Zoological Studies **49**(1): 85-93 (2010)

A High Incidence of Imposex in *Pomacea* Apple Snails in Taiwan: A Decade after Triphenyltin Was Banned

Jing-Ying Wu[1], Pei-Jie Meng[2,3], Ming-Yie Liu[4], Yuh-Wen Chiu[5], and Li-Lian Liu[1,*]

[1]*Institute of Marine Biology and Asia-Pacific Ocean Research Center - Kuroshio Research Group, National Sun Yat-sen University, Kaohsiung 804, Taiwan*

[2]*National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan*

[3]*Institute of Marine Biodiversity and Evolution, National Dong Hwa University, Pingtung 944, Taiwan*

[4]*Department of Environmental and Occupational Health, Medical College, National Cheng Kung University, Tainan 704, Taiwan*

[5]*Faculty of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

(Accepted May 5, 2009)

Jing-Ying Wu, Pei-Jie Meng, Ming-Yie Liu, Yuh-Wen Chiu, and Li-Lian Liu (2010) A high incidence of imposex in *Pomacea* apple snails in Taiwan: a decade after triphenyltin was banned. *Zoological Studies* **49**(1): 85-93. The South American apple snails *Pomacea canaliculata* and *P. scalaris* were intentionally introduced into Taiwan in the 1980s. Subsequently, *P. canaliculata* has become a serious pest to aquatic crops, and triphenyltin (TPT) was the major control agent. We conducted a nationwide survey on the imposex status of *P. canaliculata* and *P. scalaris* to evaluate the effectiveness of the ban on TPT use in agriculture since 1999. *Pomacea canaliculata* was distributed island-wide, but *P. scalaris* was only distributed in southern Taiwan, sympatrically with *P. canaliculata*. The imposex condition was found in snails from all collecting sites of the 2 species. Based on vas deferens sequence (VDS) indices, *P. scalaris* and *P. canaliculata* have the same susceptibility. Additionally, imposex-affected individuals of *P. canaliculata* were found in all types of freshwater habitats, i.e., crop drainage, reservoirs, and wastewater drainage, with respective VDS indices ranging 1.02-1.40, 0.75-2.00, and 1.00-1.88. Based on these survey results, the illegal use of TPT in agriculture has likely continued, and extra control actions are urgently needed. http://zoolstud.sinica.edu.tw/Journals/49.1/85.pdf

Key words: *Pomacea canaliculata*, *Pomacea scalaris*, Imposex, VDS, TPT.

The South American apple snails *Pomacea canaliculata* (Lamarck, 1822) and *P. scalaris* d'Orbigny, 1835 were deliberately introduced into Taiwan for aquaculture purposes in the 1980s (Chang 1986 1994, Cowie et al. 2006, Hayes et al. 2008). However because of poor market reception by local people, they were released by snail farmers. The apple snails spread into various natural waterways through irrigation drainages. Subsequently, *P. canaliculata* invaded East Asian waters, and has become a serious pest to aquatic crops in the region (Naylor 1996). By contrast, the distribution of *P. scalaris* remains limited to Taiwan (Lee and Wu 1996, Cowie et al. 2006), and so far, Taiwan is the only site where *P. scalaris* has successfully established itself outside its native range (Hayes et al. 2008).

Although various methods can be used for managing apple snail pests, e.g., hand picking, duck pasturing, and careful water control, including occasional field drainage and maintenance of low water levels (Naylor 1996, Teo 2001, Joshi and Sebastian 2006), the use of molluscicides such as metaldehyde, niclosamide, and triphenyltin (TPT) acetate (TPTA) is the most common way to control this pest in Taiwan (Cheng and Kao 2004). In 1999, the Council of Agriculture (COA), Executive Yuan banned the use of TPTA in agriculture as

*To whom correspondence and reprint requests should be addressed. Tel: 886-7-5252000 ext. 5108. Fax: 886-7-5255100. E-mail:lilian@mail.nsysu.edu.tw

a precaution against its unclear toxicological effects. However, over 70% of farmers surveyed nationwide expressed dissatisfaction with metaldehyde and niclosamide, and 27% of them still used TPTA as a pest control agent in 2004 (Cheng 2004). Tributyltin (TBT) and TPT were detected in 3.5% and 43.8% of a total of 144 rice-field irrigation sediments with concentrations of not detected (ND) ~52.4 μg TBT/kg wet wt. and ND ~319 μg TPT/kg wet wt., respectively (TACTRI 2003). The presence of imposex, with the vas deferens sequence (VDS) indices ranging 1.1-2.8, was reported in TPTA-targeted *P. canaliculata* from central and eastern Taiwan (Liu et al. 2006). Additionally, in eastern Taiwan, no TBT was detected in *P. canaliculata* with VDS stages of 0, 1, and 2, but respective TPT concentrations were 435, 918, and 1,276 ng/g dry wt. (Chuang 2005).

Imposex is an induced response from exposure to organotin compounds (e.g., TBT and TPT) (Oetken et al. 2004) that results in the formation of a penis and/or vas deferens in female gonochoristic caenogastropods (deFur et al. 1999). Imposex was identified in more than 170 caenogastropod species worldwide, but considerably more attention has been paid to marine than freshwater taxa (deFur et al. 1999, Oetken et al. 2004, Shi et al. 2005). This is also the case in Taiwan (Liu and Suen 1996, Liu et al. 1997, Hung et al. 2001, Lee et al. 2005 2006, Liu et al. 2006).

The male apple snail copulatory apparatus consists of a muscular penile sheath, penile bulb, pouch, and penis. In females, a rudimentary penis (also called a vestigial penis) develops into a penile pouch and penis when treated with TBT or testosterone (Takeda 2000). Meanwhile, a newly grown penile sheath can also be observed in imposex-induced females.

