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ARTICLE

Detection of Tetrodotoxin in the Xanthid Crab *Atergatis floridus* (Linnaeus, 1767) Collected from Jeju Island off the South Coast of Korea, Using Competitive Enzyme-Linked Immunosorbent Assay (cELISA)

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Abstract

Tropical and subtropical xanthid crabs are known for containing potent neurotoxins, such as paralytic shellfish poison and tetrodotoxin (TTX), which can cause human intoxication. Although the toxic xanthid crab *Atergatis floridus* is known to be distributed on Jeju Island on the south coast of Korea, information about TTX in this crab is yet to be available. In this study, we used a competitive enzyme-linked immunosorbent assay (cELISA) to screen for TTX levels in *A. floridus* and *A. integerrimus*, a member of the family Xanthidae recently collected from the southern coast of Jeju Island. The cELISA assay indicated *A. floridus* contained weak to moderate toxic levels of TTX in their tissues; the walking leg muscle showed the highest TTX concentration, followed by the gonad, hepatopancreas, chelipeds muscle, stomach, gills, and cephalothorax muscle, with the TTX contents per individual ranging from 29.64 to 109.06 µg. In contrast, the ELISA revealed that TTX levels in all *A. integerrimus* tissues analyzed were below the detection limit. This study first reports TTX toxin in the xanthid crab *A. floridus*, and the findings provide fundamental information for monitoring the toxicity of the xanthid crabs on the coast of Jeju Island.

Keywords Tetrodotoxin · *Atergatis floridus* · Competitive ELISA · Xanthid crab · Jeju Island

1 Introduction

Tetrodotoxin (TTX) is a water-soluble, heat-resistant, and highly potent natural toxin that blocks voltage-gated sodium channels on the surface of nerve cells, failing in the nerve impulse transmission and subsequent paralysis in the poisoned animals (Saoudi et al. 2010; Bane et al. 2014; Melnikova and Magarlamov 2022). TTX is recognized for being found in several species of pufferfish, as well as in certain other marine invertebrates, such as flatworms (Yamada et al. 2017; Okabe et al. 2019), gastropods (Noguchi et al. 1984; Hwang et al. 2005; Costa et al. 2021), octopuses (Sheumack et al. 1984; Williams and Caldwell 2009; Asakawa et al. 2019; Yamate et al. 2021), and tropical and subtropical xanthid crabs (Yasumura et al. 1986; Saito et al. 2006; Tsai et al. 2006). In coastal marine ecosystems, TTX is utilized by certain animals as an antipredator defense or a venom to

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explore the prey (Williams 2010; Nagashima and Arakawa 2014; Melnikova and Margarlamov 2022).

Most toxic crabs of the family Xanthidae occur predominantly in coral reefs and rocky shores within tropical and subtropical regions. In southern Japan, the floral egg crab *A. floridus*, *Zosimus aeneus*, and *Platypodia granulosa* have been reported as vectors containing TTX (Noguchi et al. 2011). The floral egg crab *A. floridus* is known for its high toxicity among xanthid crabs occurring in southern Japan and Taiwan and the Great Barrier Reef (Endean et al. 1983; Hwang and Tsai 1999; Noguchi et al. 1983, 1986; Tsai et al. 1997, 2002, 2006). The floral egg crab is also known to be distributed in Jeju Island. One species in the family, Xanthidae *A. integerrimus*, was recently collected from the south coast of Jeju Island, marking its first recorded occurrence in Korean waters. *A. integerrimus* has a wide geographic distribution in tropical and subtropical regions in the Indo-Pacific, including subtropical Japan (Tanaka et al. 2010), the Philippine Seas (Jiang et al. 2016; Karagozlu et al. 2018), South China Sea (Xie et al. 2018), Gulf of Thailand (Kunsook and Karinthanyakit 2021), Bay of Bengal (Elayabharathi et al. 2020), and the Persian Gulf (Naderloo et al. 2016). Information on the toxicity of xanthid crabs *A. floridus* and *A. integerrimus* in Korean waters is yet to be available.

