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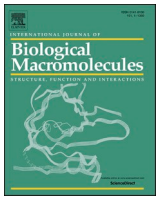

Species- and heat-dependent variability in shrimp allergens revealed by quantitative proteomics: Implications for risk assessment☆

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ABSTRACT

Shellfish allergy is a major cause of food-induced severe adverse reactions worldwide, with shrimp representing one of the most implicated triggers. Detection and monitoring of allergenic proteins in shrimp-containing foods rely largely on immunoassay-based detection, typically targeting tropomyosin without species-level specificity. However, allergen composition may vary across species and processing conditions, limiting the accuracy of existing approaches.

Here, we applied quantitative proteomics to characterise allergen profiles in five commonly consumed shrimp species under raw and heat-treated conditions. Protein extracts ($n = 3$ per species/treatment) were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following in-solution digestion. Identified proteins were mapped against curated allergen databases and species-specific transcriptomes to assess presence and relative abundance. Complementary SDS-PAGE and immunoblot analyses using allergen-specific antibodies were performed to evaluate qualitative detection across species and treatments.

Proteomic analysis revealed marked interspecies variability in allergen presence and abundance. Several allergens exhibited differential stability following heat treatment, reflecting distinct biophysical properties influencing persistence in heat-processed foods. Selected putative allergens identified by transcriptome analysis, including aldolase and enolase, were confirmed at the protein level, while others were not detected. Moreover, weak concordance between transcriptomic and proteomic abundance was observed for multiple allergens, including arginine kinase and myosin light chain. Immunoblotting for tropomyosin and myosin light chain demonstrated inconsistent detection across species and treatments, highlighting limitations of antibody-based detection systems.

These findings establish that shrimp allergen composition is species- and heat processing-dependent, with implications for risk assessment frameworks. Quantitative proteomics provides a robust platform for comprehensive allergen profiling and supports improved detection strategies for aquatic food safety.

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1. Introduction

Food allergies represent a growing global food safety concern, with shellfish consistently identified as one of the leading causes of severe allergic reactions [1,2]. Among shellfish, shrimp (prawn) is a major contributor due to its widespread consumption and incorporation into diverse food products [3,4]. In addition to clinical implications, shrimp allergenicity presents a significant challenge for food manufacturers, regulators, and risk assessors, particularly in ensuring accurate detection and labelling of allergenic components in raw and processed foods [5–7]. The diversity of commercially traded shrimp species, combined with variable allergen composition across taxa, presents a fundamental challenge for both clinical diagnosis and food safety monitoring that cannot be addressed by single-species reference approaches [6,8,9].

Current approaches to allergen monitoring and analytical detection in food systems rely predominantly on immunoassay-based techniques, such as enzyme-linked immunosorbent assays (ELISAs) [10,11]. While widely used, these methods are often designed around a narrow subset of allergenic targets, most notably tropomyosin [12,13], and may not adequately capture the broader diversity of allergenic proteins present in shrimp (**Supp. Table A.1**). Formal allergen risk assessment additionally integrates clinical threshold data, exposure assessment and reference doses, and regulatory practice is increasingly moving toward threshold-based quantitative risk assessment rather than relying on analytical detection alone [14]. Furthermore, commercially available assays frequently lack transparency regarding the specific allergen targets and are typically developed using a single reference species. This is challenging given the diversity of shrimp species entering global food supply chains, where differences in protein composition may influence allergen detection and quantification [12,15,16]. Additionally, antibody-based detection can be affected by food processing conditions, especially the most common processing technique – heat treatment, which may alter protein structure and reduce antibody recognition, thereby compromising analytical accuracy [17–19].

Five shrimp species – *Penaeus monodon, Litopenaeus vannamei, Melicertus latisulcatus*, *Fenneropenaeus merguiensis*, and *Metapenaeus endeavouri* – are among the most widely harvested and consumed across the Asia-Pacific region and contribute substantially to international seafood trade [20–22]. Despite their economic and dietary importance, comparative data on allergen composition and abundance across these species remain limited [8]. Given the increasing complexity of global food systems, understanding species-specific differences in allergen profiles is essential for improving risk assessment strategies and ensuring the reliability of allergen detection in multi-species and processed food products.

Shrimp allergen composition is inherently complex. To date, sixteen shellfish/crustacean allergens are recognised within the World Health Organisation and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database, of which eleven have been identified in shrimp species, alongside additional putative allergens reported through recent molecular studies [23–25]. Notably, these proteins differ in their structural stability and susceptibility to processing-induced modifications, which can influence their persistence and detectability in food matrices [4,6,17,26–28]. Such variability presents a significant challenge for existing detection methods that do not account for differences in allergen stability, molecular changes during heat processing and species-specific allergens.

Recent transcriptomic analyses have expanded the repertoire of known and putative shrimp allergens, enabling high-throughput identification of allergen-encoding mRNA transcripts across multiple species [15]. In addition to allergen discovery, transcriptome-derived species-specific protein databases can substantially improve proteomic identification coverage in non-model seafood species by capturing lineage-specific sequences and reducing false peptide assignments associated with generic reference databases. However, transcript abundance does not necessarily reflect protein expression levels or functional allergen presence. The translation of mRNA into protein is subject to multiple regulatory processes, and post-translational modifications further influence protein stability and detectability [29,30]. As a result, reliance on transcriptomic data alone may lead to an incomplete or inaccurate representation of allergen profiles relevant to food exposure [31].

Proteomic approaches offer a direct and quantitative framework for characterising allergenic proteins within complex biological and food matrices. In particular, mass spectrometry-based proteomics enables highly sensitive identification and relative quantification of multiple allergens, providing insights into their abundance, stability, and variation across species and processing conditions [10,32,33]. When combined with complementary techniques such as SDS-PAGE protein separation and antibody analysis via immunoblotting, proteomic analyses can also highlight inconsistencies in antibody-based detection systems, particularly under conditions where protein structure is altered [34].