Although TPT usage in agriculture was banned in 1999 and TBT use on small boats has been restricted since 2003, detectable levels of these organotins are still present in aquatic systems. A review of aquatic organotin pollution in Taiwan found slowly decreasing levels of organotin contamination during the period of 1996-2005 (Meng et al. 2009). Because the half-life of TPT in soils ranges 27-140 d (Loch et al. 1990, Paton et al. 2006), high levels of detected TPT are probably not just residues from previous use. This indicates that the illegal application of TPT by agricultural interests possibly continues. The consequent chemical risks to human health are a serious concern, both for farmers and consumers eating potentially contaminated food. Therefore, the effectiveness of the ban of TPT use in agriculture in 1999 was evaluated by a nation-wide survey on the imposex status of apple snails *P. canaliculata* and *P. scalaris*.

MATERIALS AND METHODS

Pomacea canaliculata and *P. scalaris* were collected from various freshwater habitats, i.e., crop and wastewater drainages and reservoirs in Taiwan and adjacent islands during the period of Mar. 2006 - Apr. 2007 (Fig. 1, Table 1). Samples were returned to the laboratory and frozen at -20°C for later use.

After thawing, snail shell length (between the tip of the apex and the edge of the bottom lip) was recorded before the shell was broken in a vice. Snails were sexed based on the appearance of gonads and accessory organs.

Reproductive structures (i.e., the lengths of the penile sheath, rudimentary penis, and straightened penis), if present, were measured (Figs. 2, 3). Development of imposex in females as the vas deferens sequence (VDS) follows Liu et al. (2006) (Fig. 3). The 4 stages are: stage 0 with no evidence of a male genital system; stage 1 with the appearance of a rudimentary penis; stage 2 with a rudimentary penis and a penile sheath; and stage 3 with the rudimentary penis developing into a penile pouch and penis. The VDS index was calculated as the average imposex stage of a population. The VDS data were analyzed and compared between species and among populations, using 1-way analysis of variance (ANOVA) and Tukey's multiple-comparisons test.

RESULTS

Distribution of *P. canaliculata* and *P. scalaris* in Taiwan

In total, 26 locations were surveyed (Table 1). Thirteen populations of *P. canaliculata* were from crop drainages, 10 from wastewater drainages, and 3 from reservoirs. Only 3 populations of *P. scalaris* were collected, 2 from crop drainages and 1 from wastewater drainage. Although both species were found, *P. canaliculata* was widespread in Taiwan and on adjacent islands, occurring at sites like C13 and W10 on Orchid I. and R3 and W9 in the Penghu Archipelago (Fig. 1, Table 1). *Pomacea*