The mouse bioassay (MBA) has been the primary method used for extensive testing of TTX toxicity until recent years (Hwang and Jeng 1991; Noguchi and Mahmud 2001; Yu et al. 2004; Tsai et al. 2006). However, due to ethical concerns associated with the use of animals and the lack of toxin specificity in MBA, there is an increasing need for the development of alternative analytical methods (Christian and Luckas 2008; Visciano et al. 2016; Bane et al. 2016). The enzyme-linked immunosorbent assay (ELISA) is one of the effective alternative detection methods, offering rapidity, simplicity, and specificity while requiring only minimal sample preparation and equipment (Akbora et al. 2020; Dillon et al. 2021; Turner et al. 2023). To screen TTX in the animal tissues, monoclonal or polyclonal antibodies were developed using TTX molecules as antigens, and the level of TTX in the homogenized tissues was quantified successfully using ELISA or competitive ELISA (cELISA)(Tao et al. 2010; Reverté et al. 2018; Vlasenko et al. 2020; Thuy et al. 2020). Owing to its strengths, the immunologic assays have also been widely applied to detect marine biotoxins, including azaspiracids (Samdal et al. 2015), okadaic acid (Lu et al. 2012), palytoxin (Boscolo et al. 2013), and yessotoxin (Samdal et al. 2004).

Located off the southern coast of Korea, the coastal ecosystem of Jeju Island is enriched with benthic fauna of subtropical origin (Cho et al. 2014; Noseworthy et al. 2016). In particular, the rocky shores on Jeju Island include numerous species of subtropical brachyuran crabs. Among the 40 species of xanthid crabs reported in Korean waters, 21 species of xanthid crabs have been identified on Jeju Island, including the toxic *A. floridus* (Hong et al. 2009; NIBR 2023). There is currently a lack of comprehensive toxicity data on TTX-containing or potentially TTX-containing xanthid crabs in Korean waters, necessitating toxicity information for toxin management and ecological research. In this study, we investigated TTX content in *A. floridus* collected from tidal pools on the east coast of Jeju Island and the unrecorded xanthid crab *A. integerrimus*, from the west coast of Jeju Island using ELISA, with the aim of providing critical baseline data for future toxin research on xanthid crab.

2 Materials and Methods

2.1 Sampling Effort

In November 2023, six specimens of *A. floridus* with a carapace width ranging from 1.5 to 3.8 cm were collected from the rocky tidal pools on the east coast of Jeju Island. In addition, two *A. integerrimus* trapped in a shallow subtidal gill net were obtained from the west coast of Jeju Island in October and November 2023 (Fig. 1). To understand the distribution of TTX in the tissue, we selected the two large specimens of *A. floridus* (14.4 and 31.5 g) and extracted the tissues from the walking legs, chelipeds, and cephalothorax. The gonad, hepatopancreas, stomach, and gill tissues were also isolated from the crabs, weighed, and homogenized. Alternatively, the entire body of the remaining four small *A. floridus* (3.0–12.5 g) was homogenized for the assay. The muscles in the walking leg, cheliped, and cephalothorax of the two *A. integerrimus* (205.7 and 245.5 g) were extracted from the shell separately, weighed using an electronic balance and homogenized. The gonad, hepatopancreas, stomach, and gill tissues of the two crabs were also isolated and homogenized for the assay.

2.2 DNA Extraction, PCR, and Sequencing

Using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), genomic DNA was extracted from the muscles of appendages. For species identification, the cytochrome c oxidase subunit I (COX1) gene from the mitochondrial genes of the two xanthid crabs was subsequently amplified by the polymerase chain reaction (PCR) using the primer set F: LCO1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3') and R: HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994). The PCR-reaction mixture contained 100 ng of template DNA, Taq MasterMix 2X with Dye (MGmed, Seoul, Korea), and ten pmol of each primer, with a total volume of 50 µL. The thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation

