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Cross-allergenicity of crustacean and the edible insect *Gryllus bimaculatus* in patients with shrimp allergy

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ABSTRACT

Food scarcity is a serious problem for many developing as well as developed countries. Edible insects have attracted attention recently as a novel food source. Crickets are especially high in nutritional value and easy to breed and harvest. In this study, we evaluated the risk of allergic reactions associated with cricket consumption in individuals with crustacean allergy.

We evaluated food allergy risk in the consumption of *Gryllus bimaculatus* (cricket) in patients with shrimp allergy, using enzyme-linked immunosorbent assay (ELISA) and IgE crosslinking-induced luciferase expression assay (EXiLE). Sera from individuals with shrimp allergy (positive for shrimp-specific IgE by ImmunoCAP (> 0.35 UA/mL; n = 9) or without shrimp allergy (negative for shrimp-specific IgE; n = 6) were obtained.

There was a strong correlation between shrimp- and *Gryllus*-specific IgE levels obtained by ELISA ($r_s = 0.99$; $P < 0.001$). The binding of shrimp-specific IgE on shrimp allergen was dose-dependently inhibited by *Gryllus* allergen (0–1.0 mg/mL). There was a strong correlation between shrimp- and *Gryllus*-specific IgE responses, as assessed by EXiLE assays ($r_s = 0.89$; $P < 0.001$). We determined that a protein of approximately 40 kDa reacted with the positive, but not negative, sera for shrimp-specific IgE by ImmunoCAP. Liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis identified the major allergen in shrimp and *Gryllus* to be tropomyosin.

Our data suggest that the cricket allergen has the potential to induce an allergic reaction in individuals with crustacean allergy. Therefore, allergy risk and shrimp-specific IgE levels should be considered before consumption of cricket meal.

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) reported in 2016 that nearly 515 million people worldwide lack sufficient food to live an active and healthy life, with approximately 13% of the world's population being considered undernourished (McGuire, 2015). Thus, new alternative sources of food, especially to substitute meat and fish, would be needed to alleviate the global food crisis. However, for any novel food source, assessment of potential risks for its consumption, including allergenic potential, is imperative.

Insects are currently being considered as a promising food source, owing to their high nutritional values—they are a potentially healthy food with high protein, vitamin, and mineral content (Churchward-Venne et al., 2017; Rumpold and Schluter, 2013; van Huis, 2016). In fact, insects constitute a part of the diet of an estimated 2 billion people worldwide, with over 1900 different species globally reported as food sources (Barennes et al., 2015). In Asia and Oceania, grasshoppers and locusts are among the most widely eaten insects, while options such as silkworms, wasps, caterpillars, and crickets are regarded as delicacies. Insects have been a traditional source of food in approximately 100 countries worldwide (Van Itterbeeck and van Huis, 2012).

Among edible insects, the cricket, an orthopteran, has an especially remarkable potential due to its high nutritional value and straightforward breeding and harvesting (Han et al., 2016; Lacey, 2016). In fact, the field cricket is commonly consumed as a food across Asia (Van Itterbeeck and van Huis, 2012); its composition includes approximately

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58% protein and 10% fat (Lacey, 2016; Wang et al., 2004). In a previous study, the protein from crickets was observed to be superior to that from soybeans in terms of amino acid intake, when fed to rats (Finke et al., 1989). In addition, crickets provide higher calories, protein, iron, and vitamin B_{12} than meat, suggesting that the health benefits of meat could be gained from cricket products (Lacey, 2016; Rumpold and Schluter, 2013).

Type I hypersensitivity or allergic reaction is mediated by antigen-specific IgE that binds antigens, circulates throughout the body, and binds to the Fc receptor on mast cells (Ebo and Stevens, 2001; Galli and Tsai, 2012). Upon encountering a specified antigen, IgE-receptor crosslinking results in cell degranulation; the release of mediators can lead to a wide variety of effects, including edema, vasodilation, mucous secretion, and bronchoconstriction. The major allergen in shrimp has been identified as the muscle protein tropomyosin (Ayuso et al., 2002). Furthermore, hexamerin 1B and arginine kinase were identified as novel and specific allergens in the field cricket (Srinroch et al., 2015). However, the allergic risk associated with consumption of the cricket as food has not been well described yet. The current study aimed to evaluate food allergy risk for an edible cricket, *Gryllus bimaculatus*, in patients with shrimp allergy, and to identify the allergen in this cricket species.

2. Materials and methods

2.1. Patients

This study was approved by the Ethics Committee of the Tokushima University Hospital. Written informed consent was obtained from each study participant for the use of his or her serum in this study. Sera from individuals with shrimp allergy (positive for shrimp-specific IgE by ImmunoCAP [> 0.35 UA/mL], and history of immediate-type reaction to shrimp or positive for oral food challenge of shrimp; n = 9) or without shrimp allergy (negative for shrimp-specific IgE; n = 6) were obtained (Table 1).

2.2. Gryllus breeding and allergen preparation

Adults and nymphs of *Gryllus bimaculatus* were reared at 29 °C and 50% humidity (Watanabe et al., 2014). The adults and last instar nymphs were lyophilized and ground into powder by a mixer. The *Gryllus* powder and raw shrimp meat (*Fenneropenaeus merguiensis*) were homogenized by a polytron homogenizer in PBS with 0.1% Triton X (Sigma-Aldrich, St. Louis, MO) and heated for 10 min at 95 °C. The homogenates were centrifuged at 16,215 × *g* for 15 min at 4 °C and supernatants were collected. Protein concentration was determined using the bicinchoninic acid assay (Thermo Fisher Scientific, Waltham, MA), with bovine serum albumin as the standard. The supernatants were considered as *Gryllus* or shrimp allergen in this study.

2.3. SDS-PAGE and immunoblotting

Gryllus and shrimp extracts (20 μg) were boiled in 2% SDS solution containing 10% β-mercaptoethanol, and resolved by electrophoresis using a 12% polyacrylamide gel; protein bands were visualized using Coomassie Brilliant Blue (CBB). For immunoblotting, resolved proteins were electro-transferred onto a 0.45-μm nitrocellulose membrane using a Trans-Blot semi-dry transfer cell (Bio-Rad Laboratories, Hercules, CA). Resolved proteins on the membrane were visualized by Ponceau 3R staining. The membrane was washed twice with TBS-T [50 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 0.1% Tween] and blocked with TBS-T, containing 5% skim milk. The membrane was subsequently incubated overnight with sera (diluted 1:100 in TBS-T, containing 5% skim milk) at 4 °C. After washing with TBS-T, the membrane was incubated with horseradish peroxidase (HRP)-conjugated mouse anti-human IgE (diluted 1:5000 in TBS-T) for 1 h at 25 °C. The membrane was incubated with 3,3′,5,5′-tetramethylbenzidine (TMB) ELISA substrate (Thermo Fisher Scientific, Waltham, MA) and the signal was detected using a LAS-3000 imaging system (Fujifilm, Tokyo, Japan).