In this study, we applied a quantitative proteomics approach to characterise allergen composition across five commercially important shrimp species under both raw and heat-treated conditions. Previous transcriptomic studies have expanded the repertoire of known and putative shrimp allergens and provided valuable insight into allergen-encoding genes. However, transcript abundance alone cannot determine whether allergenic proteins are expressed, nor does it reflect their abundance, stability, or persistence following food processing. By directly quantifying allergenic proteins, proteomics provides a more biologically and analytically relevant framework for allergen monitoring and risk assessment. Specifically, we aimed to (i) quantify the abundance of eleven known shrimp allergens, (ii) assess species- and processing-dependent variation in allergen profiles, and (iii) evaluate the protein-level presence of previously identified putative allergens. Through integration of proteomic, transcriptomic, and immunological analyses, this study provides new insights into the complexity of shrimp allergen composition and supports the development of more robust allergen detection and food safety monitoring strategies.

2. Materials and methods

2.1. Shrimp species selection and protein extraction

The five shrimp species analysed in this study are the Black tiger shrimp (*P. monodon*), Vannamei shrimp (*L. vannamei* or *Penaeus vannamei*), King shrimp (*M. latisulcatus* or *Penaeus latisulcatus*), Banana shrimp (*F. merguiensis*), and Endeavour shrimp (*M. endeavouri*). Shrimp muscle tissue extraction was conducted as previously described in Kamath et al. (2013) with some modifications [17]. Thirty grams of the edible meat, which is the abdominal or tail muscle tissue, from five individual shrimps of the same species, were chopped into small pieces. Shrimp tissue was homogenised using a T10 Basic Ultra-Turrax homogenizer (IKA, Staufen, Germany) in phosphate-buffered saline (PBS) (4 mL/g of tissue) and incubated overnight (16 h) at 4 °C with shaking. To assess the impact of thermal processing on allergen composition, 30 g of shrimp muscle tissue was subjected to heat treatment. The muscle tissue was heated at 95 °C for 10 min in the same amount of PBS (4 mL/g of tissue), followed by cooling to room temperature prior to homogenisation and incubation. All extractions were done in triplicate.

All shrimp protein extracts were centrifuged at 20,000 x*g* for 30 min at 4 °C, and the supernatant (protein extract) was collected. The extracts were filtered through a 0.45 μm membrane filter to remove insoluble components. Protein concentration for each extract was estimated using the bicinchoninic acid (BCA) assay (Pierce, Rockford, Illinois, USA) following the manufacturer's instructions and a set of prediluted BSA solutions used as the protein standards (Pierce).

2.2. In-solution tryptic digestion and mass spectrometric analysis

Whole protein extracts were digested in-solution using trypsin as described by Nugraha et al. (2018) with some modifications [35]. Approximately 50 μg of protein from each sample was added into an appropriate volume of 100 mM ammonium bicarbonate (ABC) to make a total volume of 50 μL solution. Samples were then reduced using 10 mM dithiothreitol (DTT) at 60 °C for 1 h and alkylated using 25 mM iodoacetamide (IAA) for 30 min at room temperature (RT) in the dark. Samples were treated with 10 mM DTT again and incubated for 30 min at RT. Consequently, all shrimp samples were digested with porcine trypsin (Thermo) dissolved in 1 mM hydrochloric acid. The trypsin to protein ratio was 1:50 (*w*/w). Tryptic digest was conducted at 37 °C and incubated for 16 h. The digested proteins were acidified using 0.5% formic acid. Peptides were desalted for mass spectrometry analysis using Bond Elut OMIX SCX pipette tips (Agilent) following the manufacturer's protocol, concentrated on SpeedVac before subjecting samples to LC-MS/MS analysis. The LC-MS/MS was carried out at the Melbourne Mass Spectrometry and Proteomics Facility at the Bio21 Institute (The University of Melbourne, Australia) using an LTQ Orbitrap Elite (Thermo Scientific) with a nanoESI interface and Ultimate 3000 RSLC nanoHPLC (Dionex Ultimate 3000). The tryptic digest and LC-MS/MS analysis were conducted for each shrimp species in three replicates.

2.3. Annotation of transcriptomes

De novo assembled transcriptomes of all five analysed shrimp species (3 replicates each, making a total of 15 transcriptomes) [15,36] were annotated using the Trinotate program (version 4.0). Prior to annotation, transcriptomes were subjected to the TransDecoder program (version 5.7.1) to extract long open reading frames (ORFs) and predict the likely coding regions. TransDecoder was applied using the command line interface and appropriate command lines from the updated GitHub protocol (https://github.com/TransDecoder/TransDecoder/wiki). Subsequently, SQLite (version 3.40), a necessary database integration software for Trinotate annotation, which compiles data resources from the most recent version of UniProt and Pfam, was downloaded from http://www.sqlite.org/. After compiling the database required for Trinotate using SQLite, Trinity transcriptome files and the corresponding TransDecoder files were both utilised in running the Trinotate software according to the author's protocols and command lines within the command line interface (https://github.com/griffithlab/rnaseq_tutorial/wiki/Trinotate-Functional-Annotation) [37]. Annotated transcriptomes were renamed by adding prefixes which reflected the contig ID and shrimp species name.

2.4. In-house database establishment for mass spectrometric analysis

For each shrimp species, the 3 replicates of annotated transcriptomes were compiled into one data file and added to a database of Penaeus proteins sourced from NCBI GenBank

($n = 172{,}452$ proteins). This combined dataset was used to generate species-specific protein databases for mass spectrometry searches. This approach substantially improves protein identification coverage compared with generic reference databases, as species-specific databases reduce false-negative identifications arising from sequence divergence between the sample organism and the reference proteome.

