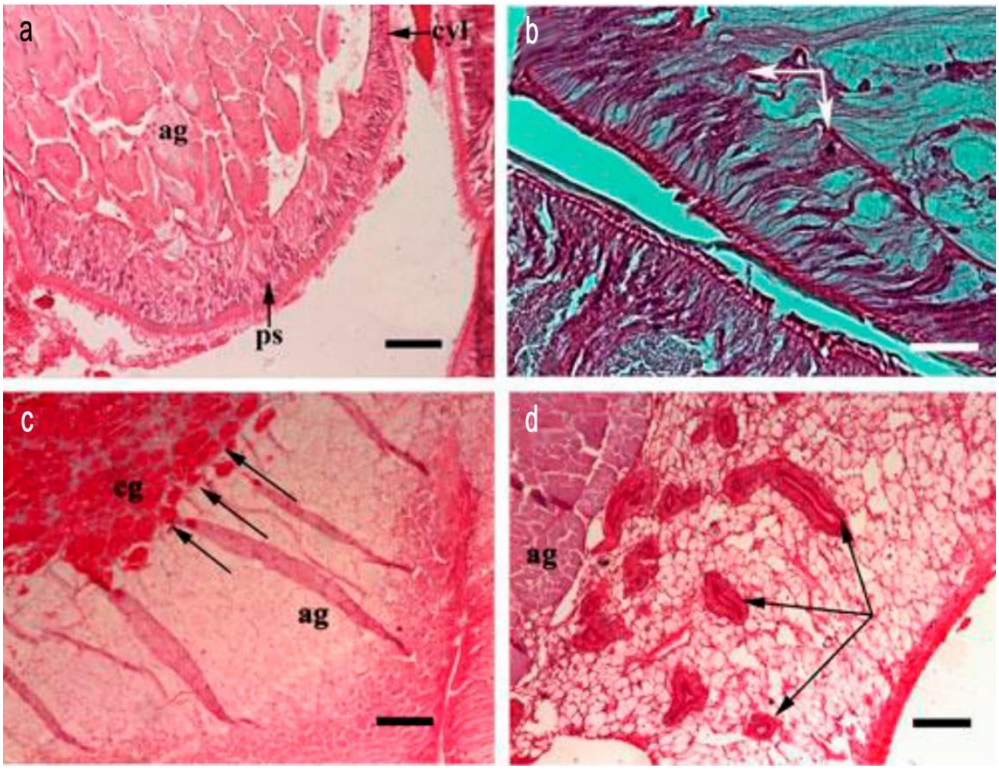

Figure 7. Albumen gland from a female *Thaisella chocolata*: transverse histological sections a) modified cylindrical epithelium of gonoduct (scale bar = 70 µm), b) gonoduct epithelium with lack of basal lamina (scale bar = 20 µm), c) diffuse demarcation (arrows) between albumen and capsule glands, d) acini of prostatic gland on the periphery of albumen gland. c-d scale bar = 200 µm, ag: albumen gland, cg: capsule gland, cyl: cylindrical, ps: pseudostratified.

pilings and farming systems). From the above, and considering the long life estimated for TBT, remaining for decades (Miloslavich *et al.*, 2007), it is expected that the sediment will have a high concentration of this organotin compound, of ancient or recent origin.

Imposex proportion and degrees

The occurrence of pseudopenis and vas deferens in females of *Thaisella chocolata* from Caldera Bay is consistent with the characterization of the imposex syndrome described by Smith (1971). The proportion of imposex determined in the sample of this study is the maximum possible (I% = 100%); taking into account: 1) the number of samples analyzed (n = 90), 2) the random nature of the sample, and 3) a collection after this study of 60 females with I% = 100% was obtained at 10 km from the original site collection, it is expected that imposex is a widespread phenomenon in the population of *T. chocolata* in this location.

Most imposex scales used in this study (Gibbs *et al.*, 1987; Stroben *et al.*, 1992; Fernandez *et al.*, 2002; Rodríguez *et al.*, 2008) coincide in extreme masculinization of *T. chocolata*. However, the scale of Stroben *et al.* (1992) is the most integrated in representing in detail macroscopically and histologically all the symptoms of imposex, especially in the area of the vagina; these characteristics were observable in the ninety females of *T. chocolata* from Caldera Bay. The scale of Barreiro *et al.* (2001) is an exception, since it classified the degree of convolution of the oviduct in females affected by imposex, and apparently reflects its gradual transformation toward a seminal vesicle (Oehlmann *et al.*, 1991). This phenomenon has been described only in *Nassarius reticulatus* (Linnaeus, 1758) (Barreiro *et al.*, 2001; Rodríguez *et al.*, 2008) and *Nucella lapillus* (Linnaeus, 1758) (Oehlmann *et al.*, 1991). Despite the occurrence of imposex advanced degrees in *T. chocolata*, convolution of the oviduct was not recorded, suggesting specific species sensitivity on this part of the system.