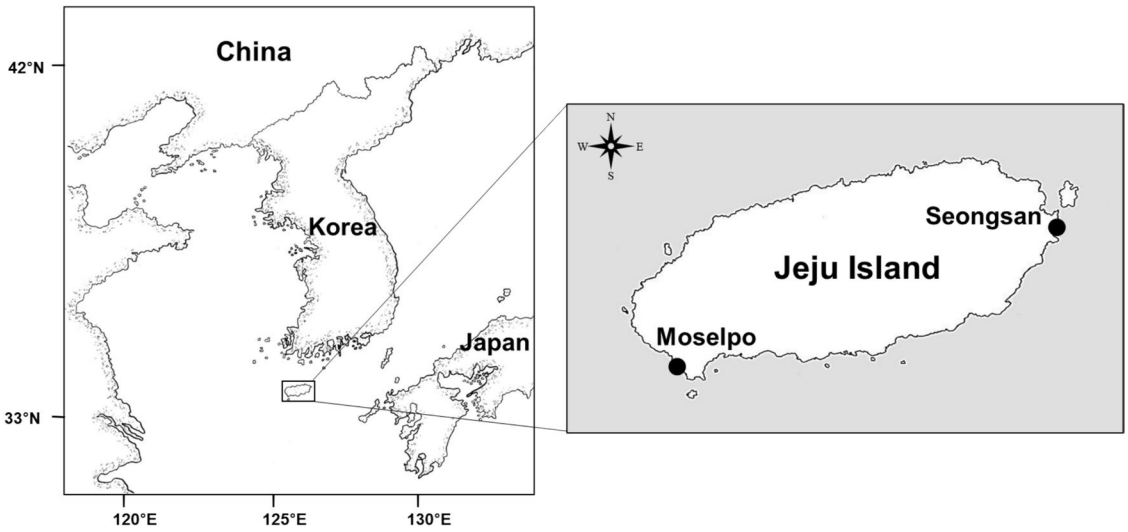

Fig. 1 Sampling site location. Two xanthid crabs were collected from rocky intertidal zones on Jeju Island, located off the south coast of Korea. *Atergatis integerrimus* and *A. floridus* were collected in Moselpo and Seongsan, respectively

at 95 °C for 30 s, annealing at 50 °C for 45 s, extension at 72 °C for 1 min, and then a final elongation at 72 °C for 10 min. After purification with the Accuprep® PCR purification kit (Bioneer, Daejeon, Korea), the obtained PCR products were sequenced by Macrogen Inc. (Seoul, Korea) using an ABI 3730xl analyzer (Applied Biosystems, Foster City, CA, USA).

2.3 Species Identification

The basic local alignment search tool (BLAST) tool in the National Center for Biotechnology Information (NCBI) database (Sayers et al. 2022) was used to analyze the nucleotide sequences of the COX1 gene isolated from the two xanthid crabs. The COX1 gene sequences were aligned with those available from the genera *Atergatis* and *Atergatopsis* using multiple alignment with the fast Fourier transform (MAFFT) software (Katoh et al. 2019). The best fit model based on the Akaike Information Criterion was TIM2 + F + I implemented in IQ-TREE (Minh et al. 2020). Finally, phylogenetic trees were constructed based on the COX1 gene sequences generated using the maximum likelihood (ML) method with 1,000 bootstrap replicates in the RAxML software (Stamatakis 2006). The phylogenetic trees were condensed by applying a 60% cut-off value.

2.4 TTX Screening Using cELISA

The TTX level in the different tissues of *A. flrodidus* and *A. integerrimus* was determined using a EuroProxima Tetrodotoxin cELISA kit (R-Biopharm Nederland B.V., Arnhem, The Netherlands) containing a mouse monoclonal antibody raised from TTX. The EuroProxiam kit has been validated for screening TTX with detection limits of 9.4 ng/g and detection capability of 20 ng/g.

According to the manufacturer's protocol, 5 mL of sodium acetate buffer (comprising 300 mL of 0.1 M $C_2H_3NaO_2$ and 200 mL of 0.1-M CH_3COOH, pH 4.8) was mixed with the tissues and homogenized using a Sonifier 450 (Branson, USA), operating at 20 kHz for 5 min. Subsequently, the homogenate was centrifuged at 4000 × g, and the supernatant was isolated and proceeded for quantitative TTX analysis. A volume of 50 µL of the supernatant and TTX standard solutions ranging from 0.6 to 20.0 ng/mL were added to wells in a microplate pre-coated with TTX. Then, 50 µL of the mouse-antiTTX primary antibody was added to each well and incubated in the dark at room temperature (20–25 °C) for 30 min. After incubation, the wells were washed with rinsing buffer, and 100 µL of anti-mouse IgG horseradish peroxidase-labeled as the secondary antibody was added to each well and incubated for 30 min. Finally, 100 µL of substrate solution containing hydrogen peroxide/tetramethylbenzidine was added to visualize the bound TTX-antibody complex. The antibody-substrate mixture was incubated for another 30 min, and sulfuric acid was added to terminate the reaction. The optical density of the TTX-antibody complex was measured at 450 nm using a VersaMax microplate spectrophotometer (Molecular Devices, San Jose, CA, USA). The TTX concentrations, expressed as the TTX weight per gram tissue (µg/g), were referred from the standards included in each microplate using RIDASOFT Win software (R-Biopharm, Darmstadt, Germany).