2.5. Protein identification and quantification using in-house curated database

Mass spectrometry data files for each shrimp species were analysed with the MaxQuant (version 2.6) search engine using the species-specific in-house curated protein database. The 3 technical replicates for each species, both raw and heated, were analysed using MaxQuant simultaneously, and the search parameters included 'oxidation' and 'deamidation' as variable modifications and 'carbamidomethyl' as the fixed modification. The analysis also included label-free quantification (LFQ) expressed in intensity-based absolute quantification (iBAQ) values, and the results were filtered to contain at least one unique peptide. iBAQ values of MaxQuant search results were totalled, and iBAQ percentages (iBAQ%) for each identified protein were calculated for relative abundance analysis. The final iBAQ% (relative abundance) for each identified protein was the average calculated from the 3 technical replicates. Relative abundance of known shrimp allergens, unreported putative allergens and non-allergenic proteins for each species was compared for interspecies differences. The known shrimp allergens, confirmed by AllerCatPro 2.0 analysis [38], include 11 proteins, namely, tropomyosin, arginine kinase (AK), myosin light chain-2 (MLC2), sarcoplasmic calcium-binding protein (SCP), myosin light chain-1 (MLC1), troponin C, troponin I, hemocyanin, triosephosphate isomerase, filamin C, and fatty acid-binding protein (**Supp. Table A.2**). The "unreported putative allergens in shrimps" include 7 proteins previously identified by Karnaneedi et al. (2021) [15], heat shock protein 70, alpha-tubulin, chymotrypsin, enolase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, and cyclophilin (**Supp. Table A.3**). Individual allergen abundance was also compared between different shrimp species. Statistical comparisons of allergen abundance between species were performed using one-way ANOVA followed by Tukey's multiple comparison test, and two-group comparisons (raw versus heat-treated) using Student's *t*-test, in GraphPad Prism (version 11). Tukey's post hoc test was used to account for multiple pairwise comparisons and control the family-wise error rate. Statistical significance was defined as $p < 0.05$.

2.6. SDS-PAGE and immunoblot analysis

Protein extracts (raw and heat-treated) were separated by SDS-PAGE under reducing conditions using standard protocols. Following electrophoresis, proteins were transferred onto membranes for immunoblot analysis. Membranes were probed with allergen-specific primary antibodies targeting tropomyosin and MLC2, followed by appropriate secondary antibodies conjugated with IR-Dye. Detection was performed using Odyssey CLx (LiCor Biosciences), and band intensities were visually assessed to compare allergen presence and relative abundance across species and treatment conditions. This study did not involve human subjects or patient-derived biological materials. All shrimp specimens were obtained from commercial sources.

2.7. Proteo-transcriptomic correlation analysis

To evaluate the relationship between transcriptomic and proteomic abundance of allergenic proteins, transcripts per million (TPM) values obtained from previous transcriptomic analyses [15] were compared with corresponding proteomic iBAQ% values for known allergens. Correlation analysis was performed separately for each shrimp species. The strength of association between mRNA (TPM) and protein (iBAQ%) abundance was assessed using both the Pearson correlation coefficient (with R^2 reported) and Spearman's rank correlation coefficient (ρ), the latter to accommodate the non-normal, compositional nature of the data. Only allergens with corresponding transcriptomic and proteomic abundance data available across all analysed species were included in the correlation analysis.

3. Results

3.1. Proteome coverage across five shrimp species

A comprehensive proteomic analysis was performed across five shrimp species (*Penaeus monodon, L. vannamei, M. latisulcatus, F. merguiensis, and M. endeavouri*), resulting in the identification of 457–551 proteins per species in the raw extracts, and 292–427 proteins in the

heat-treated shrimps (**Supp. Table A.4**). Across all species, allergenic proteins constituted a substantial fraction of the total proteome. Both known (n = 11) and putative (n = 7) shrimp allergens collectively contributed a considerable proportion of total protein abundance (57–66%), indicating that allergenic proteins represent a major component of the shrimp proteome rather than minor constituents (Fig. 1). Heat treatment substantially reduced protein recovery from mass spectrometry analysis but resulted in a higher fraction of known allergens within the whole proteome (Fig. 1, **Supp. Table A.4**).

3.2. Quantitative variability of known allergens across species (raw extracts)

All eleven known shrimp allergens (**Supp. Table A.2**) were detected across the five shrimp species; however, quantitative proteomic analysis of raw shrimp extracts revealed interspecies variability in allergen abundance (Fig. 2, Fig. 3). AK was consistently the most abundant allergen across all species except L. *vannamei* in the raw state, contributing up to ~34% of the total allergen fraction in *P. monodon*. In contrast, tropomyosin – commonly targeted in allergen detection assays – was present at relatively low levels across all species (0.14–2.59%) (Fig. 2). This finding directly challenges the widespread assumption that tropomyosin is the dominant protein in crustacean muscle extracts and has important implications for allergen detection assays calibrated against tropomyosin alone. Other allergens exhibited strong species-specific variability. For example, MLC2 was markedly enriched in L. *vannamei* (~17%) but remained comparatively low in other species, while SCP showed elevated abundance in *M. latisulcatus* (Fig. 3). These results demonstrate that allergen composition in raw shrimp is highly species-dependent and not dominated by a single allergen, with enzymatic proteins such as AK representing major contributors under non-processed conditions.