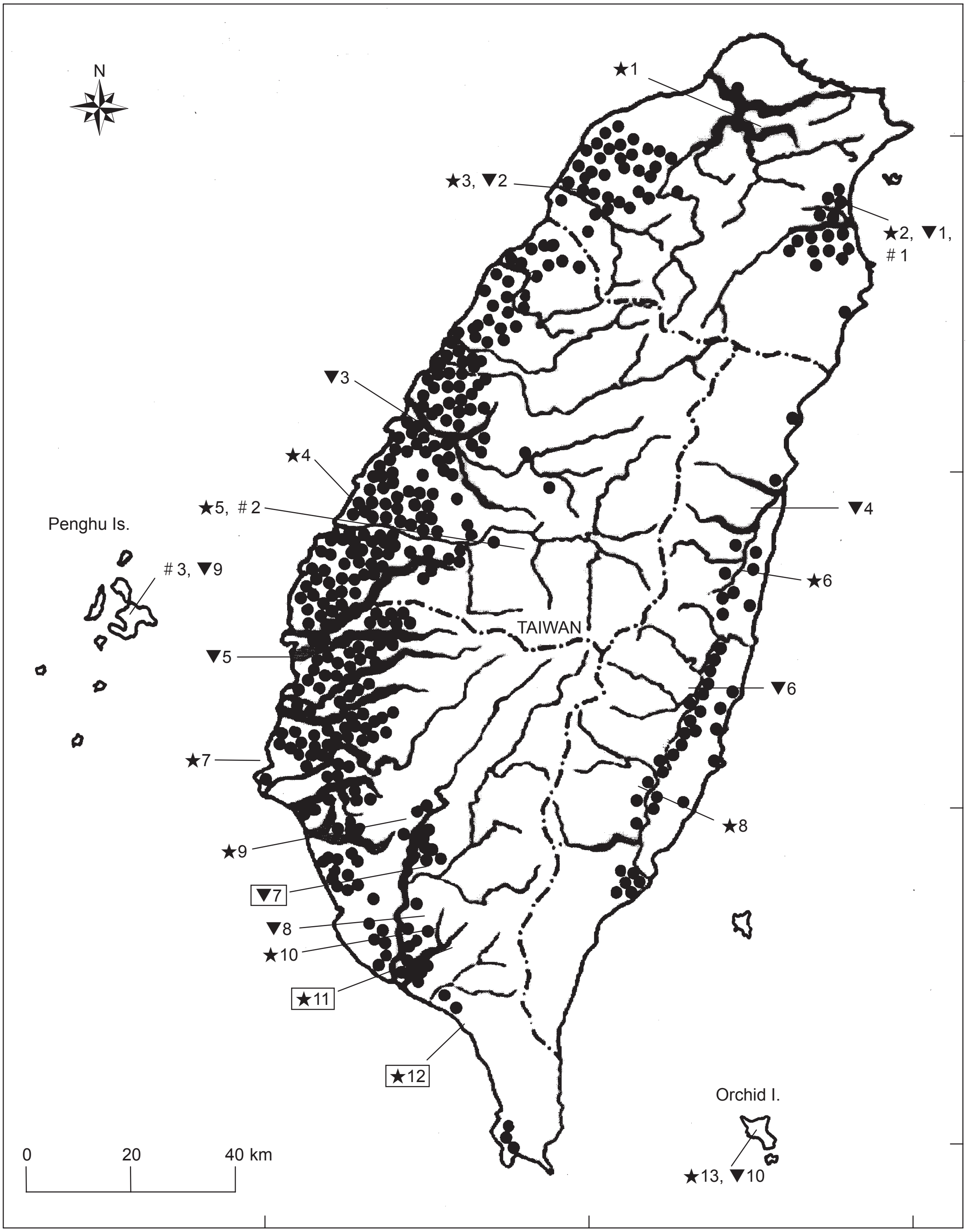

Fig. 1. Map of Taiwan showing rice fields and sampling sites for both *Pomacea canaliculata* and *P. scalaris*. ●, rice field; ★, crop drainage; #, reservoir; ▼, wastewater drainage; □, *P. canaliculata* and *P. scalaris* coexist; **★1**, Nangang; **★2**, **#1**, **▼1**, Jiaosi; **★3**, **▼2**, Hsinchu; **★4**, Fusing; **★5**, Puli; **★6**, Guanfu; **★7**, Syuejia; **★8**, Guanshan; **★9**, Cishan; **★10**, Pingdong City; **★11**, Wanluan; **★12**, Fangliao; **★13**, **▼10**, Yuren; **#2**, Puli; **#3**, **▼9**, Dongwei; **▼3**, Dali; **▼4**, Shoufong; **▼5**, Yuli; **▼6,** Douliou; **▼7**, Jiouru; **▼8**, Jioucyutan.

scalaris was only found in southern Taiwan (at sites C10, C11, and W7) and was always found sympatrically with *P. canaliculata*.

Imposex in *P. canaliculata* and *P. scalaris*

Indications of imposex were found in all collections of both *P. canaliculata* and *P. scalaris*, at VDS stages 0-2 (Tables 2, 3). No individuals in stage 3 were found. The pattern of imposex in *P. scalaris* was similar to that in *P. canaliculata*. Seriously deformed females of neither species (e.g., with split oviducts, oulva occlusion, or darkened eggs in the reproductive tract) were observed.

In all, 58% and 88% imposex-affected females of *P. canaliculata* and *P. scalaris* were in stage 1 (Tables 2, 3, Fig. 4). Although VDS indices of *P. scalaris* were usually higher than those of *P. canaliculata* in the same location, a significant difference was only found at site W7 ($p < 0.05$, Tukey's test).

Imposex-affected individuals of *P. canaliculata* were found in all examined freshwater habitats (crop and wastewater drainages and reservoirs, Fig. 4). VDS indices in each habitat had overlapping ranges, i.e., 1.02-1.40, 0.75-2.00, and 1.00-1.88, respectively (Table 3). Among

Table 1. Environmental characteristics of sampling sites in Taiwan and adjacent islands. C, crop drainage; R, reservoir; W, wastewater drainage; Pc, *Pomacea canaliculata*; Ps, *P. scalaris*

Locality	Locality code	Sampling date	Environmental characteristics
Co-existence of *Pomacea canaliculata* and *Pomacea scalaris*			
Wanluan	C11-Pc	20060326	Drainage of a betel nut field
	C11-Ps	"	
Fangliao	C12-Pc	20060527	Drainage of a pineapple field
	C12-Ps	"	
Jiouru	W7-Pc	20061020	Crop and domestic wastewater drainage
	W7-Ps	"	
Pomacea canaliculata in different environments			
Jiaosi	R1	20070327	Reservoir
Puli	R2	20070405	Reservoir
Dongwei	R3	20060427	Reservoir
Nangang	C1	20070329	Drainage of a rice field
Jiaosi	C2	20070327	Drainage of a rice field
Hsinchu	C3	20070330	Drainage of a rice field
Fusing	C4	20070403	Drainage of a rice field
Puli	C5	20070405	Drainage of water bamboo
Guanfu	C6	20070131	Drainage of a rice field
Syuejia	C7	20061020	Drainage of a rice field
Guanshan	C8	20070129	Drainage of rice field
Cishan	C9	20060429	Drainage of rice field
Pingdong city	C10	20061020	Drainage of bok-choy and banana
Yuren	C13	20060403	Drainage of a taro field
Jiaosi	W1	20070327	Crop and domestic wastewater drainage
Hsinchu	W2	20070330	Crop, domestic, and industrial wastewater drainage
Dali	W3	20070405	Crop, domestic, and industrial wastewater drainage
Shoufong	W4	20070201	Crop and domestic wastewater drainage
Yuli	W5	20070130	Crop and domestic wastewater drainage
Douliou	W6	20070403	Crop and domestic wastewater drainage
Jioucyutan	W8	20060530	Crop, domestic, and industrial wastewater drainage
Dongwei	W9	20060427	Crop and domestic wastewater drainage
Yuren	W10	20060403	Crop and domestic wastewater drainage

