Scientific Research Publishing

Open Journal of Immunology, 2022, 12, 15-39
https://www.scirp.org/journal/oji
ISSN Online: 2162-4526
ISSN Print: 2162-450X

In Silico Analysis of the Cross-Reactivity of Pacific White Shrimp (*Litopenaeus vannamei*) Allergens with Other Arthropod Species

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How to cite this paper: Soto-Muñoz, A. and Rodríguez-Canul, R. (2022) In Silico Analysis of the Cross-Reactivity of Pacific White Shrimp (*Litopenaeus vannamei*) Allergens with Other Arthropod Species. *Open Journal of Immunology*, **12**, 15-39.
https://doi.org/10.4236/oji.2022.121002

Received: January 24, 2022
Accepted: March 28, 2022
Published: March 31, 2022

Abstract

This study provided new data in the area of cross-reactivity for Pacific white shrimp (*Litopenaeus vannamei*). Although the cross-reactivity between the most prominent allergenic proteins of shrimp and other crustaceans has been extensively studied, few data are showing the frequency of arthropod-shrimp *Litopenaeus vannamei* cosensitization in an in silico analysis. A comparative analysis of "typical and non-typical" shrimp *L. vannamei* allergens with other species was achieved, revealing new allergens and previously characterized shrimp allergens, which validated the comprehensive identification approach used in this study. Importantly, up to 192, amino acid sequences were identified that had matches to shrimp *L. vannamei* allergens that matched allergenic proteins in mites, insects, fish, bacteria, mammals, birds, and plants.

Keywords

Allergens, *Litopenaeus vannamei*, Allergenicity, Immunoglobulin E, Food Allergy

1. Introduction

Allergic processes have become a public health problem. The increase in these diseases is mainly due to complications caused by environmental and/or food triggers, as well as genetic problems of patients to predisposition (atopy) [1].

The allergy caused by shrimp proteins (allergens) is one of the main ones in terms of crustaceans and can occasionally have serious/fatal episodes of an anaphylactic process [2]. The symptoms that stand out from the anaphylactic process caused by shrimp are angioedema, laryngospasm, urticaria, itching,

anaphylaxis, gastrointestinal discomfort, such as vomiting, stomach pain, and diarrhea that are usually confused with infectious problems, so the diagnosis of food allergy it is not easy [2] [3]. Countries like Australia have the highest prevalence of IgE-mediated food allergy with around 18% of their population affected. On the contrary, England and the United States have estimated between 12% and 17% [4]. While in Mexico, it is estimated that about 16% of the population suffers from a food allergy, of which 8% - 9% corresponds to an allergy caused by shellfish. The most affected population is children with 4.3% and adults with 3.8% [3] [4] [5].

In recent years, species belonging to the crustacean phylum have been characterized as potential allergenic sources. Sensitization to allergenic crustacean proteins is complicated by the highly reactive nature of some of these proteins [6]. One of the known allergens from crustaceans (including Pacific white shrimp) is tropomyosin. Tropomyosin is one of the allergens with the highest percentage of cross-reactivity reported between mollusks and crustaceans, but also with other invertebrates [6] [7]. This allergen is highly stable to heat treatment, maintaining its allergenicity. There is a diverse group of different isoforms that are found primarily in muscle cells. About 60% of shrimp-allergic patients react to this protein. And it is highly conserved among various species of crustaceans, such as shrimp, lobster, and crab with 95% - 100% identity [7] [8] [9].

Cross-reactivity between proteins from different species is proposed as one of the mechanisms that would explain the immediate hypersensitivity reactions observed in individuals exposed to an allergenic source for the first time [9]. Although exact data are not available, it is suspected that this mechanism would be involved in patients allergic to shrimp with a hypersensitivity reaction when they consume this food for the first time [10] [11]. The structural homology (amino acids) that exists between allergens can result in binding to antibodies, mainly of the IgE type, resulting in a process known as cross-sensitization, which occurs during exposure to an allergen and causes an animal or person is hypersensitive to another protein and cross-reactivity takes place resulting in an allergic process [11]. Cross-sensitization of food allergens can cause non-sensitized allergen-specific IgE resulting in a false-positive test (e.g., skin prick test) that is extremely important for clinical practice and differential diagnosis [11] [12] [13].

IgE cross-reactivity to shrimp allergens is increasingly important, not only tropomyosin but also the other outstanding allergens (myosin light chain, arginine kinase, and sarcoplasmic calcium-binding protein) and the less studied (hemocyanin, troponin C, ubiquitin, triosephosphate isomerase among others) [13]. Although the cross-reactivity between the most prominent allergenic proteins of shrimp and other crustaceans has been extensively studied [14] [15], few data are showing the frequency of arthropod-shrimp sensitization in an in silico analysis; most epidemiologic analyses have not evaluated the clinical significance of such sensitization [15]. Therefore, the main objective of this study was to analyze in silico the structural cross-reactivity of Pacific white shrimp (*Litopenaeus vannamei*) allergens with other arthropod species, as well as superficially

describing the possible form of sensitization: consumption vs. cross-reactivity and the clinical relevance of sensitization to shrimp in patients sensitized to other allergens.

2. Materials and Methods

2.1. Search for Pacific White Shrimp Allergen Sequences and Their Alignment

Sequences of known Pacific white shrimp (*Litopenaeus vannamei*) allergens were searched in the databases for proteins (NCBI and Uniprot) and in the database for allergens (Allergome). The sequences that were searched were the proteins: tropomyosin (Lit v1), arginine kinase (Lit v2), myosin light chain (Lit v3), and sarcoplasmic calcium-binding protein (Lit v4). Also, troponin C (Lit v6), hemocyanin, pyruvate kinase, and thioredoxin. Their alignment was performed using the CLUSTAL Omega program available at http://www.ebi.ac.uk/Tools/msa/clustal/; using the "Multiple Alignment" option and its identity percentage. The number of sequences selected for Lit v1 was 4 and 5 sequences for the rest of the allergens.

2.2. Cross-Reactivity Analysis between Shrimp Allergens and Allergens from Other Species

Sequence cross-reactivity of Pacific white shrimp allergens was determined using free tools such as Allermatch (http://allermatch.org/). This tool provides comparative data on cross-reactivity and IgE-binding properties of clinically important shrimp *Litopenaeus vannamei* allergens.

Allermatch contains the entries from three databases of known allergen proteins: the UniProt Protein Knowledge Base (UniProtKB), the World Health Organization Allergen Nomenclature List, and the International Union of Immunological Societies (WHO-IUIS) and the Comprehensive Protein Allergen Resource (COMPARE). Two of the three options available in the software (the 80 amino acid sliding window and the full alignment) were used to search for cross-reactivity.

2.3. Phylogenetic Analysis

The Molecular Evolutionary Genetic Analysis (MEGA) program, version X, was used to obtain phylogenetic trees, using the taxa method with Bootstrap support with 1000 repetitions as a measure of reliability and robustness under the assumption of an evolutionary minimum. In topology, this model uses a comparative matrix to find similarities between amino acid sequences to establish evolutionary proximity between species. The array was constructed with all amino acid sequences of the allergens retrieved from the UniProt database and reported to the WHO/IUIS from the Allermatch software. Alignment for phylogenetic analysis was carried out using CLUSTAL W, which performs alignments. The

parameters to perform the multiple alignments were set to use the gap opening penalty of 10.00 and extension penalty of 0.20, and the divergent cut delay was 30%.

3. Results

Table 1 shows the sequences used in the present study, which were obtained from the NCBI and Uniprot protein databases, as well as the exclusive database for allergens (Allergome). The 4 allergens already well characterized for the Pacific white shrimp (*Litopenaeus vannamei*) were used, which are: tropomyosin (Lit v1), arginine kinase (Lit v2), myosin light chain (Lit v3), and calcium-binding protein. sarcoplasmic (Lit v4). And also some others were reported such as troponin C, hemocyanin, pyruvate kinase, and thioredoxin.

Supplementary Tables S1-S6 show the identity percentages between the sequences used for each shrimp (*L. vannamei*) allergen obtained by their alignments. As can be seen, the identity percentages between each one of them vary between 97% and 99% ($\overline{x}$ = 99%), which indicates that they are quite similar between them, therefore, there is high confidence to use them for the cross-reactivity analysis.