3 Results

3.1 Identification of the Xanthid Crab

In this study, we successfully identified two *Atergatis* species through morphologic and molecular analyses. The brown-colored xanthid crabs collected from the east coast of Jeju Island exhibited carapace widths (i.e., the longest axis of the carapace) ranging from 22.0 to 51.0 mm and carapace lengths (i.e., the vertical axis of the carapace) from 15.1 to 38.4 mm. Their carapace was broadly oval with a smooth surface and displayed a brown color with intricate, lighter colored patterns resembling a lace-like structure (Fig. 2). In contrast, the red-colored xanthid crabs trapped in the gill nets had carapace widths of 98.8 and 115.7 mm and carapace lengths of 62.6 and 75.2 mm. These crabs also had a broadly oval carapace, which was reddish, with small white spots sparsely distributed across its surface (Fig. 2).

In the ML tree based on the COX1 sequences, the two *Atergatis* species sequences clustered with high similarity into distinct clades corresponding to *A. floridus* and *A. integerrimus* (Fig. 3). This clustering strongly supported the species-level identification of the specimens. Moreover, the shallow genetic divergence observed within each clade underscored the reliability of COX1 sequences for precise species identification. *A. floridus* exhibited a remarkable sequence similarity of 98.9–100% with sequences previously reported from Singapore, Bangladesh, and the Philippines. Similarly, *A. integerrimus* showed a high similarity of 99.3–100% with sequences from tropical and subtropical regions such as southern China, India, Philippines, Singapore, and Thailand (Table S1).

3.2 TTX Concentration Determined by cELISA

The cELISA demonstrated strong linearity with a coefficient of determination (r^2) of 0.99 for the TTX calibration curve (Fig. S1). Screening for TTX using cELISA revealed that the four specimens of *A. floridus*, which were analyzed as whole bodies due to their small size, exhibited TTX concentrations ranging from 5.93 to 12.05 μg/g. In the analysis of each tissue, the highest TTX concentrations were found in the walking leg muscle, with levels of 15.14 μg/g and 28.69 μg/g, followed by the gonad (4.76 μg/g and 16.14 μg/g), hepatopancreas (3.19 μg/g and 13.49 μg/g), cheliped muscle (10.50 μg/g and 12.60 μg/g), stomach (3.11 μg/g and 8.75 μg/g), gills (2.32 μg/g and 6.16 μg/g), and cephalothorax muscle (0.51 μg/g and 1.12 μg/g) (Table 1). The total TTX content per individual was up to 109.06 μg (Table 1). Conversely, the ELISA results demonstrated that TTX levels

Fig. 2 *Atergatis integerrimus* (left) and *A. floridus* (right) specimens collected from Jeju Island off the south coast of Korea. Scale bar = 1 cm