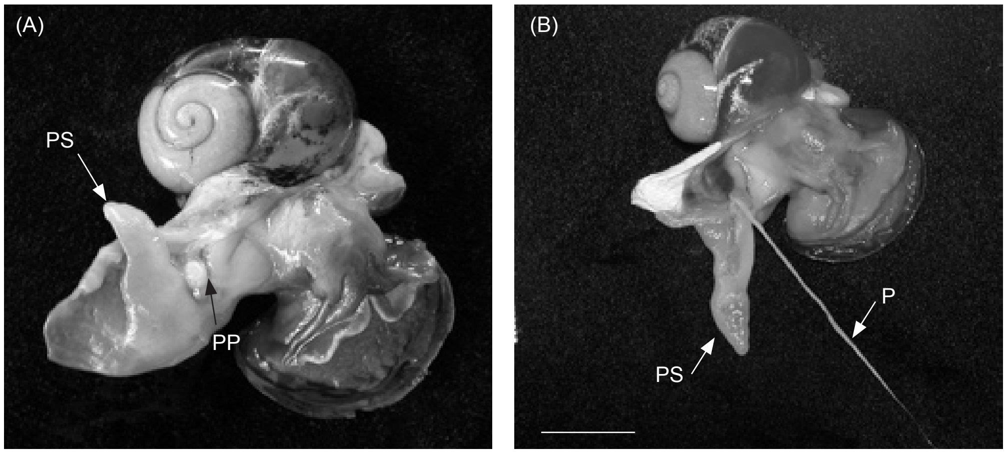

Fig. 2. General appearance of a male apple snail (*Pomacea canaliculata*). (A) Gross morphology. (B) Straightened penis. P, penis; PP, penile pouch; PS, penile sheath; scale bar: 10 mm. Photos from Liu et al. (2006).

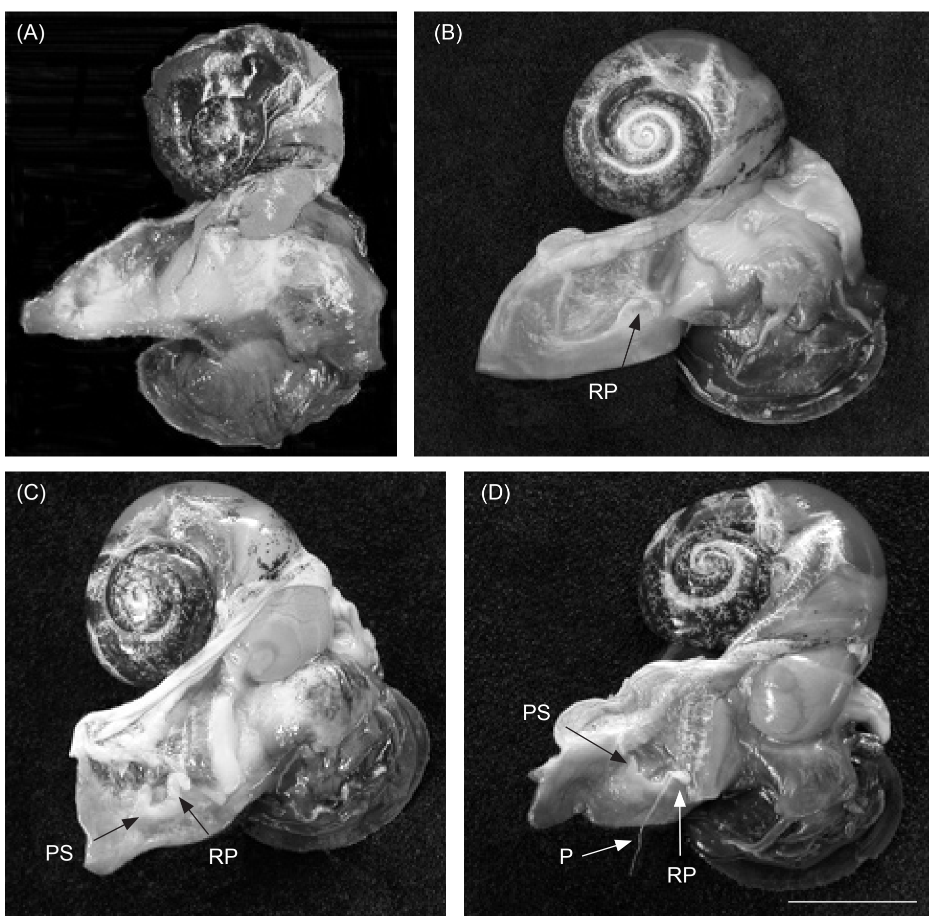

Fig. 3. General appearance of a female apple snail (*Pomacea canaliculata*). (A) Stage 0 with no evidence of a male genital system. (B) Stage 1 with the appearance of a rudimentary penis. (C) Stage 2 with a rudimentary penis and penile sheath. (D) Stage 3 with the rudimentary penis developing into a penile pouch and penis. P, penis; PS, penile sheath; RP, rudimentary penis; scale bar: 10 mm. Photos B-D are from Liu et al. (2006).

crop drainages, sites C2-C5 and C8 had higher incidences than other crop sites ($p < 0.05$, Tukey's test) (Fig. 4). Although crop drainages received irrigation water from different crops, i.e., rice (C1-C4, C6-C9), taro (C13), betel nut (C11), pineapple (C12), water bamboo (C5), bok-choy, and banana (C10), the degree of imposex was not related to the crop type.