Supplementary Tables S7-S13 show some of the allergens from other species that have amino acid sequence matches to white shrimp (*L. vannamei*) allergens and cross-reactivity. Analysis in Allermatch software identified many allergens sequence matches, primarily with known allergens in crustaceans, arachnids (mites), insects, and parasites (nematodes). After duplicate removal, the results yielded 103 allergen sequences identified for white shrimp (*L. vannamei*) tropomyosin (Lit v1) cross-reactivity (Table S7), 29 for arginine kinase allergen (Lit v2) (Table S8), 5 for myosin light chain (Lit v3) allergen (Table S9), 9 for sarcoplasmic calcium-binding protein (Lit v3) allergen (Table S10), 22 for troponin C (Table S11), 17 for hemocyanin (Table S12) and 8 for pyruvate kinase (Table S13). The remaining allergen amino acid sequences belonged to plants (pollen), fish, mammals, birds, and other allergen sources. The tables were divided by color according to the percentage of coincidences and therefore the probability of cross-reactivity. Being red (high cross-reactivity; % > 60), yellow (medium cross-reactivity; % > 30), green (low cross-reactivity; % > 20) and blue (no cross-reactivity; % < 20).

A total of 67 crustacean allergens were identified for cross-reactivity among the four major allergens (Lit v1 - v4) of white shrimp (*L. vannamei*) (Figure 1 and Figure 2). Of the 67 allergens, 48 coincide with the main shrimp allergen, which is tropomyosin (TM), 15 arginine kinase (AK), 3 myosin light chains (MLC), and 1 sarcoplasmic calcium-binding protein (SCP). For *L. vannamei* tropomyosin it has 100% amino acid sequence identity to the previously reported Lit v 1 in Uniprot (B4YAH6). 100% identities were also found for *Litopenaeus monodon* (A1KYZ2), *Litopenaeus aztecus* (Q3Y8M6), and *Litopenaeus japonicus*

Table 1. Pacific white shrimp allergen sequences.

Protein	Database	Size	Access number	Protein name	Locus
Lit v 1	NCBI	284	ACB38288.1	Lit v 1 tropomyosin	ACB38288
	Uniprot	284	B4YAH6	Lit v 1 tropomyosin	-
	Allergome	284	4029	Lit v 1	-
	Allergome	284	4030	Lit v 1.0101	-
Lit v 2	NCBI	356	ABI98020.1	arginine kinase	ABI98020
	NCBI	356	B0FRF9.1	arginine kinase Lit v 2	B0FRF9
	Uniprot	356	Q004B5	Arginine kinase Lit v 2.0101	-
	Allergome	356	3544	Lit v 2	-
	Allergome	356	3616	Lit v 2.0101	-
Lit v 3	NCBI	177	ACC76803.1	Lit v 3 allergen myosin light chain	ACC76803
	NCBI	177	ROT68323.1	Lit v 3 allergen myosin light chain	ROT68323
	Uniprot	177	B7SNI3	Lit v 3 allergen myosin light chain	-
	Allergome	177	4052	Lit v 3	-
	Allergome	177	4053	Lit v 3.0101	-
Lit v 4	NCBI	193	ACM89179.1	sarcoplasmic calcium-binding protein	ACM89179
	Uniprot	193	C7A639	Sarcoplasmic calcium-binding protein	-
	Uniprot	178	A0A3R7PHV9	SCP domain-containing protein	-
	Allergome	193	6092	Lit v 4	-
	Allergome	193	6093	Lit v 4.0101	-
Others	Allergome	150	12255	Lit v 6 (Troponin C)	-
	NCBI	117	ROT66451.1	Troponin C	-
	Allergome	662	12159	Hemocyanin	-
	NCBI	662	ART94437.1	Hemocyanin	-
	NCBI	649	ROT83548.1	Hemocyanin	-
	Allergome	591	11779	Pyruvate Kinase	-
	Allergome	N/A	10161	Thioredoxin	-

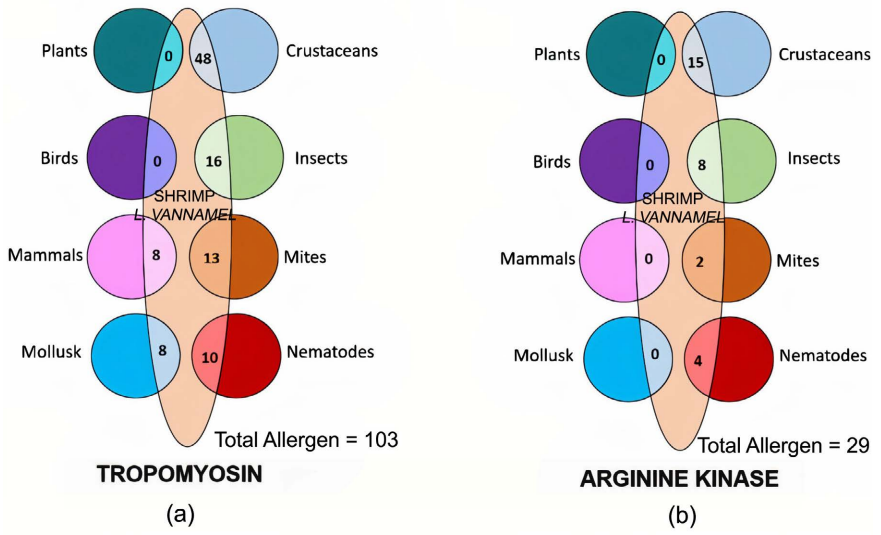

Figure 1. Venn diagram of the cross-reactivity of tropomyosin and arginine kinase from *Litopenaeus vannamei.* Similarities between the amino acid sequences of tropomyosin from other species (a) with tropomyosin from *L. vannamei* are depicted. And arginine kinases from other species (b) with the arginine kinase from *L. vannamei.* In total 103 sequences for TM and 29 for AK were analyzed. Figure made with the Microsoft Office trash can and BioRender

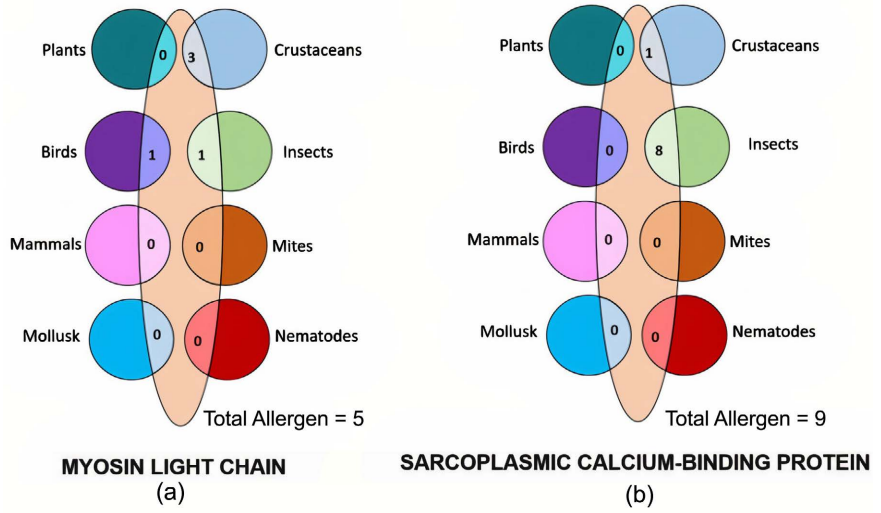

Figure 2. Venn diagram of the cross-reactivity of myosin light chain (MLC) and sarcoplasmic calcium-binding protein (SCP) from *Litopenaeus vannamei.* Similarities between the amino acid sequences of MLC from other species (a) with the MLC from *L. vannamei* are depicted. And SCP from other species (b) with the SCP from *L. vannamei.* In total 5 sequences for MLC and 9 for SCP were analyzed. Figure made with the trash can of Microsoft Office and BioRender.