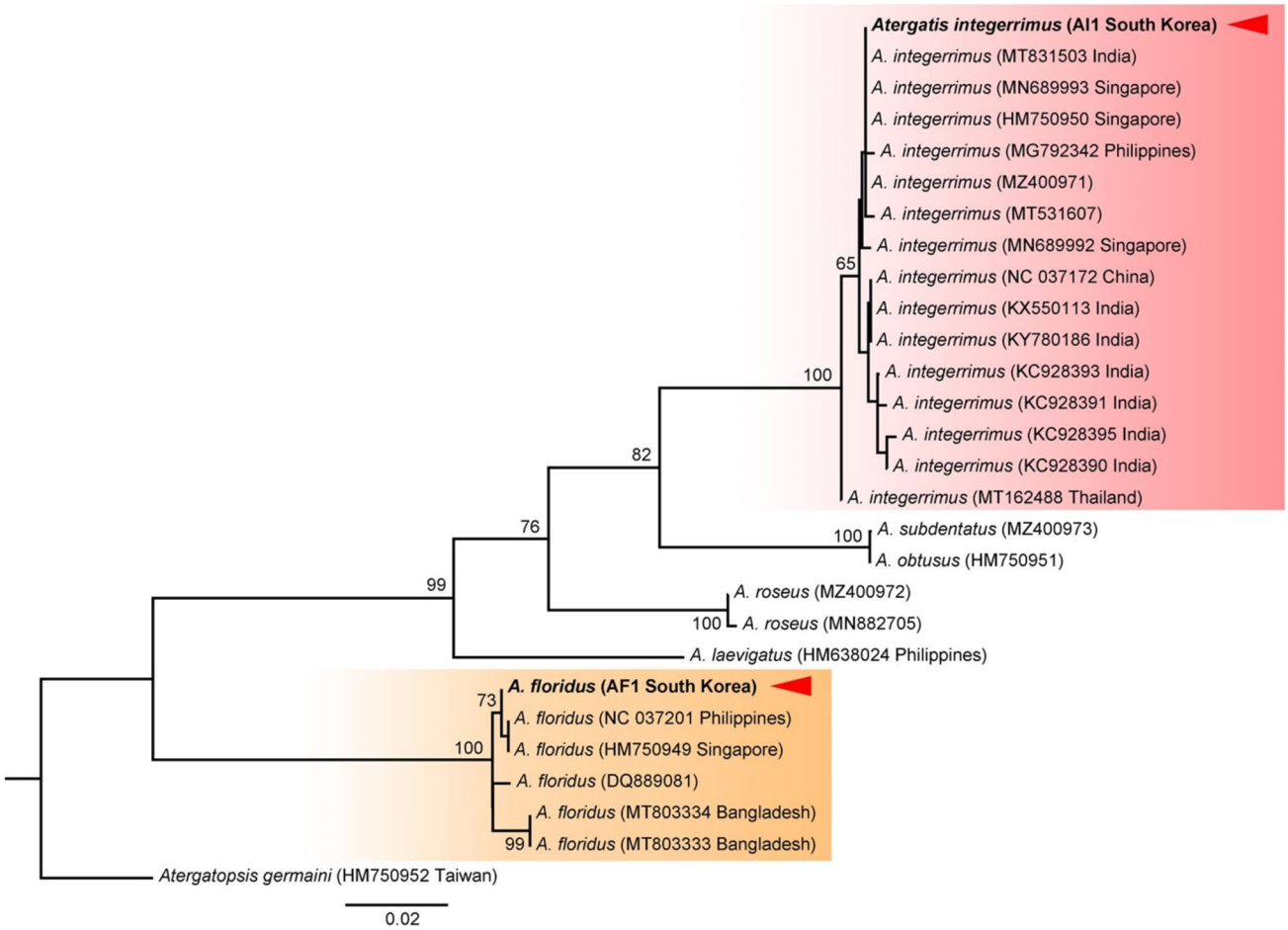

Fig. 3 Maximum likelihood tree of *Atergatis* crabs based on the cytochrome c oxidase subunit I sequence. The bootstrap values > 60 are shown on the branches. *Atergatopsis germaini* is used as an outgroup. Red arrowheads indicate the specimens analyzed in this study

Table 1 TTX concentrations (μg/g tissue) in various tissues of *Atergatis integerrimus* and *A. floridus* determined by cELISA

	TTX in *A.integerrimus* (μg/g)		TTX in *A. floridus* (μg/g)					
Sampling month	Oct	Nov	Nov					
Sex	M	M	F	M	F	M	M	M
Whole body	–	–	–	–	8.73	8.52	5.93	12.05
Chelipeds muscle	ND	ND	10.50	12.60	–	–	–	–
Walking legs muscle	ND	ND	28.69	15.14	–	–	–	–
Cephalothorax muscle	ND	ND	0.51	1.12	–	–	–	–
Hepatopancreas	ND	ND	3.19	13.49	–	–	–	–
Gills	ND	ND	2.32	6.16	–	–	–	–
Gonad	ND	ND	4.76	16.14	–	–	–	–
Stomach and mid-gut	ND	ND	3.11	8.75	–	–	–	–
TTX in individuals (μg)	ND	ND	37.86	29.64	109.06	90.34	78.29	36.14

ND not detected

- Not analyzed

in all analyzed tissues of *A. integerrimus* were below the detection limits (Table 1).