2.4. ELISA for shrimp- and Gryllus-specific IgE

For estimating allergen-specific IgE levels, microplates (Greiner Bio-One, Kremsmünster, Austria) were coated with 0.5 μg/mL of the *Gryllus* or shrimp extract, diluted in 50 μL of 100 mM carbonate/bicarbonate buffer (pH 9.6), at 4 °C overnight. After three washes with TBS-T, Pierce Protein-Free (PBS) Blocking Buffer (Thermo Fisher Scientific) was added to the microplates for 1 h to block any nonspecific binding. Following another three washes with TBS-T, the microplates were incubated with sera (diluted 1:10 in blocking buffer) for 2 h at 25 °C, washed again, and incubated with HRP-conjugated mouse anti-human IgE (100 μg/mL) diluted in Signal Enhancer HIKARI solution B (Nacalai Tesque, Kyoto, Japan) for 1 h at room temperature. Post wash, the microplates were incubated with TMB-stabilized substrate for HRP (Promega, Madison, WI), and the reaction was stopped with HCl. The chromogen produced was measured by its absorbance at 450 nm using the Infinite 200 microplate reader (Tecan Group Ltd., Männedorf, Switzerland). All samples were assayed in duplicate. IgE levels were

Table 1
Detailed characteristics of the 15 study participants.

	Gender	Age (yr)	Shrimp allergy	Rationale for diagnosis	Induced symptoms	AD	BA	AR	Shrimp IgE (U_A/mL)	Shrimp IgE (IgE U/mL)	*Gryllus* IgE (IgE U/mL)
1	M	5	+	p-OFC	E, SI	+	–	–	15.20	152.3	101.9
2	M	9	+	p-OFC	TI, U	–	–	+	1.54	4.9	5.5
3	M	8	+	p-OFC	AP, E, TI	–	+	–	35.60	420.8	251.0
4	F	8	+	p-OFC	SI, TP	+	+	+	16.60	91.5	82.9
5	M	12	+	H	A, E, TP	–	–	+	5.50	10.1	22.5
6	F	6	+	H	AP, U	–	–	+	62.00	768.1	617.4
7	M	17	+	H	C, E, U, W	+	+	–	59.70	1235.6	1347.3
8	M	9	+	p-OFC	E, SI	+	+	–	1.99	6.2	3.5
9	F	7	+	p-OFC	E, SI, TI	–	+	+	0.66	4.8	4.6
10	F	13	–	–	–	–	–	–	< .35	0.0	0.0
11	M	6	–	–	–	–	–	–	< .35	0.0	0.0
12	M	1	–	–	–	–	–	–	< .35	0.0	0.0
13	M	40	–	–	–	–	–	–	< .35	0.0	0.0
14	M	33	–	–	–	–	–	–	< .35	0.0	0.0
15	F	40	–	–	–	–	–	–	< .35	0.0	0.0

Abbreviations: A, angioedema; AD, atopic dermatitis; AP, abdominal pain; AR, allergic rhinitis; BA, bronchial asthma; C, cough; E, erythema, F, female; H, history of immediate-type reaction to shrimp; M, male; p-OFC, positive for oral food challenge; SI, skin itching; TI, throat itching; TP, throat pain; U, urticaria; W, wheeze. Shrimp-specific IgE levels were measured by ImmunoCAP (IgE U_A/mL) and ELISA (IgE U/mL); *Gryllus* IgE levels were measured by ELISA (IgE U/mL).

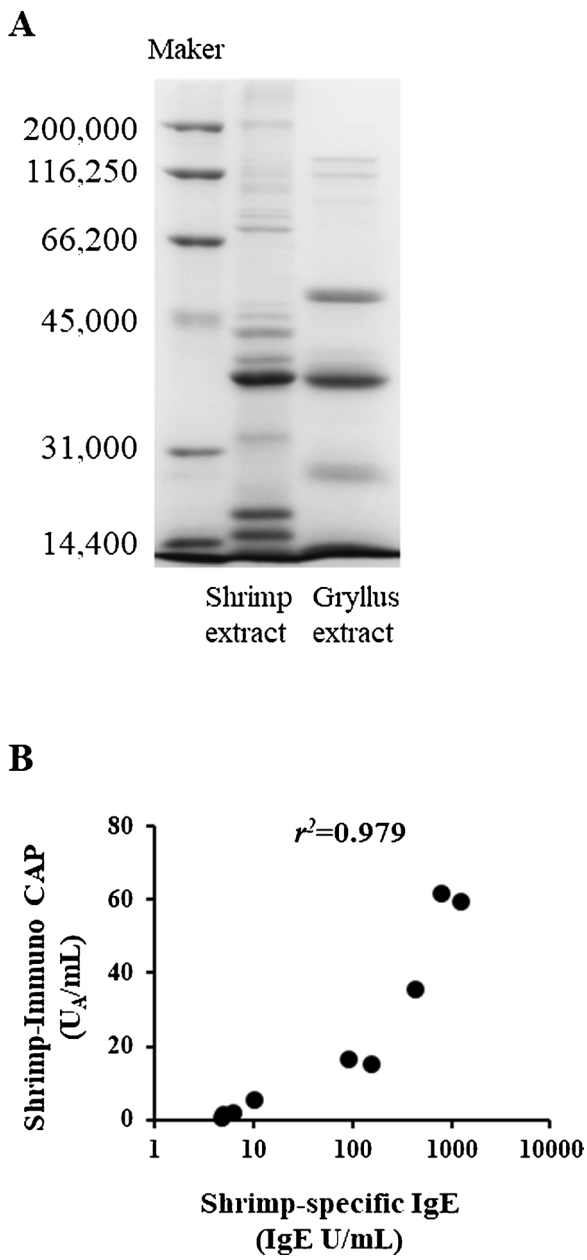

Fig. 1. Extraction of *Gryllus* and shrimp allergens. A, SDS-PAGE of the protein extracts. *Gryllus* and shrimp extracts (20 μg) were resolved by electrophoresis using a 12% polyacrylamide gel; protein bands were visualized using Coomassie Brilliant Blue (CBB). B, Comparison and correlation of shrimp-specific IgE levels obtained by ImmunoCAP (UA/mL) with that by ELISA (IgE U/mL). Spearman rank correlation test was used to assess the relationship (n = 15).

calculated from the absorbance values using the standard provided in the Human IgE ELISA Kit (Bethyl Laboratories, Montgomery, TX) and expressed as arbitrary units (IgE U/mL). The detection limit of *Gryllus*- and shrimp allergen-specific IgE in serum was 0.7 U/mL. The ImmunoCAP system has a detection limit of 0.35 U_A/mL for IgE (Ewan and Coote, 1990).