(A2V731). As well as an identity of 93% - 94% for the shrimp "*Metapenaeus ensis*" and the Malaysian jumbo shrimp "*Macrobrachium rosenbergii*" (**Table S6**, red color). With the discovery of cockroach tropomyosins, *Blattella germanica* (Q9NG56), *Periplaneta fuliginosa* (Q8T6L5), *Periplaneta americana* (P0DSM7), and *Gromphadorhina portentosa* (A0A4P8D340); as well as the moth "*Galleria*

mellonella" (A0A4P8D330) that showed that they have identities greater than 44% with the TM of *L. vannamei*, resulting in a moderate reactivity for cross-linking of the IgE receptor to occur and a process of anaphylaxis. The same occurs for mites, *Dermatophagoides farinae* (Q23939) *Sarcoptes scabiei* (A0A1M4PIX0), and the mosquito "*Aedes aegypti*" (Q17H75) (**Table S7**, yellow color). And finally, low or null identities were found for some parasites (e.g. *Ascaris lumbricoides*), insects (e.g. Tenebrio Molitor), and some mammals such as humans and pigs. The amino acid sequences found by the Allermatch software and those reported by the WHO/IUIS for tropomyosins were used for the analysis of the phylogenetic tree, revealing that the TMs of crustaceans are mostly very similar to each other and amphipods (small crustaceans) as well as some mites. In contrast, the TM sequences of some mammals, insects, and microorganisms appear to be more distantly related to the TM of shellfish including white shrimp (*L. vannamei*) (**Figure 3**).

For the arginine kinase (AK) allergen, several species of crustaceans were identified, including *Penaeus monodon*, *Penaeus Chinensis*, *Crangon Crangon*, and *Scylla paramamosain*. The 4 amino acid sequences for AK mentioned above together with that of *L. vannamei* were very similar to each other, with more than 65% identity (**Table S7**, red color), which indicated a high possibility of cross-reactivity. These sequences are also mildly like AK allergens from *Periplaneta americana* cockroaches (A1KY39) and the mites *Dermatophagoides farinae* (A0A088SAW4) and *Dermatophagoides pteronyssinus* (B2ZSY4) (30% - 39% identity). In contrast, these were different from allergens from some plants such as *Betula pendula* and some mammals such as *Bos Taurus* (**Table S6**, blue color). As with TM, the amino acid sequences found for AK from crustaceans are most closely related to each other, as well as to insects and mites, but not closely related to mollusks (**Figure 4**).

As for the myosin light chain (MLC) allergen, only 5 almost identical amino acid sequences were identified. Of the 5, 2 are from the same shrimp (*Penaeus monodon*) with 88-89% identity (high potential for cross-reactivity; **Table S8**). And a low cross-reactivity was obtained for the cockroach (*Blattella germanica*) and the rooster (*Gallus gallus*). Molecular phylogenetic tree analyses on the MLC distance between crustaceans and the shrimp *Litopeneaus vannamei* are closely related, even with some bacteria. However, they are not closely related to the MLCs of insects such as butterflies and moths (**Figure 5**).

For the sarcoplasmic calcium-binding protein allergen, a very similar amino acid sequence (100% identity) was identified between the shrimp *Litopenaeus vannamei* (C7A639) and *Penaeus monodon* (E7CGC4). And a medium identity (from 34% to 53%) for the crustaceans *Penaeus monodon* (H7CHW2), *Astacus Leptodactylus* (P05946), *Scylla paramamosain* (I2DDG2), and the *Crangon crangon* (D7F1P9) (**Table S10**; yellow color). And, finally, an identity (low reactivity) of less than 3% was identified for the *Aedes aegypti* mosquito (Q16XK7) (**Table S10**; blue color). Very similar to TM and AK, amino acid sequences of

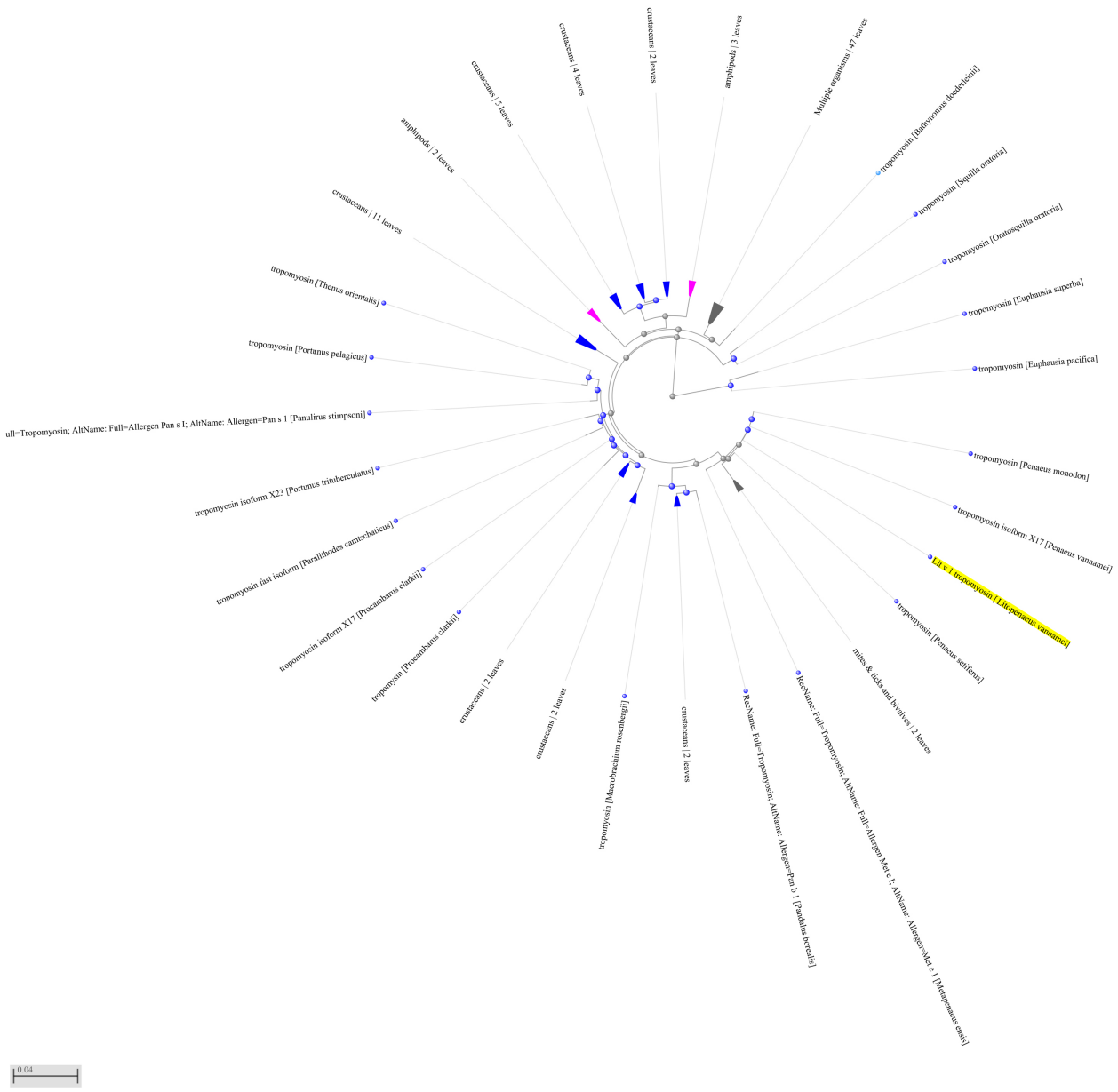

Figure 3. The phylogenetic tree between the tropomyosins of *Litopenaeus vannamei* and other species. It shows how close or far the tropomyosin from other species is to the tropomyosin from *L. vannamei* (yellow color). Tropomyosins from crustaceans (blue), small crustaceans and arthropods (pink), tropomyosins from insects, mammals, mites (grey). Figure elaborated in the MEGA-X program.

SCPs obtained by Allermatch and WHO/IUIS in a phylogenetic tree analysis showed that all edible crustacean SCPs are closely related to other species within the same phylum, but distantly related between classes of insects and bacteria (**Figure 6**). No allergen sequences were found for comparison for mites or mammals.