4 Discussion

In this study, we collected two species in the family Xanthidae from rocky intertidal in Jeju Island, and the COX1 gene sequence analysis confirmed that they are *A. floridus* and *A. integerrimus*. During the study, the floral egg crab *A. floridus* occurred in small tide pools near the low tide line during the night, where they crawled slowly and were observed to forage on seaweed. Limited studies have investigated the feeding ecology of *A. floridus*, which may provide crucial information about TTX accumulation in the crab. According to Kotaki et al. (1983), *A. floridus* collected from Ishigaki Island in Okinawa contained the calcareous coralline algae *Jania* sp., and the algae contained marine biotoxin gonyautoxins. Saisho et al. (1983) also examined the stomach contents of *A. floridus* collected from Ishigaki Island, reporting that the stomach included the red algae *Hypnea* sp, unidentified animal tissues, marine sponge, and fish fragments. In this study, no recognizable objects were identified in the stomachs of the floral egg crabs used in the analysis. The stomach contents of *A. floridus* collected in this study were not analyzed, due to the limited number of specimens collected.

The cELISA revealed that the TTX levels in the appendages and internal organs of the two male *A. integerrimus* were below the detection limit, indicating that they are not toxified with TTX. While several studies have reported the geographic distribution of the egg crab in tropical and subtropical Indo-Pacific regions (Naderloo et al. 2016; Xie et al. 2018; Kunsook et al. 2021; Karim et al. 2022), limited studies have examined TTX toxins in *A. integerrimus*. Yasumura et al. (1986) screened TTX in *A. integerrimus* collected from the central Philippines using liquid chromatography (LC), reporting that the two male egg crabs contained TTX and its analogs, including 4-*epi*tetrodotoxin and anhydrotetrodotoxin. However, the toxicity of TTX and its analogs in the crabs tested using MBA revealed that the toxicity was marginal, as two mouse units per gram (MU/g). The TTX values of *A. integerrimus* identified in this study may be attributed to the ELISA detection limit, indicating the need for further instrumental analysis to detect trace amounts of TTX and confirm the presence of TTX analogs.

Despite the long-standing recognition of *A. floridus* toxicity, few studies have quantitatively analyzed TTX levels in its tissues. Here, we present the TTX concentrations in *A. floridus* determined in our study alongside those reported from Nagasaki, Japan (Zhang et al. 2023), as summarized in Table 2. The TTX levels in individual crabs from our study ranged from 29.64 to 109.06 μg, which are comparatively lower than those reported in Nagasaki, where levels ranged from 2.16 to 421.88 μg. Notably, the highest TTX concentration reported by Zhang et al. (2023) is approximately four times greater than the maximum level observed in our study (Table 2). The dynamics of TTX accumulation in xanthid crabs remain unclear, though dietary intake is suspected to play a crucial role in its toxicification (Kotaki et al. 1983; Saisho et al. 1983).

Since previous studies primarily analyzed xanthid crab toxicity using MBA, a direct comparison with our findings is essential. To facilitate this comparison, the TTX concentrations (expressed in μg/g) from both our study on *A. floridus* and the study by Zhang et al. (2023) were converted to MU using the conversion factor 1 MU = 0.2 μg TTX (Biessy et al. 2019). Table 3 presents a summary of xanthid crab toxicity as analyzed in this and previous studies as MU. Saito et al. (2006) also assessed TTX levels in various body parts of *Atergatis floridus* crabs collected from Kanagawa and Wakayama using MBA. Their findings indicated that TTX toxicity was noticeably higher in the chelipeds muscle of all samples and walking legs muscles in several samples compared to other tissue. Notably, the muscle in the cephalothorax of crabs in Wakayama and Kanagawa Japan was either non-toxic or exhibited only marginal toxicity. In our study, we similarly observed the highest TTX contents in the walking leg muscles, with the cheliped muscles showing the second-highest average TTX content after the walking legs. In contrast, the lowest TTX content was found in the cephalothorax muscles (Table 3). These results suggest that TTX accumulation in the appendages may be utilized for defensive purposes. The low concentration of TTX in the cephalothorax may be intended to protect the chest ganglion in that region, as presumed by Saito et al (2006).