3.3. Effect of heat treatment on allergen abundance and stability

Thermal processing resulted in substantial alterations in allergen composition across all five shrimp species. A clear processing-induced redistribution of allergen abundance was observed, with shifts in the

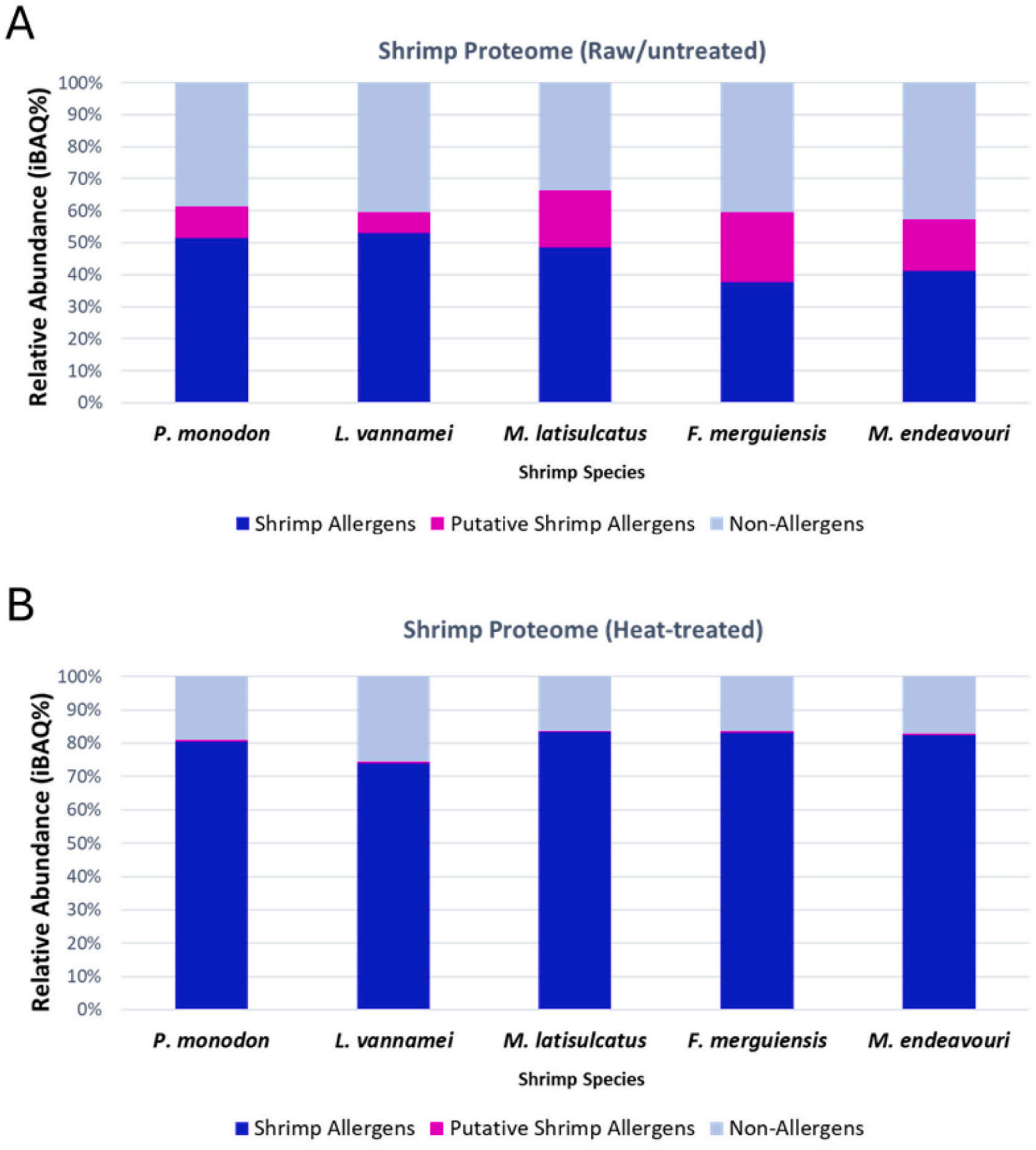

Fig. 1. Relative abundance (iBAQ%) of three classifications: eleven known shrimp allergens (blue), seven putative shrimp allergens (pink), and non-allergens (grey) within the proteome of five shrimp species. (A) Raw/untreated extracts, (B) Heat-treated extracts. iBAQ% values were obtained from MaxQuant analysis.