2.5. Gryllus- and shrimp-specific IgE elicitation in FcεRI-expressing luciferase-reporter mast cell line

For the EXiLE assay, a human FcεRI-expressing mast cell line, RS-ATL8, was used to show IgE crosslinking-induced luciferase expression, as described previously (Nakamura et al., 2010). The EXiLE test, which measures IgE crosslinking-induced mast cell activation as an indicator of clinically meaningful serum IgE, is considered an effective diagnostic method for patients who are suspected of having IgE-dependent allergy without the risk of severe systemic reactions. In fact, the EXiLE test correlates well with oral food challenge tests on patients with egg allergy (Nakamura et al., 2010). In addtion, there is a strong correlation between the EXiLE test and cell degranulation assay (Nakamura et al., 2012).

RS-ATL8 cells (5×10^4 cells/well in 50-μL volume) were plated on a clear-bottom white 96-well plate (ViewPlate; PerkinElmer, Waltham, MA) and incubated for 3 h at 37 °C in a 5% CO_2 incubator. The cells were subsequently sensitized with sera (n = 15), positive or negative for shrimp-specific IgE by ImmunoCAP. After overnight incubation, cells were washed with sterile PBS, and stimulated with 100 nM *Gryllus* or shrimp extract in MEM containing 10% FCS (50 μL/well), for 3 h at 37 °C. After stimulation, 50 μL of luciferase substrate solution containing cell lysis reagent (ONE-Glo; Promega, Tokyo, Japan) was added to the cells, and chemiluminescence was measured using an EnVision Multilabel Plate Reader (Perkin Elmer). Luciferase expression levels were presented as the fold increase of light units compared to the background expression after subtraction of a blank control (without cells). Measurements were conducted in duplicate, and the average value was used for analysis.

2.6. Identification of allergenic proteins in Gryllus and shrimp by mass spectrometry

Gryllus and shrimp protein extracts were separated on 12% SDS-PAGE. After electrophoresis, the gels were fixed with 50% methanol/10% acetic acid solution, stained with Coomassie Brilliant Blue (CBB), and de-stained with 7% acetic acid solution and distilled water. Stained gel bands were excised from whole gel, washed with distilled water and 50% acetonitrile/20 mM Tris-HCl buffer (pH 8.0), digested with 4 μg/mL trypsin in 20 mM Tris-HCl buffer (pH 8.0) at 37 °C for 20 h, and suspended in 0.1% formic acid (Kido et al., 2012).

The *Gryllus* and shrimp peptides were subjected to a mass spectrometric (MS) analysis. Briefly, the *Gryllus* and shrimp peptide samples were injected into an UltiMate 3000 RSLCnano system (Thermo Fisher Scientific) with Acclaim PepMap RSLC Nano Column (75 μm × 15 cm; Thermo Fisher Scientific). The mobile phase consisted of solvent A (0.1% formic acid solution) and solvent B (80% acetonitrile/0.08% formic acid solution). The peptides were continuously analyzed using the Orbitrap Elite hybrid mass spectrometer (Thermo Fisher Scientific). The obtained MS and MS/MS data were searched using the NCBI non-redundant database; shrimp and *Gryllus* peptides were searched against the Decapoda (81,791 sequences) and Polyneoptera (112,549 sequences), respectively, in the NCBI database. Shrimp proteins were identified with only one peptide scoring > 29; *Gryllus* proteins were identified when a score > 37 was obtained (false discovery rate < 1%).

2.7. Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (version 18.0; SPSS, Inc., Chicago, IL). Spearman rank correlation test was used to assess the relationship between different samples. Differences between groups were assessed by Wilcoxon matched pairs signed rank test. A P value < 0.05 was considered significant.

3. Results

3.1. Reactivity of Shrimp-sensitive sera against Gryllus bimaculatus extracts

The extracts from *Gryllus bimaculatus* and shrimp were subjected to SDS-PAGE and CBB staining (Fig. 1a); the pattern of protein bands was different in both, despite the same extraction method. The correlation

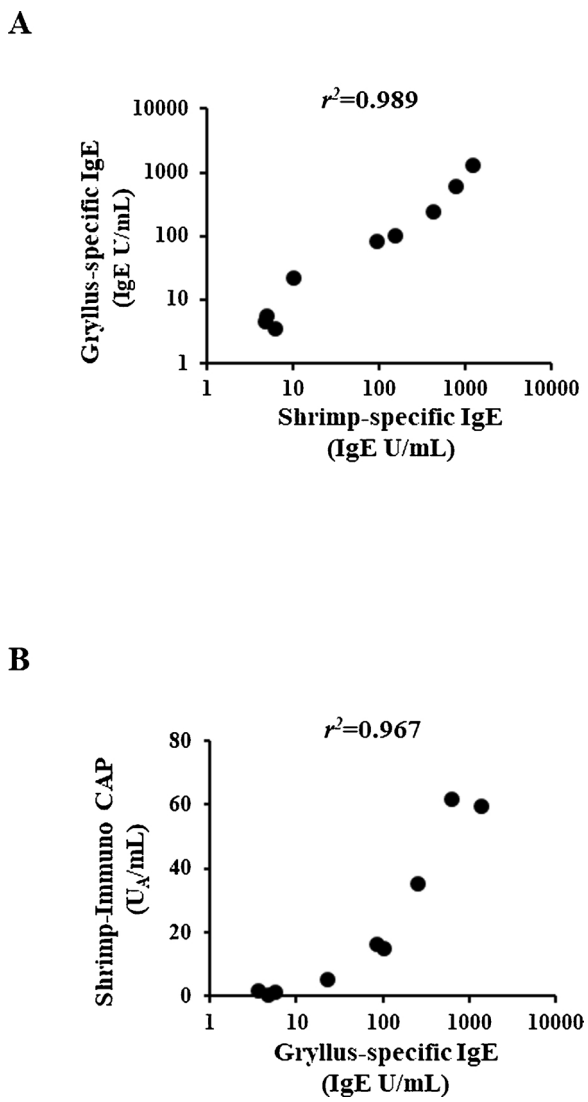

Fig. 2. Correlation between allergen-specific IgE levels analyzed by ELISA and ImmunoCAP. A, Correlation of shrimp- and *Gryllus*-specific IgE levels by ELISA (IgE U/mL). B, Correlation of *Gryllus*- (IgE U/mL) and shrimp-specific IgE (UA/mL) levels, obtained from ELISA and ImmunoCAP, respectively. Spearman's rank correlation test was used to assess the relationship.