For troponin C and pyruvate kinase allergens, amino acid sequences with low or null identities (less than 10%) were found, which would not indicate a low probability of cross-reactivity with these allergens and therefore that they can

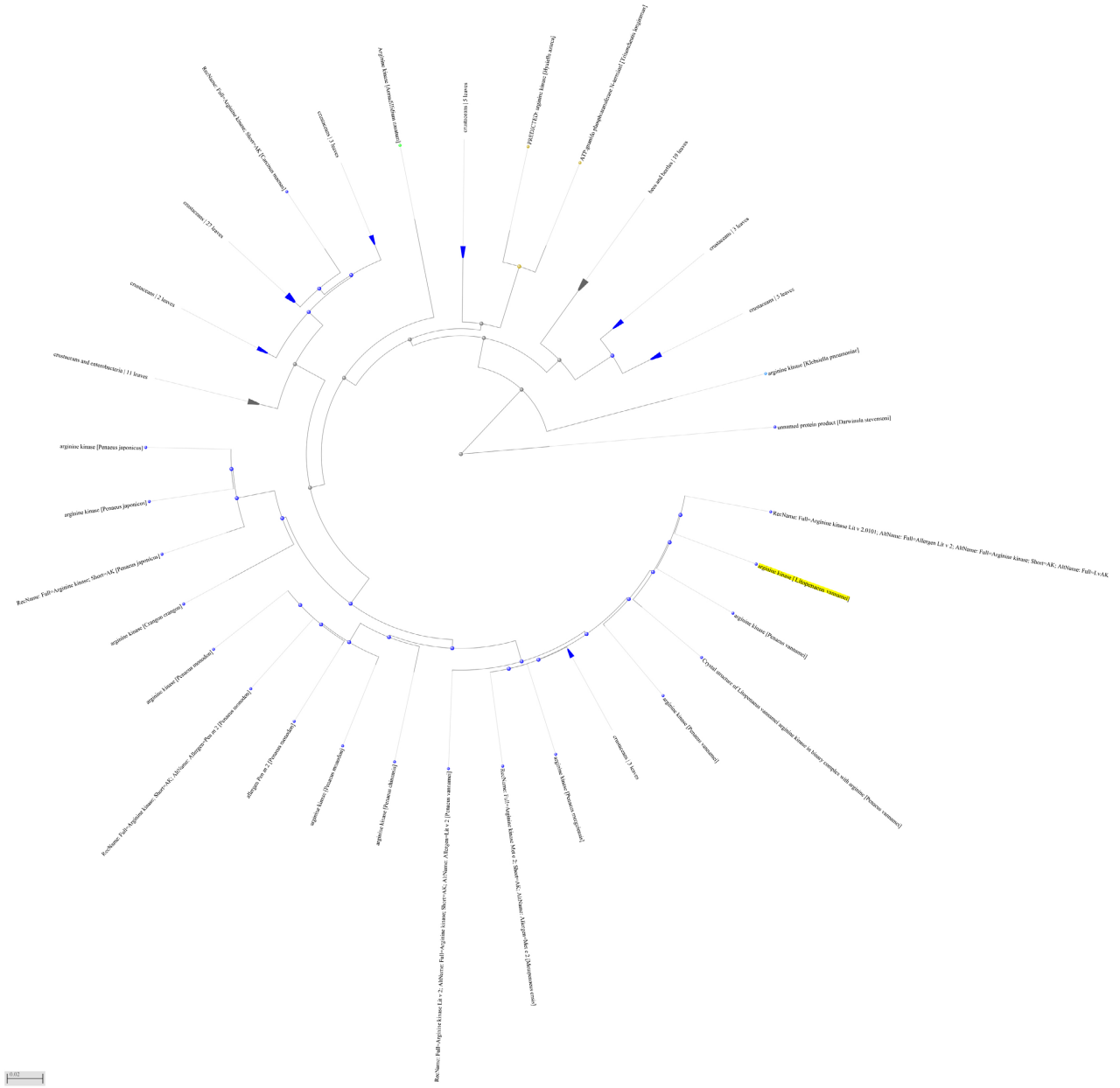

Figure 4. The phylogenetic tree between the arginine kinases of *Litopenaeus vannamei* and other species. It shows how close or far the arginine kinases of other species are to the arginine kinase of *L. vannamei* (yellow color). AK of crustaceans (blue), ectoparasites (yellow), cochineal (green), and AK of crustaceans, enterobacteria and bees (grey). Figure elaborated in the MEGA-X program.

trigger a series of anaphylactic processes. The only thing that can be saved for these allergens is that an amino acid sequence with medium identity was found for troponin C of the shrimp *Penaeus merguiensis* (S5ZHH2) and pyruvate kinase a sequence of low identity with the shrimp *Penaeus monodon* (E7CGC5). For the allergen thioredoxin, no matches were found.

In summary, many allergen sequence matches were identified, mainly with species of crustaceans, insects, mites, and nematodes. For the coincidences with the tropomyosin (TM) of *L. vannamei*, they were 46% with crustaceans,

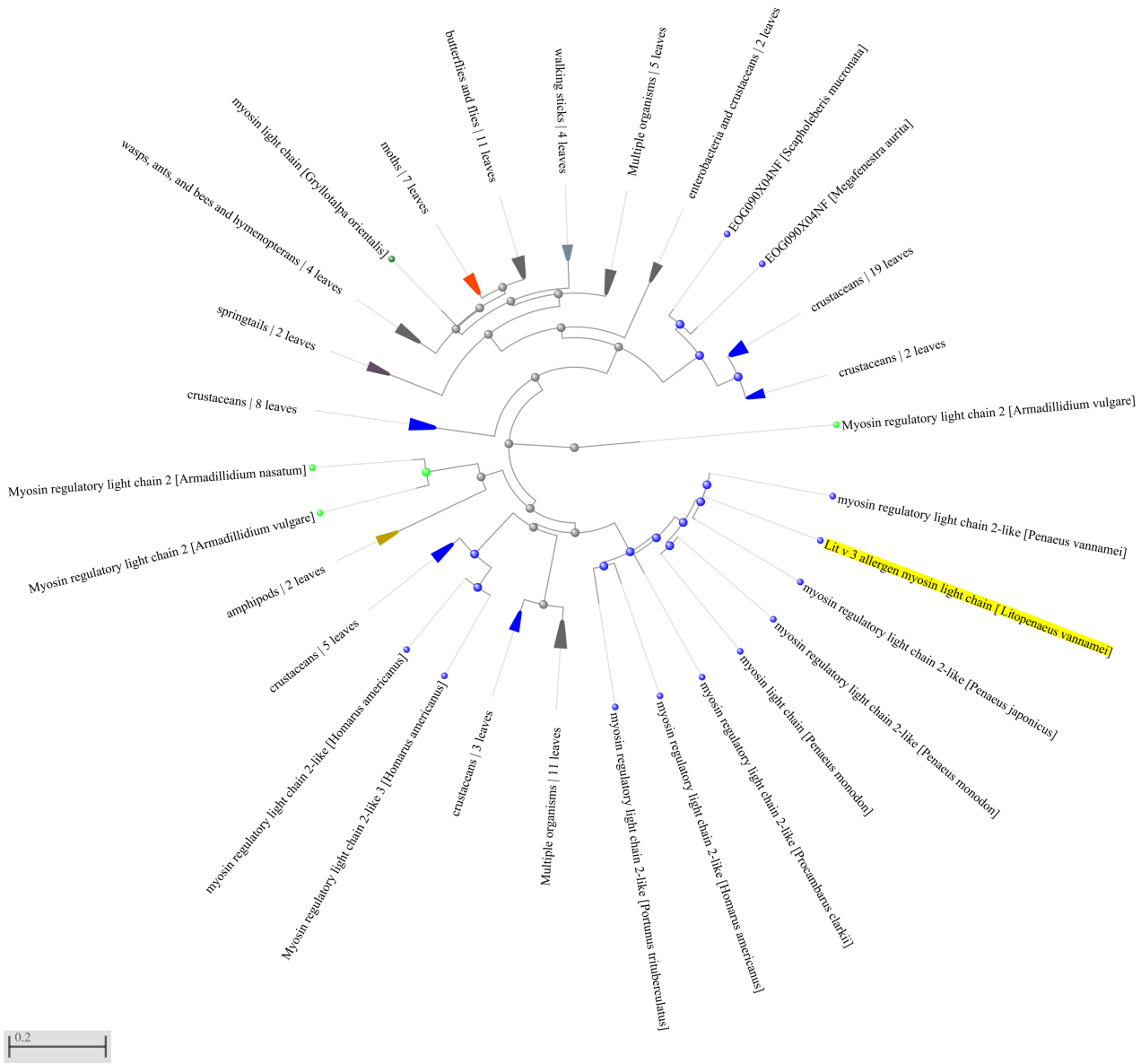

Figure 5. The phylogenetic tree between the myosin light chains of *Litopenaeus vannamei* and other species. It shows how close or far myosin light chains from other species are to the myosin light chain from *L. vannamei* (yellow color). MLC of crustaceans (blue), amphipods (yellow) crustaceans and microorganisms (purple), insects (green and red), and MLC of birds and other species (grey). Figure elaborated in the MEGA-X program.