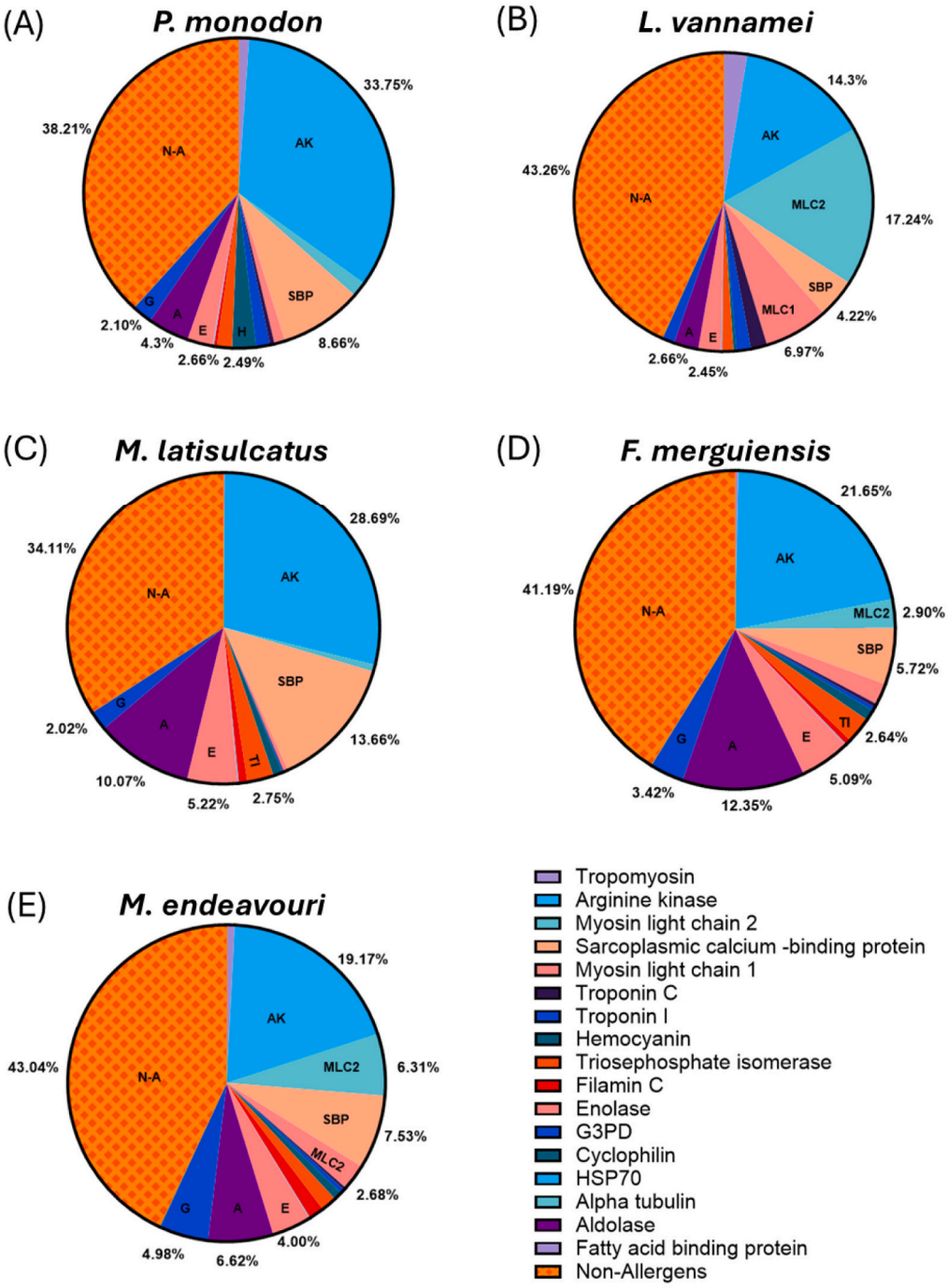

Fig. 2. Protein distribution of allergens, putative allergens and non-allergens in raw protein extracts of the five shrimp species. (A) *P. monodon*, (B) *L. vannamei*, (C) *M. latisulcatus*, (D) *F. merguiensis*, (E) *M. endeavouri*.

relative dominance of specific proteins (Fig. 4). While AK was highly abundant in raw extracts, its relative contribution decreased following heat treatment across multiple species (Fig. 5). In contrast, tropomyosin and MLC2 exhibited relative enrichment in heated extracts, indicating increased persistence and/or detectability under thermal conditions (Fig. 5). This shift suggests that structural and muscle-associated proteins are more resistant to heat-induced denaturation compared to enzymatic allergens. Additional allergens, including SCP, displayed variable thermal responses depending on species, further highlighting the complexity of processing effects. Overall, these findings demonstrate that heat treatment significantly alters allergen composition, resulting in distinct profiles between raw and processed shrimp and highlighting the influence of protein-specific physicochemical properties on allergen stability.

3.4. Abundance and validation of putative allergens

Several putative allergens previously identified at the transcriptomic level were confirmed at the protein level, including aldolase, enolase, and glyceraldehyde-3-phosphate dehydrogenase (G3PD) (**Supp. Table A.3**). These proteins were consistently detected across five shrimp species and, in some cases, contributed substantially to the allergen fraction, especially in the raw extracts (Fig. 1**.A**). Putative allergens exhibited substantial variability in both presence and abundance across