between ELISA using shrimp extract and ImmunoCAP of shrimp was examined by sera (n = 15), positive or negative for shrimp-specific IgE on ImmunoCAP. There was a strong correlation between shrimp-specific IgE levels obtained by ImmunoCAP and ELISA ($r_s > 0.98$; $P < 0.001$) (Fig. 1b), hence indicating that the allergen in shrimp meat in this study was the same as that from ImmunoCAP.

Shrimp and *Gryllus*-specific IgE levels in the serum samples were measured by ELISA; a strong correlation was observed ($r_s = 0.99$; $P < 0.001$) (Fig. 2a). In addition, there was a strong correlation between the levels of shrimp-specific IgE by ImmunoCAP and *Gryllus*-specific IgE by ELISA ($r_s = 0.98$; $P < 0.001$) (Fig. 2b). We also examined the cross-reactivity of shrimp-specific IgE against *Gryllus* allergen by a competitive inhibition assay on ELISA. Binding of shrimp-specific IgE was dose-dependently inhibited by *Gryllus* allergen (0–1.0 mg/mL) (Fig. 3).

3.2. EXiLE assays of shrimp-sensitive sera against Gryllus bimaculatus extracts

The EXiLE test is a mast cell activation test to evaluate the biological activity of binding between antigen-specific IgE and allergens. We

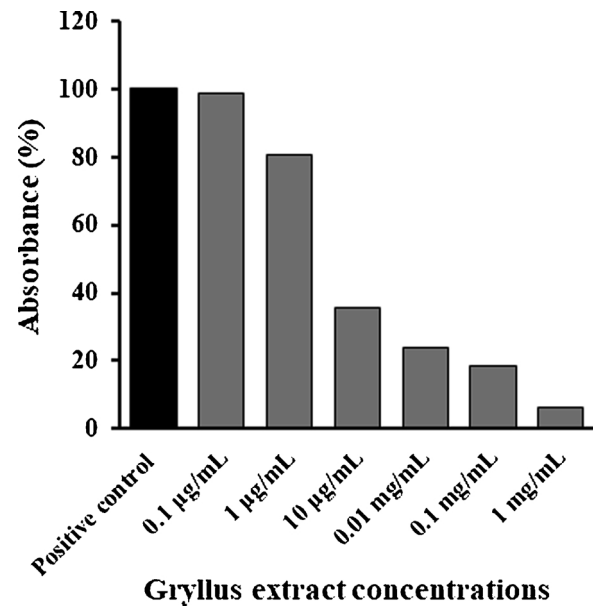

Fig. 3. Competitive inhibition of shrimp-specific IgE binding by *Gryllus* extract. Pooled sera, positive for shrimp-specific IgE by ImmunoCAP, were pre-incubated for 30 min with 0–1.0 mg/mL of *Gryllus* extract, and the level of shrimp-specific IgE was analyzed by ELISA. Positive control, without *Gryllus* extract, was considered as 100%.

conducted an EXiLE assay to evaluate the potential of *Gryllus* allergen to clinically induce allergic symptoms in individuals with shrimp allergy. There was a strong correlation between shrimp- and *Gryllus*-specific IgE responses, as assessed by EXiLE assays ($r_s = 0.89$; $P < 0.001$) (Fig. 4a). Compared to negative sera, sera positive for shrimp-specific IgE by ImmunoCAP significantly increased luciferase expression elicited by *Gryllus* allergen (Fig. 4b).

3.3. Identification of Gryllus bimaculatus allergen

Using SDS-PAGE and immunoblotting, we determined that a protein of approximately 40 kDa reacted with positive, but not with negative, sera for shrimp-specific IgE by ImmunoCAP (Fig. 5).

Liquid chromatography-tandem mass spectrometry (LC—MS/MS) analysis identified tropomyosin as the major allergen in shrimp and *Gryllus* (Tables 2 and 3). We predicted the amino acid sequence of *G. bimaculatus* tropomyosin based on transcriptome and genome sequence data of this species (Mito et al, unpublished data). Two high-molecular-weight (HMW) and one low-molecular-weight (LMW) isoforms were identified in *Gryllus* (Fig. 6). The approximate molecular mass of the immunoblot band indicated HMW tropomyosin as the antigen that induced the shrimp-specific IgE. Based on whole amino acid sequences, including reported epitopes, *Gryllus* HMW tropomyosin showed high amino acid similarity with that of a shrimp *Penaeus monodon* (Fig. 6), consistent with our result that showed cross-reactivity of shrimp and *Gryllus* allergens. *Gryllus* HMW tropomyosins also showed high amino acid similarity with the homologs from other insects (Fig. 6). Relationships among two HMWs and LMW in *Gryllus* and other species is shown in Fig. 7, indicating that *Gryllus* HMW2 is orthologous to the shrimp HMW.