15% with insects, 13% with mites, 10% with nematodes, 8% with mammals, and 8% with mollusks. For arginine kinase (AK) 57% with crustaceans, 33% with insects, 5% with mites, and 5% with nematodes. For myosin light chain (MLC) 60% with crustaceans, 20% with insects, and 20% with birds, and for sarcoplasmic calcium-binding protein 85% with crustaceans and 15% with insects (**Figure 7**).

As it was observed, the tropomyosin allergen of the shrimp *L. vannamei* is the one that has the highest number of coincidences with other species and is mostly high and medium identities (greater than 80% and 40%, respectively).

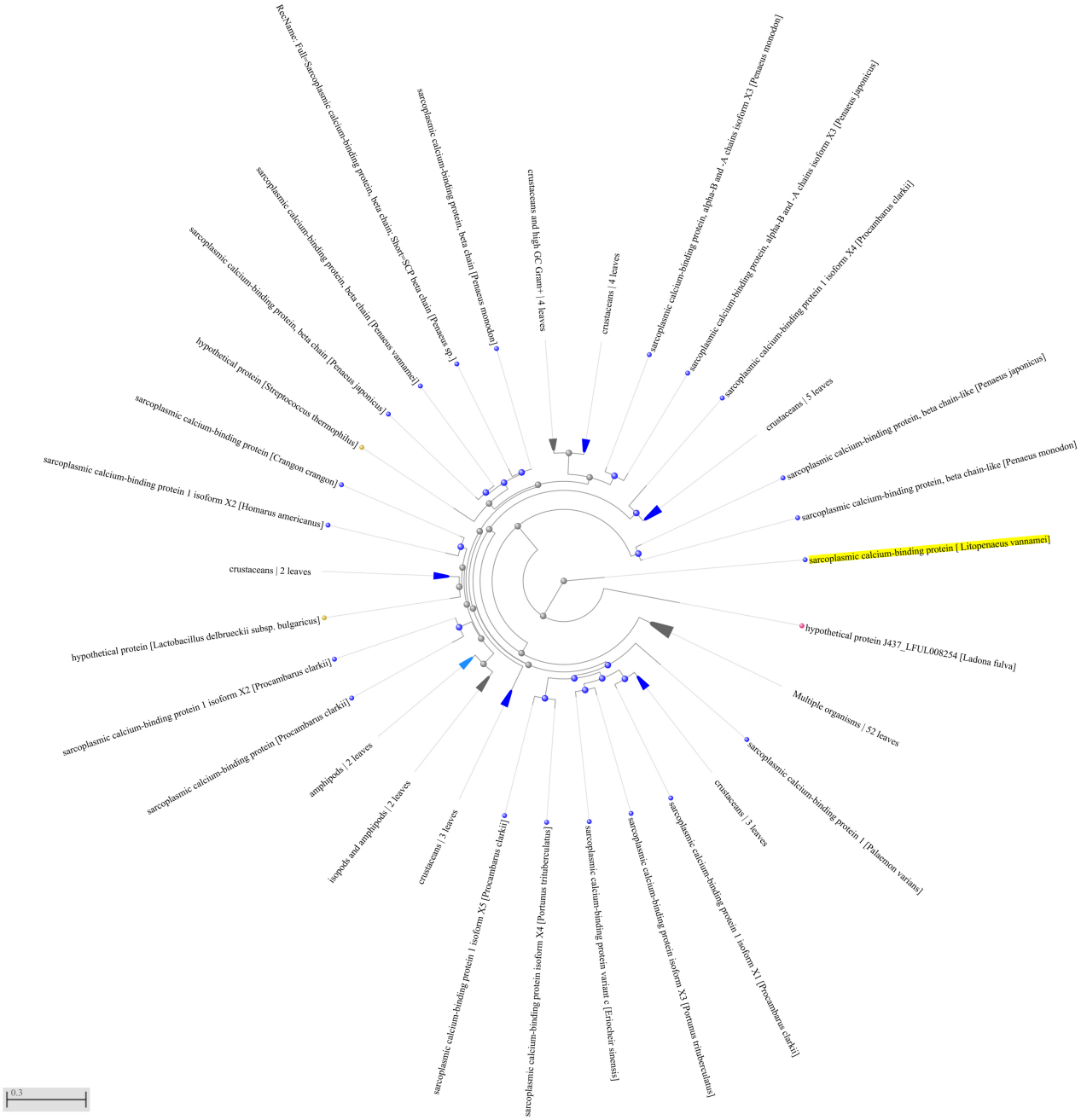

Figure 6. The phylogenetic tree between the sarcoplasmic calcium-binding proteins of *Litopenaeus vannamei* and other species. It depicts how close or far sarcoplasmic calcium-binding proteins from other species are to the sarcoplasmic calcium-binding protein from *L. vannamei* (yellow color). Crustacean SCPs (blue), microorganisms (ping and yellow), amphipods (sky blue) Figure elaborated in the MEGA-X program.

Which would confirm the theory or hypothesis that tropomyosin is the major shrimp allergen. Figure 8 describes the 6 shrimp (*L. vannamei*) allergens and some of the species that had coincidences in common. As can be seen, tropomyosin is the one with the highest identities (red, yellow, and brown), followed by arginine kinase and myosin light chain. And in lower percentages of identity troponin C and hemocyanin (blue color). This would not indicate that

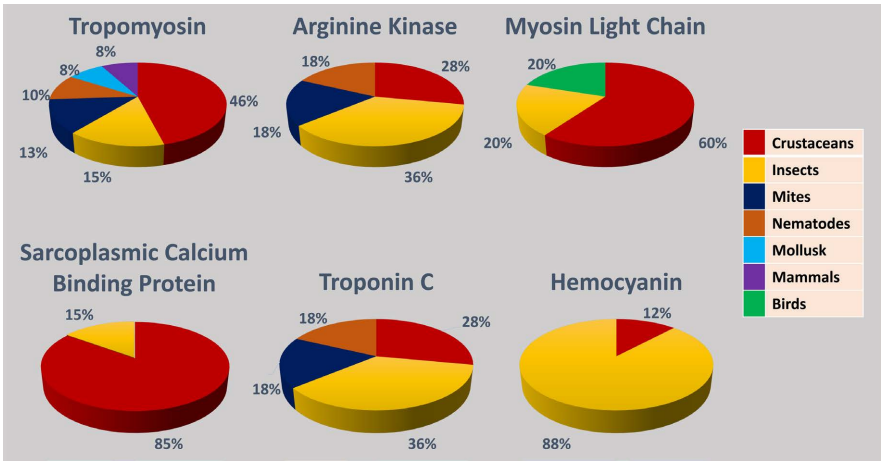

Figure 7. Total allergens identified from cross-reactivity analysis. With each of the white shrimp *Litopenaeus vannamei* allergens. The distribution between the different groups of allergen sources is shown in percentages and arranged in descending order. Figure made with the trash can of Microsoft Office and BioRender.

Organism	Access number	#	Tropomyosin *L. vannamei* (Percentage of identity %)	Arginine kinase *L. Vannamei* (Percentage of identity %)	Myosin light chain *L. Vannamei* (Percentage of identity %)	Sarcoplasmic calcium binding protein *L. Vannamei* (Percentage of identity %)	Troponin C *L. Vannamei* (Percentage of identity %)	Hemocyanin *L. Vannamei* (Percentage of identity %)
Litopenaeus vannamei	B4YAH6	1	100	99	100	100	100	100
Penaeus monodon	A1KYZ2	2	100	84	88	90	27	55
Penaeus aztecus	Q3Y8M6	3	100	82	89	98	13	0
Macrobrachium rosenbergii	D3XNR9	4	93	0	62	0	0	0
Periplaneta fuliginosa	Q8T6L5	5	45	39	0	0	1.7	0.15
Dermatophagoides farinae	Q23939	6	41	30	0	0	1.7	0
Aedes aegypti	Q17H75	7	27	22	0	3	0.86	0.15
Anisakis simplex	G4XTD3	8	27	24	0	0	0.86	0
Ascaris lumbricoides	C0L3K2	9	27	16	0	0	0.12	0
Blattella germanica	Q9NG56	10	46	0	5	0	1.7	0.15
Onchocerca volvulus	Q25632	11	27	0	0	0	0	0
Perna viridis	Q9GZ70	12	5	0	0	0	0	0
Homo sapiens	P02538	13	0.72	0	0	0	0	0.15
Sus scrofaa	P08835	14	0.36	0	0	0	0	0

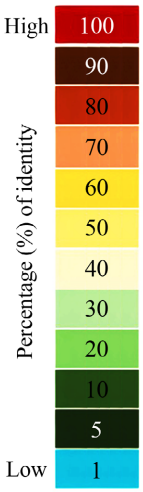

Figure 8. Percentages of identity between the allergens of *Litopenaeus vannamei* and other species. Some of the amino acid sequences had matches to Pacific white shrimp allergens. Being an identity or cross-reactivity high (red), medium (yellow), low (green), and null (blue). Figure made with the trash can of Microsoft Office and BioRender.

tropomyosin, arginine kinase, myosin light chain, and sarcoplasmic calcium-binding protein from other species (mainly crustaceans) could trigger an anaphylactic response in people sensitized to Pacific white shrimp.