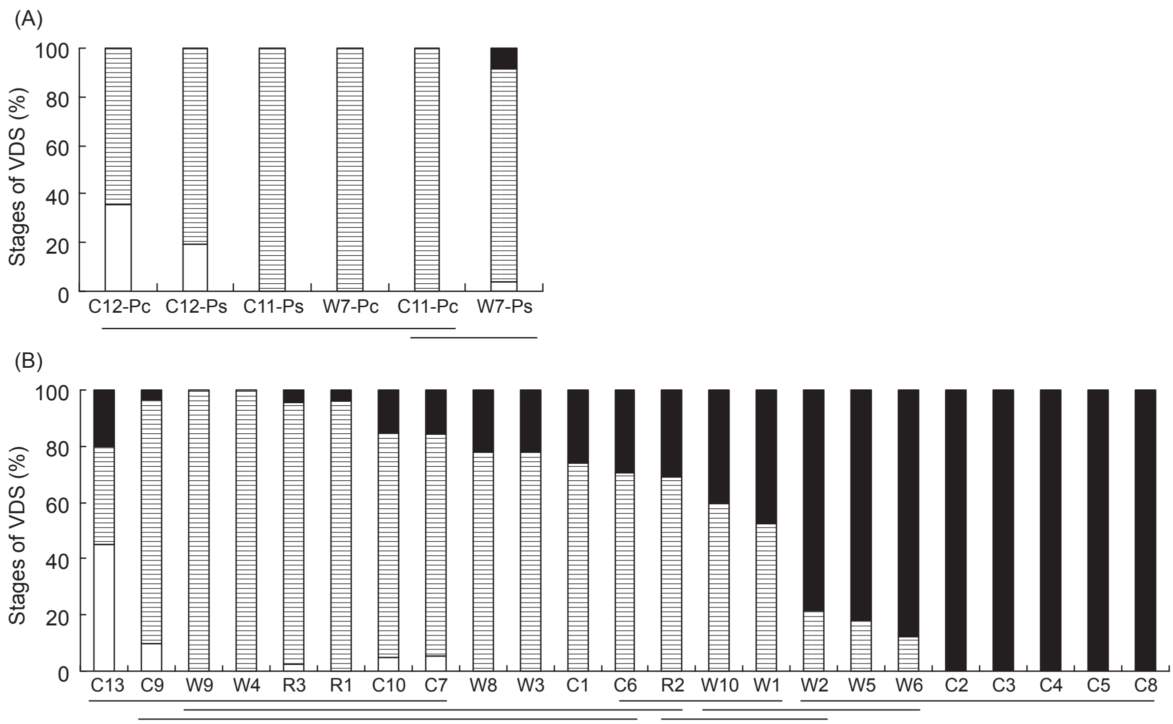

Fig. 4. Relative occurrence ratio of vas deferens sequence (VDS) stages in *Pomacea canaliculata* and *P. scalaris*. (A) VDS stages of *P. canaliculata* and *P. scalaris* when both species coexist. (B) *Pomacea canaliculata* collected from various freshwater habitats. C, crop drainage; R, reservoir; W, wastewater drainage; ■, stage 0; ≡, stage 1; □, stage 2. Horizontal bars signify populations that do not significantly differ (ANOVA, $p > 0.05$; Tukey's test).

Table 2. Summary results of imposex surveys in localities where both *Pomacea canaliculata* and *P. scalaris* coexist. C, crop drainage; W, wastewater drainage; Pc, *P. canaliculata*; Ps, *P. scalaris*; *n*, sample size; VDS, vas deferens sequence; Stage 0, with no evidence of a male genital system; Stage 1, with a rudimentary penis; Stage 2, with a rudimentary penis and penile sheath; values are the mean ± SD

Locality code	Sex	*n*	Shell length (mm)	Weight (g)	Penile sheath length (mm)	Penis length (mm)	Rudimentary penis length (mm)	VDS stages 0	VDS stages 1	VDS stages 2	VDS index
C11-Pc	F	21	40.6 ± 3.7	16.5 ± 4.2	0		2.01 ± 0.35	0	21	0	1.00
	M	9	41.4 ± 3.1	16.3 ± 4.0	17.01 ± 1.87	33.7 ± 9.8					
C11-Ps	F	18	38.3 ± 2.5	14.2 ± 3.3	0		1.52 ± 0.30	0	18	0	1.00
	M	10	37.9 ± 1.9	13.2 ± 2.0	12.66 ± 3.12	29.4 ± 5.3					
C12-Pc	F	31	33.1 ± 3.6	6.4 ± 2.2	0		1.41 ± 1.19	11	20	0	0.65
	M	21	33.5 ± 3.5	6.7 ± 2.2	12.58 ± 3.33	36.1 ± 10.8					
C12-Ps	F	26	37.2 ± 3.5	9.2 ± 2.3	0		1.71 ± 0.95	5	21	0	0.81
	M	16	38.0 ± 2.2	9.0 ± 1.2	12.75 ± 2.77	31.0 ± 8.3					
W7-Pc	F	16	31.6 ± 1.8	6.4 ± 1.2	0		2.29 ± 0.48	0	16	0	1.00
	M	17	31.9 ± 3.2	5.8 ± 1.6	11.49 ± 2.63	35.0 ± 4.8					
W7-Ps	F	24	36.7 ± 2.1	9.5 ± 1.4	0.09 ± 0.31		2.52 ± 0.73	1	21	2	1.04
	M	4	37.0 ± 2.6	8.3 ± 1.3	12.57 ± 4.17	26.3 ± 13.0					

Table 3. Summary results of imposex surveys in localities from freshwater habitats in Taiwan and adjacent islands where only *Pomacea canaliculata* existed. C, crop drainage; R, reservoir; W, wastewater drainage; *n*, sample size; VDS, vas deferens sequence; Stage 0, with no evidence of a male genital system; Stage 1, with a rudimentary penis; Stage 2, with a rudimentary penis and penile sheath; values are the mean ± SD