4. Discussion

Crickets form a highly nutritious and healthy source of food (Churchward-Venne et al., 2017). However, Barennes et al (2015) reported allergic symptoms in 7.6% people following the consumption of insects in Laos (Barennes et al., 2015). In addition, Meng et al (2012) showed that 18% of lethal anaphylaxis and anaphylactic shock in China

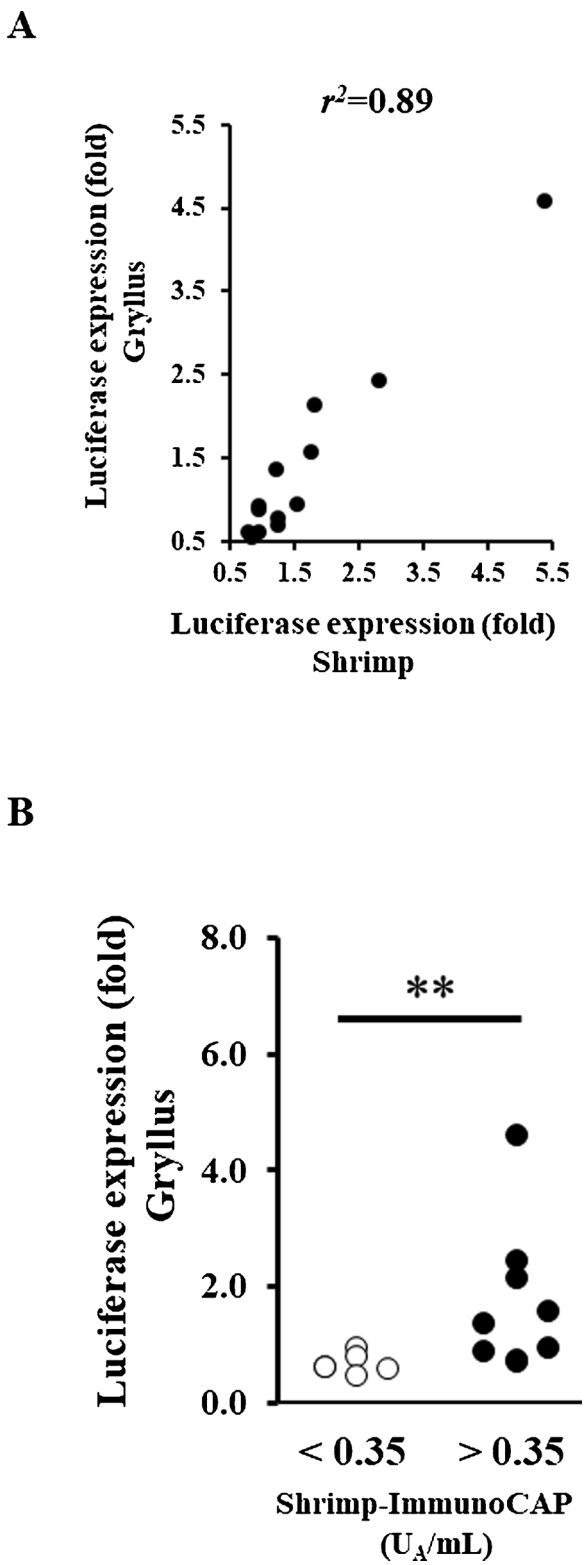

Fig. 4. IgE-mediated human FcεRI crosslinking-induced luciferase expression in RS-ATL8 cells by *Gryllus*. A, Correlation of luciferase reporter expression induced by shrimp- and *Gryllus*-specific IgE responses, as analyzed by IgE crosslinking-induced luciferase expression (EXiLE) assays. B, The difference in *Gryllus* allergen-induced luciferase expression between cells sensitized with positive and negative sera for shrimp-specific IgE by ImmunoCAP. Data are presented as the fold increase relative to non-stimulated control (taken as 1.0). ** $P < 0.01$.

were caused by insect eating (Meng et al., 2012). Moreover, inhalant allergy to insects has also been reported (de Gier and Verhoeckx, 2018; Witteman et al., 1994). Therefore, more information on food allergy following the consumption of insects is required for safe consumption of insect meal.

Several earlier studies have reported the possibility of inducing sensitization to insects. Leung et al (1996) had shown co-sensitization between several insects, cockroach, fruit fly, and shrimp (Leung et al., 1996). In addition, Broekman et al (2016) demonstrated clinically relevant co-sensitization of mealworm in patients with shrimp allergies, using ImmunoCAP, skin prick test, and basophil activation test (Broekman et al., 2016). We examined risk of allergy to cricket meal in individuals with shrimp allergy by EXiLE assays and mast cell activation tests. A strong correlation was seen between shrimp- and *Gryllus*-specific IgE levels obtained by ELISA, which also had a strong correlation with ImmunoCAP against shrimp allergen ($r_s = 0.99$; $P < 0.001$). In addition, a strong correlation between shrimp- and *Gryllus*-specific IgE responses was recognized by EXiLE assays ($r_s = 0.89$; $P < 0.001$). Therefore, patents with shrimp allergy may be at high risk of cricket meal-induced food allergy.

The allergens on edible insects have been shown in several studies. Broekaman et al. (2017) have recently reported that mealworm-allergic subjects recognized several allergenic proteins such as arginine kinase, tropomyosin, myosin heavy and light chains, larval cuticle protein A1A, A2B, and A3A (Broekaman et al., 2017). Srinroch et al (2015) demonstrated binding of cricket with IgE from patients with shrimp allergy and identified arginine kinase as the major allergen. In addition, Witteman et al (1994) and Leung et al (1996) showed co-sensitization across several insects and shrimp (Leung et al., 1996; Witteman et al., 1994). The studies showed the binding of grasshopper, cockroach, and fruit fly allergens to IgE from patients with shrimp allergy, and identified tropomyosin as the major allergen. Moreover, IgE-binding tropomyosins from mite and *Aedes aegypti* were identified and characterized (Cantillo et al., 2016). Comparative analysis of amino acid sequence of the IgE-binding epitope of tropomyosin from *Aedes aegypti*, mite, and cockroach showed remarkable similarity with that of shrimp (Cantillo et al., 2016; López-Matas et al., 2015).

We detected a protein of approximately 40 kDa that reacted with positive sera for shrimp-specific IgE by ImmunoCAP. In addition, the allergen was identified as HMW tropomyosin. Therefore, HMW tropomyosin was concluded to be a cross-reactive allergen between shrimp and *Gryllus*. In our immunoblotting analysis, we detected only the HMW tropomyosin band. Although not clearly understood, one reason could be that the allergen solution used this study was extracted using a different method than in other studies, and sera used in our study from individuals with shrimp allergy were focused on food allergy, not on inhalant allergy. Therefore, the major allergen in cricket-derived food may be tropomyosin. However, we cannot exclude the possibility that additional allergens exist, but were not detected due to sensitivity limitations of the method. In addition, we showed that *Gryllus* HMW tropomyosins had high amino acid similarity with homologs from other insects, which indicated that although various insect foods are eaten worldwide, a patient with allergy to one type of insect or shrimp may be at a risk of developing allergy against other edible insects as well.