4. Discussion

As previously mentioned, there are already studies on the cross-reactivity of

shrimp allergens [16] [17] [18] [19], but not exclusively on shrimp *Litopenaeus vannamei*. Also, no "non-typical" allergens such as troponin C, pyruvate kinase, and thioredoxin have been reported in this species because all the allergens were identified in various species of main crustaceans [20].

With the increase in clinical reports on anaphylactic processes to shellfish, shrimp being the main source of allergies, as well as the cross-reactivity of people sensitized to shellfish and who react to other species, there is already an analysis of potential allergens [21]. The results obtained in the present study on typical and non-typical allergens of *L. vannamei* indicate that sensitization to shellfish may be difficult due to the highly reactive nature of some allergenic proteins [21]. As it was possible to observe, the allergens of *L. vannamei* have several similarities with other species, which would indicate that the cross-reactivity of the Pacific white shrimp is wide and therefore the possibility of carrying out a process of respiratory, gastrointestinal, and even hypersensitivity. Skin is high (**Figure 9**).

Tropomyosin is the primary allergen for clinically reported cross-reactivity among shellfish, but also cockroaches, mites, and even parasites [22] [23]. As previously reported, tropomyosin has epitopes that are exclusive to IgE in a linear fashion and that are of great importance in determining the degree of cross-reactivity in different types of crustaceans [23]. This allergen is highly conserved in various crustaceans such as lobsters, crabs, and shrimp with identities between 95% and 100%. Thus, cross-reactivity of mainly IgE antibodies is quite frequent [24] [25]. The 100% demonstrated identity percentage between *L. vannamei* and *L. monodon* has been previously reported and validates the in silico approach used in this study. Furthermore, it was shown for the first time that crustaceans such as *M. ensis* and *M. rosenbergii* also have 100% amino acid sequence identity with the TM of *L. vannamei*. And *L. aztecus* and

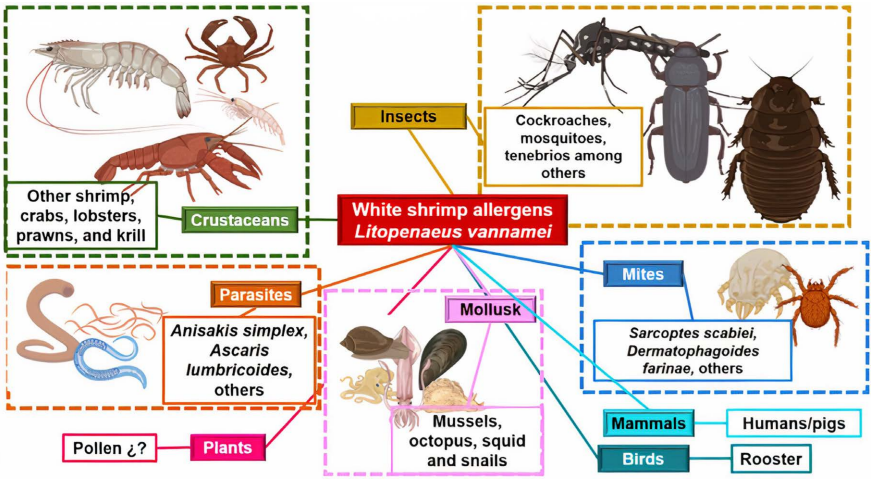

Figure 9. Cross-reactivity of white shrimp *Litopenaeus vannamei* allergens. It is represented that other species allergens of *L. vannamei* have homology in the amino acid sequence, which can cause cross-reactivity. Figure made with the trash can of Microsoft Office and BioRender.

L. japonicus sequences were previously reported with 100% identities [26] [27] [28]. It is important to mention that most of the tropomyosin that resulted from the Allermatch software and that have coincidences with the *L. vannamei* tropomyosin are potentially allergenic. According to the IUIS nomenclature, and is allergen is a protein from the same species, or family of species, with the same biological function and similar biochemical properties, including greater than 67% amino acid sequence identity and similar size [29]. The high identity (<70%) between the amino acid sequences of tropomyosins from crustaceans (**Table S7**, red color) and Pacific white shrimp indicates a high probability of immunological cross-reactivity. As already reported, an identity in the amino acid sequence greater than 70% would demonstrate a very probable possibility of binding IgE antibodies with cross-reactivity to these allergens [30] [31] [32]. Here we have demonstrated conclusive molecular data on the amino acid sequence similarity of a major allergen (tropomyosin) of Pacific white shrimp with other invertebrate species [28] [29] [30].

The allergen arginine kinase (AK) is a monomeric phosphagen ATP phosphotransferase that is generally found in invertebrates and is the key to energy metabolism. It has been identified in more than 6 crustaceans including shrimp. It is heat unstable, but IgE-AK binding has been shown in cooked shrimp, which may be due to intact IgE epitopes on expressed AK [33]. Amino acid sequence similarity is high among crustaceans (70% - 100%), indicating that patients allergic to Pacific white shrimp sensitized to arginine kinase would likely react to the other crustacean species (*L. monodon*, *L. chinensis*, *C. crangon*, and *S. paramamosain*). Arginine kinase is considered a pan-allergen [34] [35] and is important among insects and mites. In our results, about 37% of the amino acid sequences that had coincidences with the AK of *L. vannamei* are from insects and mites. These species then represent a medium identity (>30%) for cross-reactivity to be carried out by parts of the allergic patients. In addition, the similarities of the arginine kinases found for Pacific white shrimp of effort indicate that they are potential variants of is allergens because their PI is greater than 90% [29].

Myosin light chain (MLC) allergen is found primarily in smooth muscle in complexes with myosin heavy chain motor domains. It has a molecular weight of 17 to 20 kDa and is heat stable [36]. Its form of sensitization is by ingestion, it regulates the function of smooth muscle when it is phosphorylated by the kinase. 50% of shellfish-allergic patients have cross-reactivity against this protein. Light chain myosin from *L. vannamei* was reported as an allergen with a molecular weight of 20 kDa and was named Lit v 3 [37] [38]. The very low amino acid sequence PI (<20%), MLC1, and MLC 2 are not considered isoforms but rather two different proteins [39]. We mention in this study for the first time that the MLC allergen has (low) similarities with some birds among them (*Gallus gallus*), in addition to the previously reported MLC 2 allergen in *L. vannamei* and *L. monodon* shrimp. Furthermore, this study also suggests that MLCs of the cockroach (*Blattella germanica*) are probably MLC 2, respectively, which explains the close molecular phylogenetic relationship with crustaceans, but not with insects.

Finally, the sarcoplasmic calcium-binding protein (SCP) allergen is a protein that promotes muscle relaxation by translocating calcium from the myofibrils to the endoplasmic reticulum. It has a molecular weight of 20 to 22 kDa. It is thermostable and resists treatment with acids and alkanes. Until now, three isoforms are known (SCP-I, II, III) with isoelectric points of 5.05, 4.90, and 4.75 respectively [40] [41]. We identified 100% amino acid sequences for the shrimp *Penaeus monodon* implying the presence of SCP is allergens. However, the significantly average abundance among the other crustaceans (30% - 50%) concludes that the amino acid sequence is relatively unlikely to cross-react by allergic patients. Other identified muscle regulatory proteins include the protein troponin. Troponin is made up of three subunits, suffixed C, I, and T, and troponin C is listed as an allergen. Our study first demonstrated this allergen [42], together with hemocyanin for medium cross-reactivity in *L. merguiensis* and *L. monodon* shrimp.