Locality code	Sex	*n*	Shell length (mm)	Weight (g)	Penile sheath length (mm)	Penis length (mm)	Rudimentary penis length (mm)	VDS stages 0	1	2	VDS index
R1	F	27	37.5 ± 4.8	13.8 ± 5.9	0.04 ± 0.19		2.68 ± 0.59	0	26	1	1.04
	M	6	32.9 ± 4.2	9.4 ± 4.1	13.66 ± 3.13	46.0 ± 9.8					
R2	F	20	48.4 ± 6.5	23.99 ± 10.18	0.56 ± 0.72		3.53 ± 0.84	0	12	8	1.40
	M	8	48.9 ± 6.5	23.1 ± 9.6	20.77 ± 3.30	54.5 ± 11.9					
R3	F	45	43.1 ± 4.7	18.9 ± 6.9	0.05 ± 0.23		3.23 ± 0.89	1	42	2	1.02
	M	7	38.1 ± 4.2	11.9 ± 3.9	17.49 ± 2.91	40.6 ± 16.2					
C1	F	23	33.9 ± 2.8	10.0 ± 2.8	0.38 ± 0.68		2.59 ± 0.47	0	17	6	1.26
	M	8	31.7 ± 3.3	7.6 ± 2.6	17.93 ± 2.37	47.3 ± 5.6					
C2	F	19	49.7 ± 5.7	25.4 ± 7.6	2.88 ± 0.64		3.78 ± 0.68	0	0	19	2.00
	M	11	44.6 ± 3.8	17.4 ± 4.2	19.36 ± 2.85	48.0 ± 6.2					
C3	F	21	34.0 ± 3.9	9.0 ± 3.2	2.08 ± 0.60		3.00 ± 0.55	0	0	21	2.00
	M	10	31.9 ± 2.5	7.5 ± 2.0	17.07 ± 2.06	41.9 ± 7.2					
C4	F	23	42.8 ± 4.9	16.8 ± 5.5	2.27 ± 1.00		3.71 ± 0.86	0	0	23	2.00
	M	11	40.3 ± 5.0	13.7 ± 5.5	23.02 ± 5.36	53.5 ± 8.6					
C5	F	15	33.6 ± 1.8	8.3 ± 1.8	1.83 ± 0.68		3.07 ± 0.46	0	0	15	2.00
	M	16	35.4 ± 3.8	8.6 ± 2.7	17.23 ± 2.43	50.4 ± 6.9					
C6	F	13	45.3 ± 5.7	20.2 ± 7.4	0.25 ± 0.39		3.88 ± 0.92	0	9	4	1.31
	M	16	41.8 ± 4.8	15.5 ± 4.2	17.57 ± 3.15	49.3 ± 9.7					
C7	F	19	30.5 ± 4.4	7.0 ± 2.3	0.15 ± 0.35		2.55 ± 0.97	1	15	3	1.11
	M	10	31.4 ± 3.1	6.4 ± 1.5	9.60 ± 3.67	29.5 ± 9.1					
C8	F	21	36.4 ± 3.6	10.9 ± 3.1	2.15 ± 0.95		3.92 ± 0.66	0	0	21	2.00
	M	9	36.2 ± 3.0	9.4 ± 1.5	13.45 ± 3.30	44.4 ± 8.4					
C9	F	31	26.8 ± 4.3	4.6 ± 2.1	0.04 ± 0.22		2.29 ± 1.15	3	27	1	0.94
	M	37	27.8 ± 3.7	4.2 ± 1.3	12.15 ± 3.67	40.1 ± 13.3					
C10	F	20	29.9 ± 3.1	6.1 ± 1.7	0.11 ± 0.29		2.11 ± 0.62	1	16	3	1.10
	M	10	30.1 ± 1.9	6.5 ± 1.4	8.53 ± 1.79	30.7 ± 6.0					
C13	F	20	25.5 ± 4.6	3.7 ± 1.9	0.28 ± 0.59		1.14 ± 1.13	9	7	4	0.75
	M	30	25.1 ± 4.0	3.2 ± 1.4	8.87 ± 3.96	26.2 ± 9.8					
W1	F	17	38.5 ± 6.5	14.7 ± 8.1	0.99 ± 0.84		3.00 ± 0.97	0	12	5	1.29
	M	14	34.4 ± 4.2	9.6 ± 3.4	14.25 ± 3.27	40.4 ± 7.2					
W2	F	28	41.6 ± 4.1	17.1 ± 4.0	1.22 ± 0.75		3.00 ± 0.58	0	6	22	1.79
	M	6	36.4 ± 3.5	12.0 ± 3.0	17.17 ± 3.05	46.6 ± 5.2					
W3	F	18	40.9 ± 1.8	13.2 ± 2.4	0.25 ± 0.47		3.02 ± 0.52	0	14	4	1.22
	M	13	41.1 ± 5.6	14.5 ± 6.4	20.95 ± 2.78	52.1 ± 6.3					
W4	F	12	40.3 ± 4.4	14.3 ± 4.2	0		3.64 ± 0.83	0	12	0	1.00
	M	19	42.4 ± 5.4	14.9 ± 6.2	18.66 ± 3.76	55.0 ± 11.0					
W5	F	22	37.1 ± 4.2	13.2 ± 4.4	1.70 ± 1.32		3.38 ± 0.97	0	4	18	1.82
	M	8	38.7 ± 6.9	14.0 ± 8.2	17.02 ± 2.88	52.1 ± 10.7					
W6	F	24	47.3 ± 3.5	21.0 ± 4.2	1.37 ± 0.82		3.34 ± 0.48	0	3	21	1.88
	M	8	43.5 ± 2.1	16.6 ± 2.7	21.22 ± 4.23	55.3 ± 7.4					
W8	F	18	50.2 ± 7.6	23.6 ± 8.3	0.30 ± 0.60		4.58 ± 1.41	0	14	4	1.22
	M	11	46.1 ± 6.9	19.3 ± 8.1	19.90 ± 3.95	50.7 ± 9.3					
W9	F	33	41.4 ± 4.4	13.8 ± 4.8	0		3.60 ± 0.96	0	33	0	1.00
	M	38	39.8 ± 5.6	11.3 ± 4.0	11.74 ± 4.75	31.2 ± 10.1					
W10	F	19	34.5 ± 3.9	8.2 ± 1.9	0.53 ± 0.62		2.66 ± 0.66	0	10	9	1.47
	M	26	31.6 ± 4.1	6.6 ± 2.4	15.52 ± 2.66	37.5 ± 7.9					

More than 95% of all examined females of *P. canaliculata* and 90% of females of *P. scalaris* had a rudimentary penis with mean length ranges of 1.5-2.5 and 1.1-4.6 mm, respectively (Tables 2, 3). The mean value of the penile sheath length in males was longer than that in females for both species, with respective ranges of 8.5-21.2 and 0-2.9 mm, in *P. canaliculata*, and 12.6-12.8 and 0-0.1 mm in *P. scalaris*.