Although previous reports had indicated cross-reaction between crustacean and insect allergic responses, clinical studies are insufficient. In this study, we showed for the first time the potential of *Gryllus* allergens to cause allergic responses in individuals with shrimp allergy. Therefore, if cricket meal is to be introduced widely as food, the individual measurement of shrimp-specific IgE may lead to the prevention of cricket allergy.

In conclusion, using ELISA and EXiLE, we demonstrated that the crickets contain a potential allergen against individuals allergic to

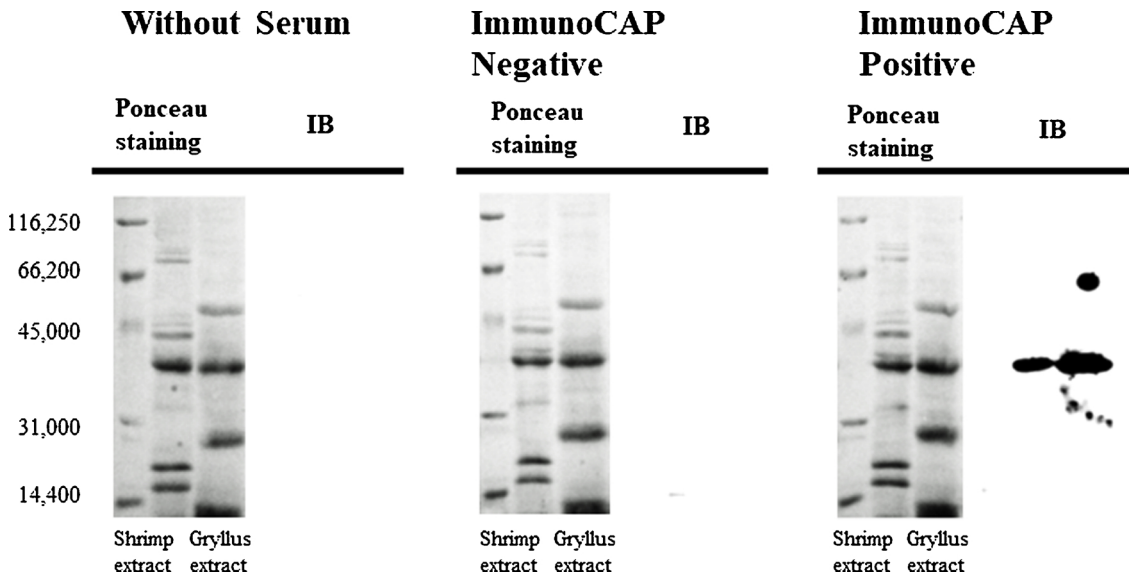

Fig. 5. SDS-PAGE pattern and immunoreactive bands from *Gryllus* and shrimp extracts. Protein bands on SDS-PAGE were visualized by Ponceau 3R staining; left lane, molecular weight; middle lane, shrimp extract; right lane, *Gryllus* extract. For immunoblotting (IB), the membrane was incubated without serum (TBS-T containing 5% skim milk only) or with pooled serum samples (positive or negative for shrimp-specific IgE by ImmunoCAP).

Table 2
Protein allergens from shrimp extract identified by mass spectrometry in this study.

Family	Protein name	Accession number	Molecular mass (Da)	Mascot score	Coverage (%)	emPAI
1	RecName: Full = Tropomyosin	sp|A1KYZ2.1|TPM_PENMO	32830	42496	99	4072810.5
1	Tropomyosin, partial [*Metapenaeus ensis*]	AAA60330.1	31686	40247	98	1666561.64
1	Tropomyosin [*Crangon crangon*]	ACR43473.1	32752	26325	89	218433.14
1	Tropomyosin [*Melicertus latisulcatus*]	AGF86397.1	32665	26217	95	259194.41
1	Tropomyosin [*Procambarus clarkii*]	ACN87223.1	32794	23479	89	24201.66
1	Tropomyosin [*Jasus lalandii*]	AFY98827.1	32861	22397	95	57724.08
1	Tropomyosin [*Portunus trituberculatus*]	ABS12234.1	32767	21569	80	11132.79
1	Slow-tonic S2 tropomyosin [*Macrobrachium nipponense*]	AHJ10946.1	32880	20465	95	74712.08
1	Hypothetical protein PF00261.16 [*Scylla olivacea*]	JAI59190.1	32798	16778	77	3952.81
2	Myosin heavy chain type 2 [*Litopenaeus vannamei*]	BAM65722.1	219056	6452	54	9.68
2	Myosin heavy chain type 1 [*Litopenaeus vannamei*]	BAM65721.1	219576	6198	54	6.92
2	Myosin heavy chain type 2 [*Penaeus monodon*]	BAM65720.1	219033	4906	48	5.81
2	Myosin heavy chain type b [*Marsupenaeus japonicus*]	BAK61430.1	218870	4130	37	3.52
2	Myosin heavy chain type 1 [*Penaeus monodon*]	BAM65719.1	219450	4043	43	3.77
2	Myosin heavy chain type a [*Marsupenaeus japonicus*]	BAK61429.1	219368	3638	40	3.08

Proteins with a mascot score of > 1000 are shown. emPAI, exponentially modified protein abundance index.

Table 3
Protein allergens from *Gryllus* extract identified by mass spectrometry in this study.

Family	Protein Name	Accession number	Molecular mass (Da)	Mascot score	Coverage (%)	emPAI
1	RecName: Full = Tropomyosin	sp|Q8T6L5.1|TPM_PERFU	32717	14380	77	644.91
1	Tropomyosin	prf||1709237A	32420	12248	72	185.6
1	Tropomyosin isoform X17 [*Zootermopsis nevadensis*]	XP_021928065.1	32751	9193	80	54.25
1	Tropomyosin isoform X16 [*Zootermopsis nevadensis*]	XP_021928064.1	32848	8768	79	61.02
1	Tropomyosin [*Zootermopsis nevadensis*]	KDR15166.1	32290	7955	58	19.43
1	Tropomyosin isoform X1 [*Zootermopsis nevadensis*]	XP_021928073.1	32338	7527	52	9.6
1	Tropomyosin isoform X18 [Zootermopsis nevadensis]	XP_021928066.1	32771	7312	77	31.92
2	Myosin heavy chain isoform G [*Locusta migratoria*]	ANS83651.1	224740	2989	33	2.02
2	Myosin heavy chain, muscle [*Zootermopsis nevadensis*]	XP_021941393.1	80839	1733	48	5.03
3	Hypothetical accessory gland protein, partial [*Gryllus firmus*]	ABG75835.1	35327	1399	39	3.23

Proteins with a mascot score of > 1000 are shown. emPAI, exponentially modified protein abundance index.