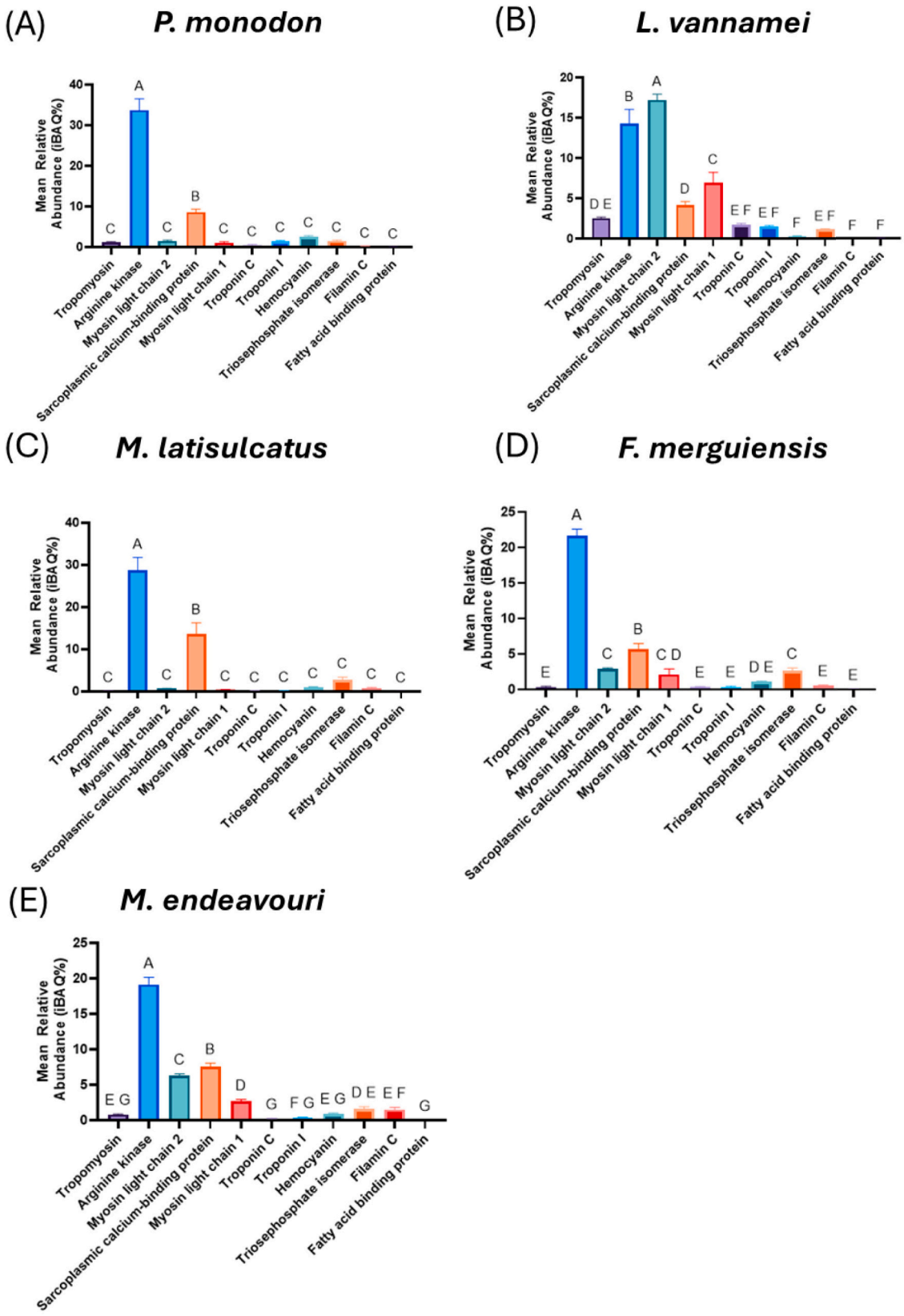

Fig. 3. Mean relative abundance (iBAQ %) of known shrimp allergens in raw protein extracts of the five shrimp species. (A) *P. monodon*, (B) *L. vannamei*, (C) *M. latisulcatus*, (D) *F. merguiensis*, (E) *M. endeavouri*. Statistical significance: different letters indicate $p < 0.05$.

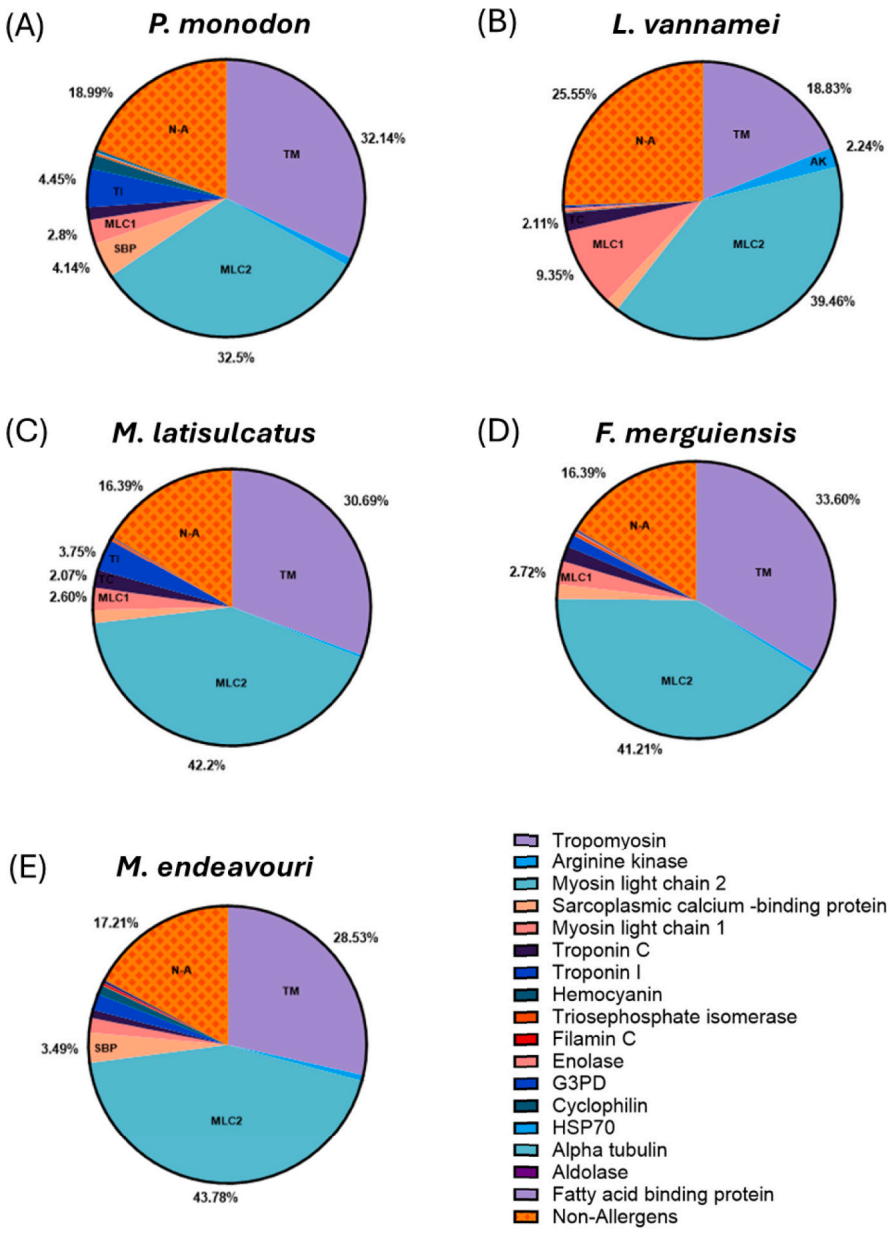

Fig. 4. Protein distribution of allergens, putative allergens and non-allergens in heat-treated protein extracts of the five shrimp species. (A) *P. monodon*, (B) *L. vannamei*, (C) *M. latisulcatus*, (D) *F. merguiensis*, (E) *M. endeavouri*.