Using data collected from populations of *P. canaliculata*, signs of imposex such as the mean rudimentary penile length and mean penile sheath length were positively correlated, i.e. y = 0.47× -0.65 ($R^2 = 0.18$; $p < 0.05$, $n = 26$).

DISCUSSION

Herein, we provide the first report of the prevalence of imposex in *P. scalaris*, which exhibited a pattern similar to that of *P. canaliculata*. Although imposex was reported in more than 170 mollusk species, freshwater studies are rare, e.g., the ramshorn snail *Marisa cornuarietis* (Schulte-Oehlmann et al. 1995 2000, Shi et al. 2005), the mud snail *Potamopyrgus antipodarum* (Duft et al. 2003), and the golden apple snail *P. canaliculata* (Takeda 2000, Liu et al. 2006). In the present study, 1 more freshwater snail *P. scalaris* was added to this list.

The occurrence of imposex at all of our collecting sites for both *P. canaliculata* and *P. scalaris* is likely related to the continued illegal use of TPT by agricultural interests. The Taiwan Agriculture Industry Association (TAIA 1997) reported that more than 150 metric tons (mt) of 45% and 20 mt of 2% TPTA were used annually for agriculture. In 1997, the use of mixed pesticides, containing TPTA, benthiocarb, and chlornitrofen, were banned, and all TPT usage in agriculture was completely prohibited in 1999. The half-life of TPT in various soil types is 27-140 d, and degradation is thought to be via diphenyltin (DPT) and monophenyltin (MPT) intermediates by means of biological, physical, and chemical processes (Loch et al. 1990, Paton et al. 2006). A decrease in TPT concentrations in soils was expected after the 1999 ban.

However, butyltins (BTs) and phenyltins (PTs) continue to be detected in freshwater environments. In general, patterns of organotin occurrence in freshwater sediments and biota are similar, with PTs being dominant (Meng et al. 2009). Based on a rice-field study in eastern Taiwan, BTs and PTs were in the ranges of ND and < 80-700 μg/kg dry wt. in sediments and ND-321 and 815-1658 ng/g dry wt. in *P. canaliculata* (Chuang 2005). The bivalve *Corbicula fluminea* in culture ponds next to the rice fields also contained BTs and PTs, i.e., ND-247 and 85-615 ng/g dry wt., respectively.

Both BTs and PTs were also detected in river sediments and fish during a survey of major rivers of Taiwan in 2005 (Lee 2005). TBT in sediments was < 0.05 μg/g dry wt., and TPT was not detected in Mar. 2005. In Aug. 2005, TPT (0.06 μg/g dry wt.) was the only compound detected. In fish (*Tilapia zillii*) muscle, respective concentrations of MBT, DBT, TBT, MPT, DPT, and TPT were 0.33, 0.48, < 0.05, 0.19, 1.90, and 0.43 μg/g dry wt. in Mar. 2005. In Aug. 2005, these same compounds in fish muscle (*Cyprinus carpio*) were ND, 0.12, < 0.05, 0.14, 0.82, and 17.00 μg/g dry wt., respectively. Obviously, the concentration of TPT in summer was much higher than that in winter. Based on those reports, it is known that organotin pollution in freshwater environments is dominated by PTs, and their origin is possibly agriculturally related.

This is also supported by our island-wide survey results of imposex in TPTA-targeted apple snails. The snails' VDS indices were 0.65-2.00 which was slightly lower than a previous study of *P. canaliculata* from central and eastern Taiwan, i.e., 1.1-2.8 (Liu et al. 2006). Evidently, the illegal use of TPT as a molluscicide still exists, and the effectiveness of the regulations is highly questionable. There is no doubt that more-constructive control actions to stop the illegal application of TPT are urgently needed in Taiwan.

Acknowledgments: We are grateful to the anonymous reviewers for their constructive comments which substantially improved the manuscript. We thank F.J. Chuang, Y.T. Wu, Z.H. Hong, C.W. Wang, Y.H. Chang, and C.F. Chang for sample collection. This study was supported by the National Science Council, Taiwan (NSC97-2621-M-110-001 and NSC96-2611-M-291-002), the Integrative Project (Catchment-estuary Linkages of Food Web Dynamics in the Lanyang River) funded by the Research Center for Biodiversity, Academia Sinica, the Bureau of Animal and Plant Health Inspection and Quarantine, the Council of Agriculture of Taiwan, and the Asia-Pacific Ocean Research Center - Kuroshio Research Group, National Sun Yat-sen University supported by the Ministry of Education, Taiwan.

REFERENCES

Chang KM. 1986. Large populations of *Pomacea canaliculata* in Taiwan. Peiyo **10:** 34-42. (in Chinese)

Chang KM. 1994. The findings of *Pomacea scalaris* Orbigny, 1835 in Taiwan. Peiyo **20:** 5-7. (in Chinese)

Cheng EY. 2004. Prospects of golden apple snail control in Taiwan. APEC Symposium on the Management of the Golden Apple Snail, 6-11 Sept. 2004, Pingtung, Taiwan. Taipei: Council of Agriculture, 5 pp.