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Tc_HMW1_XP_967128       MDAIKKKMQAMKLEKDNAQDKADAMEGQAKDANLRVEKLNEELRELQKKLSQVEGDLTTTKNSLEQANKDLEEKEKTLTNAEAEMASLNRKVQTIEEDLE 100
Tc_HMW2-like_EEZ97595   MDAIKKKMQAMKLEKDNAQDKADAMEGQAKDANLRVEKLNEELRELQKKLSQVEGDLTTTKNSLEQANKDLEEKEKTLTNAEAEMASLNRKVQTIEEDLE 100
Gb_HMW2                 MDAIKKKMQAMKLEKDNAMDKADTCEGQAKDANNKADKINEDVQELTKKLAQVENDLITTKANLEQANKDLEDKEKALQAAESEMAALNRKVQLVEEDLE 100
Pm_HMW1_A1KY2           MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQVQESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLE 100
Pf_HMW1_Q8T6L5          MDAIKKKMQAMKLEKDNAMDRALLCEQQARDANLRAEKAEEEARSLQKKIQQIENDLDQTMEQLMQVNAKLDEKDKALQNAESEVAALNRRIQLLEEDLE 100
Lm_HMW1_1709237A        MDAIKKKMQAMKLEKDNALDRALLCEQQARDANLRAEKAEEEARALQKKIQTIENDLDQTQESLGQVMAKLEEKEKALQNAESEVAALNRRIQLLEEDLE 100
Zn_HMW1_XP_021928065    MDAIKKKMQAMKLEKDNAMDRALLCEQQARDANLRAEKAEEEARSLQKKIQQIENDLDQTMEQLMQVNAKLDEKDKALQNAESEVAALNRRIQLLEEDLE 100
Gb_HMW1                 MDAIKKKMQAMKLEKDNAMDRALLCEQQARDANLRAEKAEEEARGLQKKIQTIENELDQTQEQLMQVNAKLEEKDKAFQTAEGEIAALNRRIQLLEEDLE 100
Tc_HMW2_XP_008198923    MDAIKKKMQAMKVDKDGALERALVCEQEARDANTRAEKAEEEARQLQKKIQTVENELDQTQEALTLVTGKLEEKNKALQNAESEVAALNRRIQLLEEDLE 100
Dm_HMW1_NP_001262591    MDAIKKKMQAMKVDKDGALERALVCEQEARDANTRAEKAEEEARQLQKKIQTVENELDQTQEALTLVTGKLEEKNKALQNAESEVAALNRRIQLLEEDLE 100
Zn_LMW1_XP_021928071    --------MTTNIQQGTLLD------------------------VLKKKMRQTKEDMEKYKDECEEYHKRLQVELMRREEAESEVAALNRRIQLLEEDLE  68
Gb_LMW1                 --------MTTNVQQGTLLD------------------------VLKKKMRQTKEEMEKYKDECEEFHKRLQVEIMRREEAEGEIAALNRRIQLLEEDLE  68
Dm_LMW1_NP_524360       --------MTTSIPQGTLLD------------------------VLKKKMRQTKEEMEKYKDECEEFHKRLQLEVVRREEAESEVAALNRRIQLLEEDLE  68
                                 : .: :.   :                        * *::   : ::             *  :      **.*:*:***::* :*****

Tc_HMW1_XP_967128       RSEERLATATTKLAEASQAADESSRMCKVLENRSQQDEERMDQLTNQLKEARLLAEDADNKSDEVSRKLAFVEDELEVAEDRVKGGDAKIMELEEELKVV 200
Tc_HMW2-like_EEZ97595   RSEERLATATTKLAEASQAADESSRMCKVLENRSQQDEERMDQLTNQLKEARLLAEDADNKSDEVSRKLAFVEDELEVAEDRVKGGDAKIMELEEELKVV 200
Gb_HMW2                 RSEERAATAATKLHEASEAADEAQRMCKVLENRSQQDEERMDQLTNQLKEARLLAEDADGKSDEVSRKLAFVEDELEVAEDRVKSGDSKIMELEEELKVV 200
Pm_HMW1_A1KY2           RSEERLNTATTKLAEASQAADESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAEERAETGESKIVELEEELRVV 200
Pf_HMW1_Q8T6L5          RSEERLATATAKLAEASQAADESERARKILESKGLADEERMDALENQLKEARFMAEEADKKYDEVARKLAMVEADLERAEERAESGESKIVELEEELRVV 200
Lm_HMW1_1709237A        RSEERLATATAKLAEASQAADESERARKILENRSLADEERMDALENQLKEARFLAEEADKKYDEVARKLAMVEADLERAEERAEAGESKIVELEEELRVV 200
Zn_HMW1_XP_021928065    RSEERLATATAKLAEASQAADESERIRKALENRTNMEDDRVAILEAQLSQAKLIAEEADKKYEEVARKLVLMEQDLERAEERAEQGDGKIVELEEELRVV 200
Gb_HMW1                 RSEERLATATAKLAEASQAADESERIRKALENRTNMEDDRVAILEAQLSQAKLIAEEADKKYEEVARKLAMVEADLERAEERAEAGESKIVELEEELRVV 200
Tc_HMW2_XP_008198923    RSEERLGSATAKLSEASQAADESERIRKALENRTNMEDDKVALLENQLAQAKLIAEEADKKYEEVARKLAMVEADLERAEERAEQGENKIVELEEELRVV 200
Dm_HMW1_NP_001262591    RSEERLGSATAKLSEASQAADESERIRKALENRTNMEDDKVALLENQLAQAKLIAEEADKKYEEVARKLAMVEADLERAEERAEQGENKIVELEEELRVV 200
Zn_LMW1_XP_021928071    RSEERLATATAKLAEASQAADESERIRKALENRTNMEDDRVAILEAQLSQAKLIAEEADKKYEEVARKLVLMEQDLERAEERAEQGDGKIVELEEELRVV 168
Gb_LMW1                 RSEERLATATAKLAEASQAADESERIRKALENRTNMEDDRVAILEAQLSQAKLIAEEADKKYEEVARKLVMMEQDLERAEERAEHSESKIVELEEELRVV 168
Dm_LMW1_NP_524360       RSEERLGSATAKLSEASQAADESERIRKALENRTNMEDDKVALLENQLAQAKLIAEEADKKYEEVARKLVLMEQDLERSEEKVELSESKIVELEEELRVV 168
                        *****  :*::** ***:****:.*  * **.:   :::::  *  ** :*:::**:** * :**:***.::* :** :*::.: .: **:******:**