The indices of the Vas Deferens Stages Index (VDSI), Relative Penis Length Index (RPLI) and Relative Penis Size Index (RPSI) were adequate to represent the severity of imposex in *T. chocolata*, since high values reflected proportionally a greater develop-

Table 1. Percent (%) and number (n) of imposex degree in 90 females of *Thaisella chocolata* on the scales of Gibbs *et al.* (1987), Stroben *et al.* (1992), Barreiro *et al.* (2001), Fernandez *et al.* (2002) and Rodríguez *et al.* (2008).

Scales	Characterization of the vas deferens degree	Degree	%	n
Gibbs *et al.* (1987)	Complete development of vas deferens.	4	34	31
	Vagina blocked by vas deferens.	5	44	40
	Aborted ovicapsules in the capsule gland.	6	22	19
Stroben *et al.* (1992)	Pseudopenis with penis duct and vas deferens continuous. Also includes the occurrence of two pseudopenises in females.	4	40	36
	Vagina reduced or absent. Prostate gland of variable length arises in the mantle cavity.	5a	10	9
	Vagina occluded by the vas deferens, often forming nodules.	5b	34	30
	Vagina reduced or absent. Occurrence of aborted ovicapsules in the capsule gland.	6a	4	4
	Vagina occluded by the vas deferens. Occurrence of aborted ovicapsules in the capsule gland.	6b	12	11
Barreiro *et al.* (2001)	Rectilinear, transparent and narrow oviduct.	0	100	90
Fernandez *et al.* (2002)	Pseudopenis (>1 mm) and/or vas deferens incomplete.	3	10	9
	Vas deferens continued.	4	30	27
	Vagina blocked by the vas deferens.	5	44	40
	Aborted ovicapsules in the capsule gland.	6	16	14
Rodríguez *et al.* (2008)	Hard aborted ovicapsules impossible to break.	4	16	14

ment of pseudopenis, vas deferens and occlusion of the vulva compared to those reported for other gastropods with similar values in these indexes, VDSI = 4.0 (Fernandez *et al.*, 2002), RPLI = >50 points (Lahbib *et al.*, 2008; Castro & Fillmann, 2012) and RPSI = >20 points (Spence *et al.*, 1990). We recommended the VDSI index for *T. chocolata*; this index provides a realistic measure of the virilization and reproductive potential of the females of gastropods affected, as well as also its value represents a estimating the concentrations of TBT in the environment (Fioroni *et al.*, 1991; Schulte-Oehlmann *et al.*, 1997), and would not be affected by the sigmoid shape and size of the penis (Vasconcelos *et al.*, 2011).

The occurrence of imposex has been reported in seven other species of gastropods in Chile (Gooding *et al.*, 1999; Huaquín *et al.*, 2004; Panes, 2004; Collado *et al.*, 2010; Vásquez, 2015; Batista *et al*., 2016). Gooding *et al*. (1999) used the Stroben *et al.* (1992) scale to establish the degree of imposex in *Chorus giganteus* (Lesson, 1830), *Nucella crassilabrum* (Lamarck, 1816) [=*Acanthina monodon* (Pallas, 1774)] and *Xanthochorus cassidiformis* (Blainville, 1832). The authors determined degrees <4, that is, different degrees of development of pseudopenis and vas deferens that would not prevent normal reproduction. By contrast, the degree of masculinization determined in the present study with the same scale is associated with total sterility of *T. chocolata* from Caldera Bay.

Castro & Fillmann (2012) determined, in several localities from Peru, that *T. chocolata* suffered degrees of imposex of 1-5 on the scale of Gibbs *et al.* (1987). The authors found a positive relationship between the concentration of organotin compounds in tissues (23.5 -662 ng Sn/g-1 dry weight) and the degree of imposex. According to data reported by Castro & Fillmann (2012), one would expect that the concentration of TBT in specimens of *T. chocolata* from Caldera will exceed 662 ng Sn/dry weight. This will be confirmed by analyzing the concentrations of TBT in sediments and tissues of this species (work in progress).

CONCLUSIONS

Thaisella chocolata is the eighth species described with imposex in Chile and the first reported for the northern area. The degree of imposex determined in Caldera Bay corresponds to the most advanced stage and is associated with the total sterility of this species in this locality.

The scale of Stroben *et al.* (1992) is the most appropriate to characterize imposex, describing the most observable and conspicuous alterations of this syndrome in the 90 females of *T. chocolata* analyzed. VDSI is suggested as the best imposex index since it would not be affected by the shape and/or size of the male penis.

Considering the abundance and wide geographic distribution of *T. chocolata*, we believe it would be advisable to use this species as a potential bioindicator of pollution by organotin compounds on the coast from northern Chile. Currently, it is working on determining

the concentration of butyltin compounds in sediments and tissues of *T. chocolata* from sites with different grades of antropic activity.

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