As shown in Table 3, the maximum total toxicity of *A. floridus* determined in this study (545 MU) was relatively lower than the toxicities reported for various xanthid crab

Table 2 Comparison of TTX levels in the whole body of *A. floridus* specimens analyzed in this study and those collected from Nagasaki, Japan (Zhang et al. 2023)

Location	Sampling date	Body weight (g)	TTX levels (μg/g)	Total TTX (μg)	References
Jeju, Korea	Nov-2023	3.00–31.50 (14.20 ± 9.40)	0.94–12.05 (6.32 ± 4.33)	29.64–109.06 (63.56 ± 33.37)	Present study
Nagasaki, Japan	Dec-2020	2.96–11.59 (7.53 ± 3.40)	0.6–45.46 (11.93 ± 13.80)	2.16–421.88 (110.05 ± 131.30)	Zhang et al. 2023

Table 3 The highest levels of TTX toxicity in xanthid crabs reported in this study and previous studies

Species	Toxin type	Tissue	Maximum toxicity (MU/g)	Maximum total toxicity (MU)	Method	Locality	References
Actaeodes tomentosus	TTX	Whole body	4	40	HPLC; LC–MS; GC–MS	Taiwan	Ho et al. 2006
Atergatis floridus	TTX	Whole body	60*	545*	cELISA	Korea	Present study
		Chelipeds muscle	63*				
		Walking legs muscle	143*				
		Cephalothorax muscle	6*				
		Hepatopancreas	67*				
		Gills	31*				
		Gonad	81*				
		Stomach	44*				
	TTX	Whole body	227*	2,109*	MBA; HPLC; LC–MS/MS	Japan	Zhang et al. 2023
		Appendages	128*				
	TTX	Chelipeds muscle	237		MBA	Japan	Saito et al. 2006
		Chelipeds outer shell	27				
		Walking legs muscle	325				
		Other shell	57				
		Liver	25				
		Gills	95				
		Gonad	107				
Atergatopsis germaini	TTX	Appendages	25	1,148	MBA; LC–MS; GC–MS	Taiwan	Tsai et al. 2006
		Cephalothorax	3.1				
		Viscera	23				
Demania cultripes	TTX; 4-*epi*TTX; 4,9-anhydroTTX; PSP	Appendages	7.7		MBA; HPLC-FLD; GC–MS	Philippines	Asakawa et al. 2010
		Viscera	52.1				
	TTX; anh-TTX	Appendages	3.5	800	MBA; LC–MS; GC–MS	Taiwan	Tsai et al. 2006
		Cephalothorax	5.3				
		Viscera	25				
D. reynaudi	TTX	Appendages	4.3	511	MBA; LC–MS; GC–MS	Taiwan	Tsai et al. 2006
		Cephalothorax	4.5				
		Viscera	9.8				
	TTX; anh-TTX; 4-*epi*TTX	Soft tissue	195		MBA; HILIC/MS–MS	Vietnam	Ha et al. 2023
D. toxica	TTX	Appendages	3.1	71	MBA; LC–MS; GC–MS	Taiwan	Tsai et al. 2006
		Cephalothorax	3				
		Viscera	5				
Lophozozymus incisus	TTX	Appendages	6.5	300	MBA; LC–MS; GC–MS	Taiwan	Tsai et al. 2006
		Cephalothorax	3.9				
		Viscera	8.4				
L. pictor	TTX	Appendages	3.1	247	MBA; LC–MS; GC–MS	Taiwan	Tsai et al. 2006

Table 3 (continued)