species. Among these, aldolase and enolase were consistently detected at relatively high levels, particularly in *M. latisulcatus* and *F. merguiensis*, where aldolase contributed up to ~12% of the allergen fraction (Fig. 6). In contrast, proteins such as HSP70, cyclophilin, and alpha tubulin were detected at very low abundance (<0.1%) across all species. Chymotrypsin was absent in several species (Fig. 6). This selective validation indicates that transcriptomic predictions do not uniformly translate into detectable protein expression, highlighting differences between gene expression and protein-level presence. Heat treatment influenced the relative abundance of several putative allergens, with some proteins showing apparent increases in relative contribution (Fig. 7), likely reflecting redistribution effects following the reduction of other protein fractions or changes in detectability after denaturation.

3.5. Immunoblot analysis reflects allergen trends but highlights limitations

SDS-PAGE and immunoblot analysis using anti-tropomyosin and anti-MLC2 antibodies qualitatively reflected species- and processing-dependent variation observed in the proteomic dataset (Fig. 8). In raw extracts, differences in band intensity were observed across species, reflecting variability in allergen abundance (Fig. 8**.A**). Consistent with the proteomic analysis, MLC2 showed stronger band intensity in L. *vannamei*, where it was identified as a relatively abundant allergen, while tropomyosin exhibited a weaker signal overall and was lowest in *M. latisulcatus* (Fig. 8**.B, 8.C**). Following heat treatment, both tropomyosin and MLC2 displayed stronger and more consistent band intensities across species, in agreement with their relative enrichment in heated samples observed in the proteomic analysis (Fig. 8**.D-F**).

Despite this general concordance, notable inconsistencies in band intensity and detectability were evident across species and treatment conditions. In some instances, variations in signal did not proportionally reflect differences in protein abundance, suggesting that antibody recognition may be influenced by factors such as epitope accessibility, protein conformation, and processing-induced structural changes. These findings indicate that while immunoblotting can capture broad trends in allergen stability and enrichment, it lacks the resolution required for

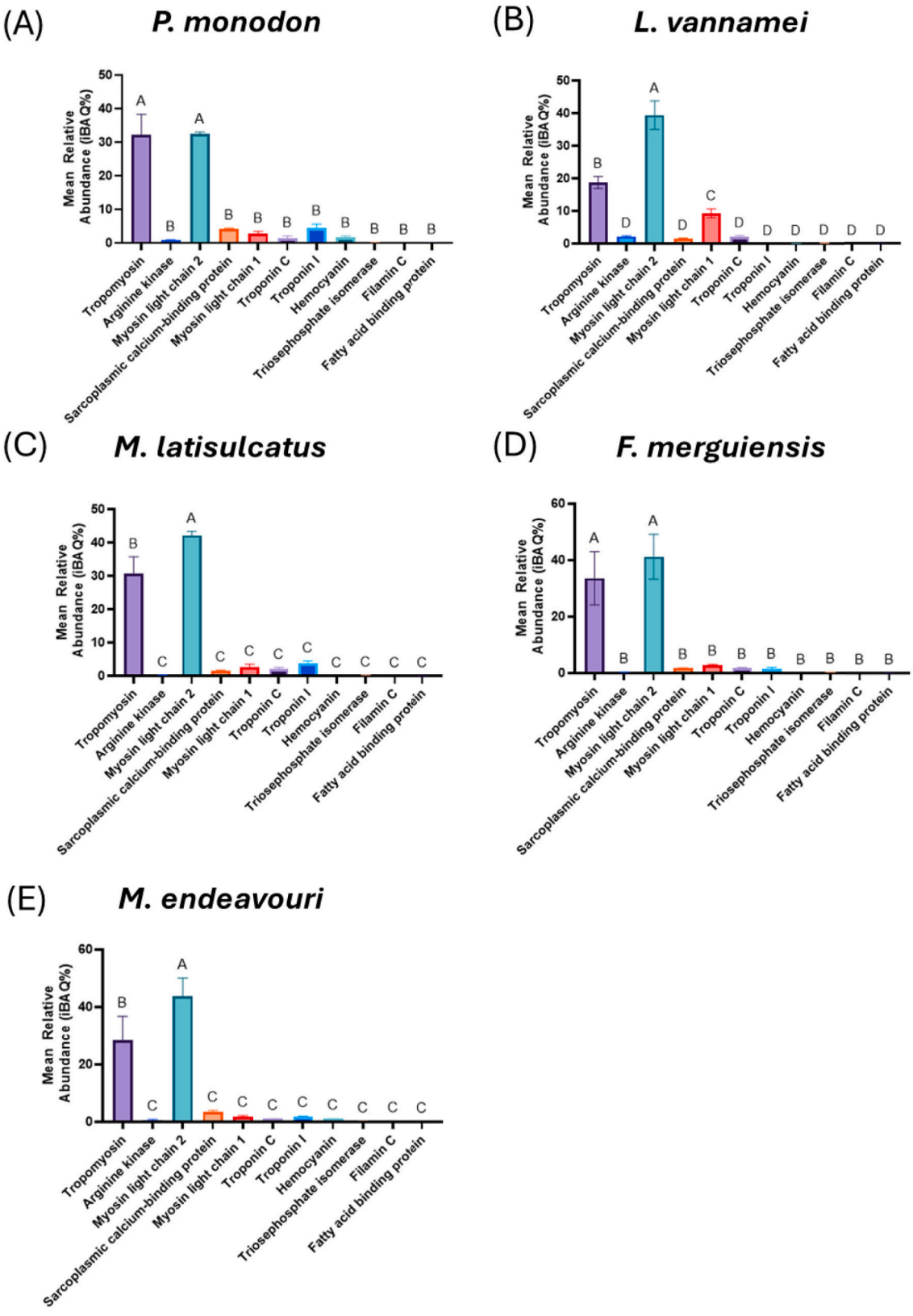

Fig. 5. Mean relative abundance (iBAQ %) of known shrimp allergens in heat-treated protein extracts of the five shrimp species. (A) *P. monodon*, (B) *L. vannamei*, (C) *M. latisulcatus*, (D) *F. merguiensis*, (E) *M. endeavouri*. Statistical significance: different letters indicate $p < 0.05$.