Tc_HMW1_XP_967128       GNSLKSLEVSEEKANQRVEEFKKQLKTLTVKLKEAEARAEYAEKTVKKLQKEVDRLEDELGINKDRYKSLADEMDSTFAELAGY---- 284
Tc_HMW2-like_EEZ97595   GNSLKSLEVSEEKANQRVEEFKKQLKTLTVKLKEAEARAEYAEKTVKKLQKEVDRLEDFVLREKAKYHNIVTDIDKTFVEMSSLSSQK 288
Gb_HMW2                 GNSLKSLEVSEEKANQRVEEYKRQIKTLSVKLKEAEARAEYAEKTVKKLQKEVDRLEDELGINKDRYKSLADEMDSTFAELAGY---- 284
Pm_HMW1_A1KY2           GNNLKSLEVSEEKANQREEAYKEQIKTLTNKLKAAEARAEFAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY---- 284
Pf_HMW1_Q8T6L5          GNNLKSLEVSEEKANLREEAYKQQIKTLTTRLKEAEARAEFAERSVQKLQKEVDRLEDELVHEKEKYKFICDDLDMTFTELIGN---- 284
Lm_HMW1_1709237A        GNNLKSLEVSEEKANQREEEISNRLRTLTTRLKEAEARAEFAERSVQKLQKEVDRLEDELVIEKEKYKIIGDDLDSAFVELIL----- 283
Zn_HMW1_XP_021928065    GNNLKSLEVSEEKANLREEEYKQQIKTLTTRLKEAEARAEFAERSVQKLQKEVDRLEDELVIEKEKYKIIGDDLDSAFVELYGY---- 284
Gb_HMW1                 GNNLKSLEVSEEKANQREEEYKQQIKTLNTRLKEAEARAEFAERSVQKLQKEVDRLEDELVHEKEKYKFICDDLDMTFTELIGN---- 284
Tc_HMW2_XP_00819892     GNNLKSLEVSEEKANQREEEYKNQIKTLNTRLKEAEARAEFAERSVQKLQKEVDRLEDDLVLEKERYKDIGDDLDTAFVELILKE--- 285
Dm_HMW1_NP_001262591    GNNLKSLEVSEEKANQREEEYKNQIKTLNTRLKEAEARAEFAERSVQKLQKEVDRLEDDLVLEKERYKDIGDDLDTAFVELILKE--- 285
Zn_LMW1_XP_021928071    GNNLKSLEVSEEKATQREETFEEQVKSLSGSLKEAEARAEFAERSVQKLQKEVDRLEDDLVAEKEKNKLLQEEMEATLHDIQNM---- 252
Gb_LMW1                 GNNLKSLEVSEEKATQREENFEEQVKSLAGSLKEAEARAEFAERSVQKLQKEVDRLEDDLLAEKEKNKLLQEEMEATLHDIQNM---- 252
Dm_LMW1_NP_524360       GNNLKSLEVSEEKATQKEETFETQIKVLDHSLKEAEARAEFAERSVQKLQKEVDRLEDDLLNVRGKNKLLQEEMEATLHDIQNM---- 252
                        **.***********. : *  . ::: *   ** ******:**::*:********** :   : : : :  ::: :: ::
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Fig. 6. Multiple sequence alignment of tropomyosin proteins from shrimp and *Gryllus*.
Cross reactive epitopes previously desribed are shaded in blue (Ayuso et al., 2002).
Tc: *Tribolium castaneum*; Gb: *Gryllus bimaculatus*; Pm: *Penaeus monodon*; Pf: *Periplaneta fuliginosa*; Lm; *Locusta migratoria*; Zn: *Zootermopsis nevadensis*; Dm; *Drosophila melanogaster*.

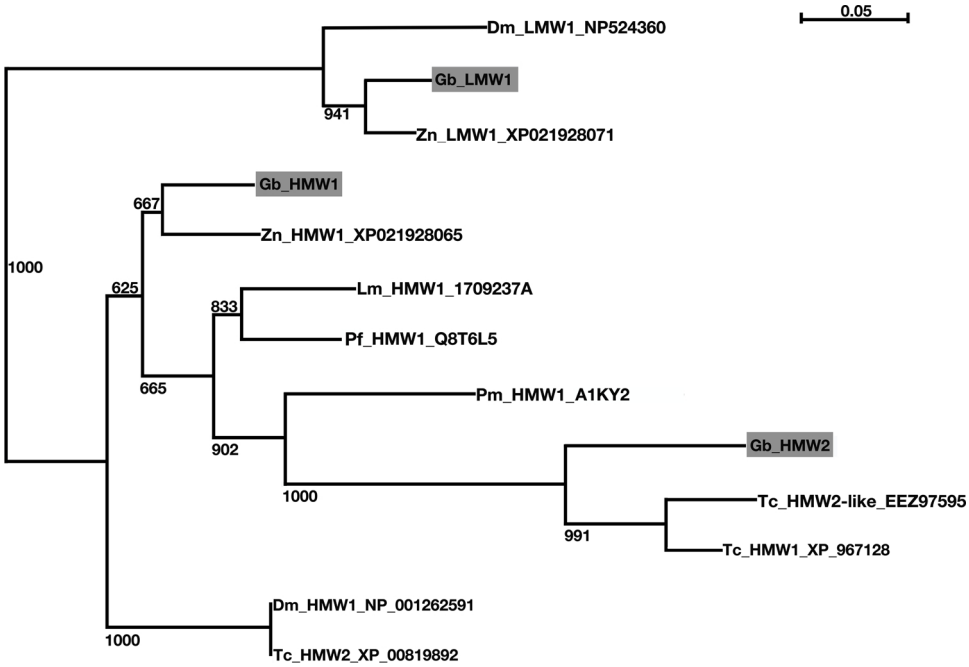

Fig. 7. Phylogenetic tree of tropomyosin proteins. The neighbor-joining algorithm was used with 1000 bootstraps.

shrimp. We also showed cross-reactivity between cricket and shrimp HMW tropomyosins, which are known as a major food allergen for individuals with shrimp allergy. Our study provides basic knowledge on the allergenicity of edible crickets, which is required for proceeding with their widespread use as a new food source.

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