In addition to the identified amino acid sequences, as well as the comparison of known crustacean allergens with Pacific white shrimp (*L. vannamei*), another type of allergen repertoire was also identified in this study. About 15 new potential shrimp allergens were successfully identified with low matches such as RNA-dependent polymerase, vitellogenin, and aldehyde dehydrogenase among others. These three proteins are allergens recorded in different species of mites and insects [43] [44]. Clinical studies frequently report cross-allergic patient reactions to crustaceans as well as mites and insects, but it remains unclear whether shrimp sensitization occurs through the respiratory or gastrointestinal tract, or, in other words, whether it is a secondary phenomenon following mite/insect/nematode sensitization or a true primary allergy [44].

In addition, we found allergens that are possibly responsible for the cross-reactivity between shellfish and fish. Aldose-A allergen was found in low identities for fish (*Pangasianodon hypophthalmus*), this allergen was not included in the main results of this study as they are phylogenetically distant to Pacific white shrimp. However, there is emerging evidence that patients suffering from shrimp allergy are also sensitized to pollen and fish allergens [45].

5. Conclusions

This study provides new data in the area of cross-reactivity for Pacific white shrimp (*L. vannamei*). A comparative analysis of "typical and non-typical" shrimp *L. vannamei* allergens with other species was achieved, revealing new allergens and previously characterized shrimp allergens, which validated the comprehensive identification approach used in this study.

From previous studies, it is known that shrimp is one of the main causes of allergy and that it causes greater sensitization in patients with a high risk of atopy; however, the relationship of sensitization between these invertebrates and their relevance has not yet been explored clinic. Therefore, the difference in the proteomic abundance of different allergens between arthropod species, including the shrimp *L. vannamei*, may have clinical and diagnostic importance. Im-

portantly, up to 192, amino acid sequences were identified that had matches to shrimp *L. vannamei* allergens that matched allergenic proteins in mites, insects, fish, bacteria, mammals, birds, and plants. These include shrimp proteins that have a high probability of being potential allergens such as beta-enolase and aldolase-A; however, these have not yet been identified as true shrimp allergens. Future studies will have to evaluate the abundance of proteins of known and potential allergens of shrimp *L. vannamei*, as well as analyses that allow evaluating, through inhibition tests, the role of allergens to examine the binding capacity of IgE antibodies and thus show clinical sensitization in patients with a shellfish allergy and determine its allergenic potential.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary

Table S1. Percentage of identity between the sequences of the white shrimp (*Litopenaeus vannamei*) tropomyosin allergen (Lit v 1) created in Clustal 2.1.

1: ACB38288.1	100.00	99.35	98.57	99.78
2: B4YAH6	99.85	100.00	99.36	99.87
3: 4029	99.67	99.38	100.00	98.96
4: 4030	99.92	99.54	99.88	100.00

Table S2. Percentage of identity between the sequences of the white shrimp (*Litopenaeus vannamei*) arginine kinase allergen (Lit v 2) created in Clustal 2.1.

1: ABI98020.1	100.00	99.16	98.96	99.58	99.08
2: B0FRF9.1	99.85	100.00	99.69	99.68	99.45
3: Q004B5	99.98	99.34	100.00	99.11	98.67
4: 3544	97.58	99.94	99.46	100.00	99.18
5: 3616	99.88	99.78	99.67	98.59	100.00

Table S3. Percentage of identity between white shrimp (*Litopenaeus vannamei*) allergen myosin light chain sequences (Lit v 3) created in Clustal 2.1.

1: ACC76803.1	100.00	98.97	98.75	99.36	99.58
2: ROT68323.1	98.78	100.00	99.28	99.87	99.69
3: B7SNI3	99.87	99.68	100.00	97.58	98.58
4: 4052	99.36	99.87	99.28	100.00	99.48
5: 4053	99.81	99.26	99.87	99.47	100.00

Table S4. Percentage of identity between the sequences of the sarcoplasmic calcium-binding protein allergen (Lit v 4) of the white shrimp (*Litopenaeus vannamei*) created in Clustal 2.1.

1: ACM89179.1	100.00	98.68	99.58	99.68	99.49
2: C7A639	99.85	100.00	99.69	98.69	98.75
3: A0A3R7PHV9	99.38	99.25	100.00	99.41	98.49
4: 6092	99.67	98.97	99.62	100.00	99.67
5: 6093	99.99	99.87	99.74	99.84	100.00

Table S5. Percentage of identity between the sequences of the allergen troponin C (Lit v 6) of the white shrimp (*Litopenaeus vannamei*) created in Clustal 2.1.

1: 12255	100.00	96.56
2: ROT66451.1	96.89	100.00

Table S6. Percentage of identity between the sequences of the white shrimp (*Litopenaeus vannamei*) hemocyanin allergen created in Clustal 2.1.

1: 12159	100.00	99.45	97.68
2: ART94437.1	99.89	100.00	98.56
3: ROT83548.1	99.67	99.58	100.00

Table S7. Cross-reactivity between tropomyosin from *Litopenaeus vannamei* and other species.

No	Db	Description	Size	% of exact matches	External db	Scientific Name
1	UniProt	Tropomyosin	279	100.00	A1KYZ2	*Litopenaeus monodon*
2	UniProt	Tropomyosin Pen a 1.0102	279	100.00	Q3Y8M6	*Litopenaeus aztecus*
3	UniProt	Lit v 1 tropomyosin	279	100.00	B4YAH6	*Litopenaeus vannamei*
4	UniProt	Tropomyosin fast isoform	279	100.00	href="http://www.uniprot.org/uniprot/A2V731" A2V731	*Litopenaeus japonicus*
5	UniProt	Tropomyosin	263	94.27	Q25456	*Metapenaeus ensis*
6	UniProt	Tropomyosin	260	93.19	D3XNR9	*Macrobrachium rosenbergii*
31	UniProt	Tropomyosin	131	46.95	Q9NG56	*Blattella germanica*
32	UniProt	Tropomyosin	127	45.52	Q8T6L5	*Periplaneta fuliginosa*
33	UniProt	Tropomyosin Per a 7.0102	126	45.16	P0DSM7	*Periplaneta americana*
34	UniProt	Tropomyosin	125	44.80	A0A4P8D340	*Gromphadorhina portentosa*
35	UniProt	Tropomyosin 1	123	44.09	A0A4P8D330	*Galleria mellonella*
39	UniProt	Tropomyosin	116	41.58	Q23939	*Dermatophagoides farinae*
41	UniProt	AAEL002761-PC	116	41.58	Q17H75	*Aedes aegypti*
42	UniProt	RNA dependent RNA polymerase	113	40.50	A0A1M4PIX0	*Sarcoptes scabiei*
52	UniProt	Tropomyosin	78	27.96	G4XTD3	*Anisakis simplex*
53	UniProt	Tropomyosin	78	27.96	C0L3K2	*Ascaris lumbricoides*
54	UniProt	Tropomyosin	75	26.88	Q9NAS5	*Anisakis simplex*
55	UniProt	Tropomyosin	75	26.88	A0A4P8D339	*Zophobas atratus*
56	UniProt	Tropomyosin	71	25.45	Q25632	*Onchocerca volvulus*
57	UniProt	Tropomyosin 2	60	21.51	A0A4P8D346	*Tenebrio molitor*
88	UniProt	Tropomyosin	19	6.81	B7XC63	*Turbo cornutus*
89	UniProt	Tropomyosin	18	6.45	B7XC67	*Fulvia mutica*
90	UniProt	Tropomyosin	16	5.73	Q9GZ70	*Perna viridis*
91	UniProt	Tropomyosin	16	5.73	B7XC65	*Anadara broughtonii*
95	UniProt	Keratin, type II cytoskeletal 6A	2	0.72	P02538	*Homo sapiens*
101	GenBank	albumin precursor	1	0.36	NP001005208	*Sus scrofa*
103	UniProt	Albumin	1	0.36	P08835	*Sus scrofa*

Table S8. Cross-reactivity between arginine kinase from *Litopenaeus vannamei* and other species.