Cheng EY, CH Kao. 2004. Review and suggestions for the control strategy of golden apple snail *Pomacea canaliculata* (Lamarck). APEC Symposium on the Management of the Golden Apple Snail, 6-11 Sept. 2004, Pingtung, Taiwan. Taipei: Council of Agriculture, 8 pp.

Chuang YF. 2005. The study on investigation of organotins contaminated levels and sources for aquaculture of *Corbicula fluminea*. Master's thesis, Department of Environmental and Occupational Health, Medical College, National Cheng Kung Univ., Taichung, Taiwan,159 pp. (in Chinese)

Cowie RH, KA Hayes, SC Thiengo. 2006. What are apple snails? Confused taxonomy and some preliminary resolution. *In* RC Joshi, LS Sebastian, RC Joshi, LS Sebastian, eds. Global advances in ecology and management of golden apple snails. Nueva Ecija, the Philippines: Philippine Rice Research Institute, pp. 3-23.

deFur PL, M Crane, C Ingersoll, L Tattersfield. 1999. Endocrine disruption in invertebrates: endocrinology, testing, and assessment. *In* Proceedings of the Workshops on Endocrine Disruption in Invertebrates, 12-15 Dec. 1998, Noordwijkerhout, the Netherlands. Pensacola, FL: SETAC Press.

Duft M, U Schulte-Oehlmann, M Tillmann, B Markert, J Oehlmann. 2003. Toxicity of triphenyltin and tributyltin to the freshwater mudsnail *Potamopyrgus antipodarum* in a new sediment biotest. Environ. Toxicol. Chem. **22:** 145-152.

Hayes KA, RC Joshi, SC Thiengo, RH Cowie. 2008. Out of South America: multiple origins of non-native apple snails in Asia. Divers. Distrib. **14:** 701-712.

Hung TC, WK Hsu, PJ Meng, A Chuang. 2001. Organotins and imposex in the rock shell, *Thais clavigera*, from oyster maricultural areas in Taiwan. Environ. Pollut. **112:** 145-152.

Joshi RC, LS Sebastian. 2006. Global advances in ecology and management of golden apple snails. Nueva Ecija, the Philippines: Philippine Rice Research Institute, 588 pp.

Lee CC. 2005. The establishment of the environmental distributions survey of toxic chemicals. Project report (EPA-94- J103-02-102). Taipei, Taiwan: Environmental Protection Agency of Taiwan, 590 pp. (in Chinese)

Lee CC, CY Hsieh, CJ Tien. 2006. Factors influencing organotin distribution in different marine environmental compartments, and their potential health risk. Chemosphere **65:** 547-559.

Lee CC, T Wang, CY Hsieh, CJ Tien. 2005. Organotin contamination in fishes with different living patterns and its implications for human health risk in Taiwan. Environ. Pollut. **137:** 198-208.

Lee YC, WL Wu. 1996. Study on *Pomacea canaliculata* (Lamark, 1819) in Taiwan. Bull. Malacol. (Taiwan) **20:** 43-50. (in Chinese)

Liu LL, SJ Chen, WY Peng, JJ Hung. 1997. Organotin concentrations in three intertidal neogastropods from the coastal waters of Taiwan. Environ. Pollut. **98:** 113-118.

Liu LL, IJ Suen. 1996. Prosobranch gastropods imposex in the west coast of Taiwan. Venus **55:** 207-214.

Liu WH, YW Chiu, DJ Huang, MY Liu, CC Lee, LL Liu. 2006. Imposex in the golden apple snail *Pomacea canaliculata* in Taiwan. Sci. Total Environ. **371:** 138-143.

Loch JPG, PA Greve, S Van der Berg. 1990. Accumulation and leaching of the fungicide fentin acetate and intermediates in sandy soils. Water Air Soil Pollut. **53:** 119-129.

Meng PJ, J Lin, LL Liu. 2009. Aquatic organotin pollution in Taiwan. J. Environ. Manage. **90:** S8-S15.

Naylor R. 1996. Invasions in agriculture: assessing the cost of golden apple snails in Asia. Ambio **25:** 443-448.

Oetken M, J Bachmann, U Schulte-Oehlmann, J Oehlmann. 2004. Evidence for endocrine disruption in invertebrates. Int. Rev. Cytol. **236:** 1-44.

Paton GI, W Cheewasedtham, IL Marr, JJC Dawson. 2006. Degradation and toxicity of phenyltin compounds in soil. Environ. Pollut. **144:** 746-751.

Schulte-Oehlmann U, C Bettin, P Fioroni, J Oehlmann, E Stroben. 1995. *Marisa cornuarietis* (Gastropoda, Prosobranchia): a potential TBT bioindicator for freshwater environments. Ecotoxicology **4:** 372-384.

Schulte-Oehlmann U, M Tillmann, B Markert, J Oehlmann. 2000. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part II: Triphenyltin as a xeno-androgen. Ecotoxicology **9:** 399-412.

Shi HH, CJ Huang, SX Zhu, XJ Yu, WY Xie. 2005. Generalized system of imposex and reproductive failure in female gastropods of coastal waters of mainland China. Mar. Ecol.-Prog. Ser. **304:** 179-189.

TACTRI. 2003. Survey of pesticide residues in the sediment of irrigation systems of paddy field. Research report. Taipei, Taiwan: Taiwan Agricultural Chemicals and Toxic Substances Research Institute (TACTRI), Council of Agriculture, Executive Yuan, 24 pp. (in Chinese)

TAIA. 1997. Domestic manufacturers production and sale of pesticides in 1996. Taipei, Taiwan: Taiwan Agriculture Industry Association (TAIA). (in Chinese)

Takeda N. 2000. Development of a penis from the vestigial penis in the female apple snail, *Pomacea canaliculata*. Biol. Bull. **199:** 316-320.

Teo SS. 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. Crop Prot. **20:** 599-604.