Species	Toxin type	Tissue	Maximum toxicity (MU/g)	Maximum total toxicity (MU)	Method	Locality	References
		Cephalothorax	3				
		Viscera	5.2				
Xanthias lividus	TTX; PSP	Whole body	4.5	118	HPLC; LC–MS; GC–MS	Taiwan	Ho et al. 2006
	TTX; PSP	Appendages	7	430	MBA; HPLC; GC–MS	Taiwan	Tsai et al. 2002
		Cephalothorax	17				
		Viscera	17				
Zosimus aeneus	TTX; 11-norTTX-6(R)-ol; 11-deoxyTTX; 5-deoxyTTX; 4-*epi*TTX; 11-oxoTTX	Whole body	11	390	LC–MS	Japan	Sagara et al. 2009
	TTX; PSP	Whole body	24	1,258	HPLC; LC–MS; GC–MS	Taiwan	Ho et al. 2006

*To compare with the toxicity reported in previous studies, the TTX level in ug/g was converted into mouse unit (MU) according to Biessy et al. (2019), in which 1 MU corresponds to 0.2 µg TTX

species in Taiwan and Japan, which range from 800 to 1148 MU. According to Noguchi and Arakawa (2008), TTX toxicity levels are categorized into three groups: (1) weakly toxic (10–100 MU/g tissue), (2) moderately toxic (100–1000 MU/g tissue), and (3) strongly toxic (> 1000 MU/g tissue). Based on this classification, the TTX levels in the whole body of *A. floridus* from Jeju Island, which range from 29.65 to 60.25 MU/g, are considered weakly toxic. However, it is noteworthy that the highest TTX concentration recorded in the walking leg muscle of one crab, at 143.45 MU/g, falls within the moderately toxic range.

TTX acquisition and distribution regulation mechanisms within the tissues of xanthid crabs remain poorly understood. The observed variations in toxin levels among individuals and across regions within the same species support the hypothesis that xanthid crabs, similar to other marine organisms such as pufferfish and blue-ringed octopuses, acquire their toxins exogenously through the food chain (Noguchi et al. 1986; Zhang et al. 2021). Moreover, the origin of paralytic shellfish toxins in xanthid crabs distributed in Ishigaki Island has been identified as the calcareous red alga *Jania* sp. (Kotaki et al. 1983). The amount of toxin acquired through exogenous pathways is directly influenced by the quantity of toxin-bearing prey consumed, resulting in inter-individual variations in toxin levels (Noguchi et al. 2006). Compared with previously reported levels, the lower toxicity observed in xanthid crabs collected from Jeju Island may be attributed to the lower abundance of TTX-bearing prey in the Jeju region. Further research on the dietary sources of *Atergatis* species is required to analyze the causative organisms comprehensively.

Another factor to consider when assessing toxicity is the variability in toxin content depending on the collection season. Both Saito et al. (2006) and this study identified high concentrations of TTX in the walking leg muscles, suggesting a regulatory mechanism that governs the distribution of TTX within tissues. This mechanism may allocate TTX according to physiologic or ecological needs, potentially contributing to seasonal fluctuations in toxin levels. In Australia, the toxicity of *A. floridus* varies significantly by collection area and season, with the highest levels recorded in autumn and the lowest in winter (Llewellyn and Endean 1991). Integrating toxin analysis with the reproductive cycle of xanthid crabs could provide valuable insights into the variability of toxin content in these toxic species.

In addition, ELISA analysis cannot provide information on TTX analogs, as TTX is found alongside its analogs within toxic organisms. Although most TTX analogs generally show lower toxicity than TTX, oxoTTX exhibits toxicity that is comparable to or even greater than that of TTX (Taniyama et al. 2009) and has been detected in *A. floridus* specimens collected from Ishigaki Island, Japan (Arakawa et al. 1994). This analogue may be also present in populations distributed in South Korea, and further instrumental analyses are necessary to determine the toxicity of these specimens accurately.

In conclusion, we employed cELISA to assess TTX levels in various tissues of the xanthid crabs *A. floridus* and *A. integerrimus* collected from Jeju Island. The highest TTX

concentration found in the whole body of *A. floridus* was 60.25 MU/μg, which indicate weak toxicity. Although the results show low TTX levels, the potential for seasonal variability in toxicity underscores the need for further research. These findings provide essential data for monitoring the toxicity of *Atergatis* species along the coast of Jeju Island.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12601-024-00191-w.

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Data availability Data will be made available on request.

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