No	Db	Description	Size	% of exact matches	External db	Scientific Name
1	UniProt	Arginine kinase Lit v 2.0101	350	99.72	Q004B5	*Litopenaeus vannamei*
2	UniProt	Arginine kinase	297	84.62	C7E3T4	*Litopenaeus monodon*
3	UniProt	Arginine kinase	282	80.34	Q4KY22	*Litopenaeus chinensis*
4	UniProt	Arginine kinase	254	72.36	D7F1J5	*Crangon crangon*
5	UniProt	Arginine kinase Scy p 2.0101	232	66.10	H6VGI3	*Scylla paramamosain*
10	UniProt	Arginine kinase	138	39.32	A1KY39	*Periplaneta americana*
11	UniProt	Arginine kinase	137	39.03	B1A7S7	*Periplaneta americana*
15	UniProt	Arginine kinase	115	32.76	A0A088SAW4	*Dermatophagoides farinae*
16	UniProt	Arginine kinase	114	32.48	B2ZSY4	*Dermatophagoides pteronyssinus*
17	UniProt	Arginine kinase	108	30.77	A7XZJ2	*Dermatophagoides farinae*
18	UniProt	Arginine kinase	108	30.77	A1KXC3	*Dermatophagoides farinae*
25	UniProt	Major allergen Bet v 1	1	0.28	Q39430	*Betula pendula*
26	UniProt	Major allergen Bet v 1	1	0.28	Q39425	*Betula pendula*
27	UniProt	Major pollen allergen Aln g 1	1	0.28	P38948	*Alnus glutinosa*
28	UniProt	Allergen Cop c 5	1	0.28	Q9UW00	*Coprinus comatus*
29	UniProt	Albumin	1	0.28	P02769	*Bos taurus*

Table S9. Cross-reactivity between the myosin light chain from *Litopenaeus vannamei* and other species.

No	Db	Description	Size	% of exact matches	External db	Scientific Name
1	UniProt	Lit v 3 allergen myosin light chain	172	100.00	B7SNI3	*Litopenaeus vannamei*
2	UniProt	Myosin light chain	154	89.53	E7CGC3	*Litopenaeus monodon*
3	UniProt	Myosin light chain	153	88.95	E1A683	*Litopenaeus monodon*
4	UniProt	Allergen Bla g 8	9	5.23	A0ERA8	*Blattella germanica*
5	UniProt	Vitellogenin-2	1	0.58	P02845	*Gallus gallus*

Table S10. Cross-reactivity between sarcoplasmic calcium-binding protein from *Litopenaeus vannamei* and other species.

No	Db	Description	Size	% of exact matches	External db	Scientific Name
1	UniProt	Sarcoplasmic calcium binding protein	188	100.00	E7CGC4	*Litopenaeus monodon*
2	UniProt	Sarcoplasmic calcium-binding protein	188	100.00	C7A639	*Litopenaeus vannamei*

Continued

3	UniProt	Sarcoplasmic calcium-binding protein	170	53.43	H7CHW2	*Litopenaeus monodon*
4	UniProt	Sarcoplasmic calcium-binding protein 1	76	40.43	P05946	*Astacus leptodactylus*
5	UniProt	Sarcoplasmic calcium-binding protein	65	34.57	I2DDG2	*Scylla paramamosain*
6	UniProt	Sarcoplasmic calcium-binding protein	64	34.04	D7F1P9	*Crangon crangon*
7	UniProt	AAEL008844-PA	6	3.19	Q16XK7	*Aedes aegypti*

Table S11. Cross-reactivity between troponin C from *Litopenaeus vannamei* and other species.

No	Db	Description	Size	% of exact matches	External db	Scientific Name
1	UniProt	Troponin C	31	27.68	E7CGC5	*Litopenaeus monodon*
2	UniProt	Troponin C, isoform 2B	15	13.39	P29291	*Homarus americanus*
3	UniProt	Troponin C	11	9.82	D7F1Q2	*Crangon crangon*
4	UniProt	HDM allergen	2	1.79	Q6Y2F9	*Dermatophagoides pteronyssinus*
5	UniProt	Per a 6 allergen	2	1.79	Q1M0Y3	*Periplaneta americana*
6	UniProt	Paramyosin	2	1.79	Q967Z0	*Dermatophagoides farinae*
7	UniProt	RNA dependent RNA polymerase	2	1.79	A0A1M4PIW9	*Sarcoptes scabiei*
8	UniProt	Der f 11 allergen	2	1.79	A0A088SCQ4	*Dermatophagoides farinae*
9	UniProt	Troponin C, isoallergen Bla g 6.0201	1	0.89	Q1A7B2	*Blattella germanica*
10	UniProt	Troponin C, isoallergen Bla g 6.0101	1	0.89	Q1A7B3	*Blattella germanica*
11	UniProt	High molecular weight allergen M-177	1	0.89	Q9U785	*Euroglyphus maynei*
12	UniProt	Troponin-like protein	1	0.89	Q9U3U5	*Anisakis simplex*
13	UniProt	Group 14 allergen protein	1	0.89	Q8N0N0	*Dermatophagoides pteronyssinus*
14	UniProt	Bee-milk protein	1	0.89	Q4ZJX1	*Apis mellifera*
15	UniProt	Group 15 allergen protein	1	0.89	Q4JK70	*Dermatophagoides pteronyssinus*
16	UniProt	Group 15 allergen protein short isoform	1	0.89	Q4JK69	*Dermatophagoides pteronyssinus*
17	UniProt	Polcalcin Nic t 1	1	0.89	Q8VWY6	*Nicotiana tabacum*
18	UniProt	Polcalcin Bra n 1	1	0.89	P69196	*Brassica napus*

Continued

19	UniProt	Polcalcin Bra r 1	1	0.89	P69197	*Brassica campestris*
20	UniProt	Paramyosin	1	0.89	Q8MUF6	*Blomia tropicalis*
21	UniProt	Cysteine protease Amb a 11.0101	1	0.89	V5LU01	*Ambrosia artemisiifolia*
22	UniProt	Calcium-binding allergen Ole e 8	1	0.89	Q9M7R0	*Olea europaea*

Table S12. Cross-reactivity between hemocyanin from *Litopenaeus vannamei* and other species.

No	Db	Description	Size	% of exact matches	External db	Scientific Name
1	UniProt	Hemocyanin	366	55.71	S5ZHH2	*Litopenaeus merguiensis*
2	UniProt	Major pollen allergen Pha a 5.1	2	0.30	P56164	*Phalaris aquatica*
3	GenBank	PREDICTED: collagen alpha-1(I) chain	1	0.15	XP_014059932	*Salmo salar*
4	GenBank	PREDICTED: collagen alpha-1(I) chain-like	1	0.15	XP_014048044	*Salmo salar*
5	UniProt	Vitellogenin-2	1	0.15	P02845	*Gallus gallus*
6	UniProt	Toxic shock syndrome toxin-1	1	0.15	P06886	*Staphylococcus aureus*
7	UniProt	HDM allergen	1	0.15	Q6Y2F9	*Dermatophagoides pteronyssinus*
8	UniProt	Paramyosin	1	0.15	Q967Z0	*Dermatophagoides farinae*
9	UniProt	Paramyosin	1	0.15	Q8MUF6	*Blomia tropicalis*
10	UniProt	Keratin, type II cytoskeletal 6A	1	0.15	P02538	*Homo sapiens*
11	UniProt	Glutathione-S-transferase	1	0.15	Q6R4B4	*Alternaria alternata*
12	UniProt	Endochitinase Ziz m 1.0101	1	0.15	Q2VST0	*Ziziphus mauritiana*
13	UniProt	Tropomyosin	1	0.15	B7XC71	*Ruditapes philippinarum*
14	UniProt	Lysosomal aspartic protease	1	0.15	Q03168	*Aedes aegypti*
15	UniProt	Tropomyosin	1	0.15	A2V716	*Balanus rostratus*
16	UniProt	Der f 11 allergen	1	0.15	A0A088SCQ4	*Dermatophagoides farinae*
17	UniProt	Group 2 allergen Sor h 2.0100	1	0.15	A0A077B7S9	*Sorghum halepense*

Table S13. Cross-reactivity between pyruvate kinase from *Litopenaeus vannamei* and other species.

No	Db	Description	Size	% of exact matches	External db	Scientific Name
1	GenBank	pyruvate kinase M1/2b /A aldose	66	11.26	XP_026775867	*Pangasianodon hypophthalmus*
2	UniProt	Vitellogenin	1	0.17	Q868N5	*Apis mellifera*
3	UniProt	Vicilin Pis v 3.0101	1	0.17	B4X640	*Pistacia vera*
4	UniProt	Protein disulfide-isomerase	1	0.17	Q00002	*Alternaria alternata*

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5	UniProt	NADP-dependent mannitol dehydrogenase	1	0.17	P0C0Y5	*Davidiella tassiana*
6	UniProt	Bet v 1 related allergen	1	0.17	D1YSM5	*Actinidia deliciosa*
7	UniProt	Allergen Ani s 10	1	0.17	D2K835	*Anisakis simplex*
8	UniProt	Aldehyde dehydrogenase	1	0.17	P42041	*Alternaria alternata*