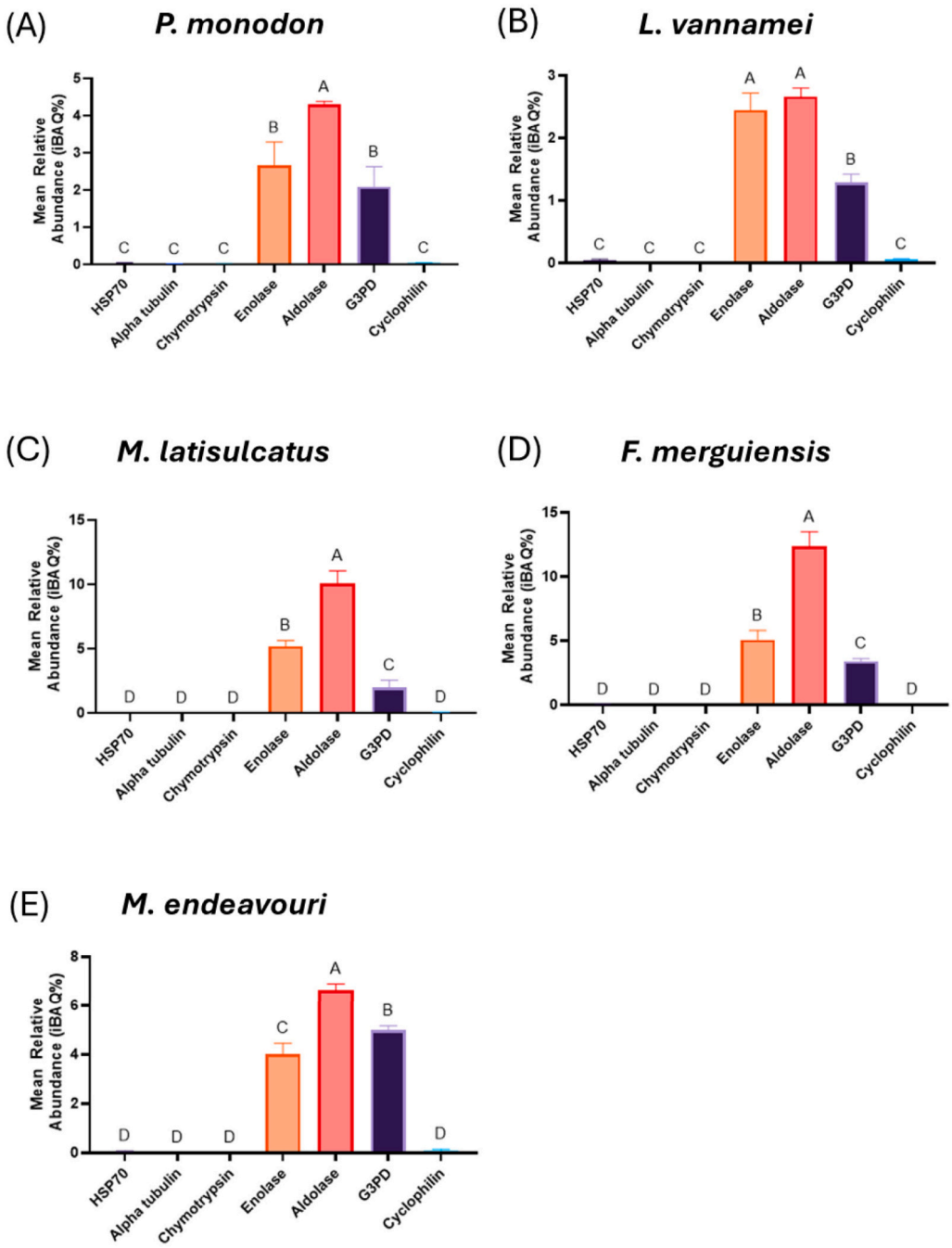

Fig. 6. Mean relative abundance (iBAQ %) of putative shrimp allergens in raw protein extracts of the five shrimp species. (A) *P. monodon*, (B) *L. vannamei*, (C) *M. latisulcatus*, (D) *F. merguiensis*, (E) *M. endeavouri*. Statistical significance: different letters indicate $p < 0.05$.

accurate quantitative comparison across species and processing conditions.

3.6. Limited correlation between transcriptomic and proteomic abundance

Correlation strength varied markedly across species, ranging from weak ($R^2 = 0.16$, *M. endeavouri*) to strong ($R^2 = 0.79$, *P. monodon*), suggesting that proteo-transcriptomic concordance is itself species-dependent (Fig. 9). Comparing between the allergens, while some showed moderate agreement between mRNA and protein levels, others, including tropomyosin, AK and MLC1, exhibited clear discrepancies, with high transcript abundance not corresponding to high protein abundance and vice versa (Fig. 9). This lack of concordance highlights the limitations of transcriptomic data in predicting functional allergen abundance and highlights the importance of direct protein-level quantification for accurate allergen profiling.

4. Discussion

Quantitative proteomic analysis of five commercially relevant shrimp species generated four principal insights. Allergen abundance differed markedly between species, indicating that shrimp allergenic protein profiles cannot be considered uniform across taxonomically related shrimp. In addition, thermal processing substantially reshaped

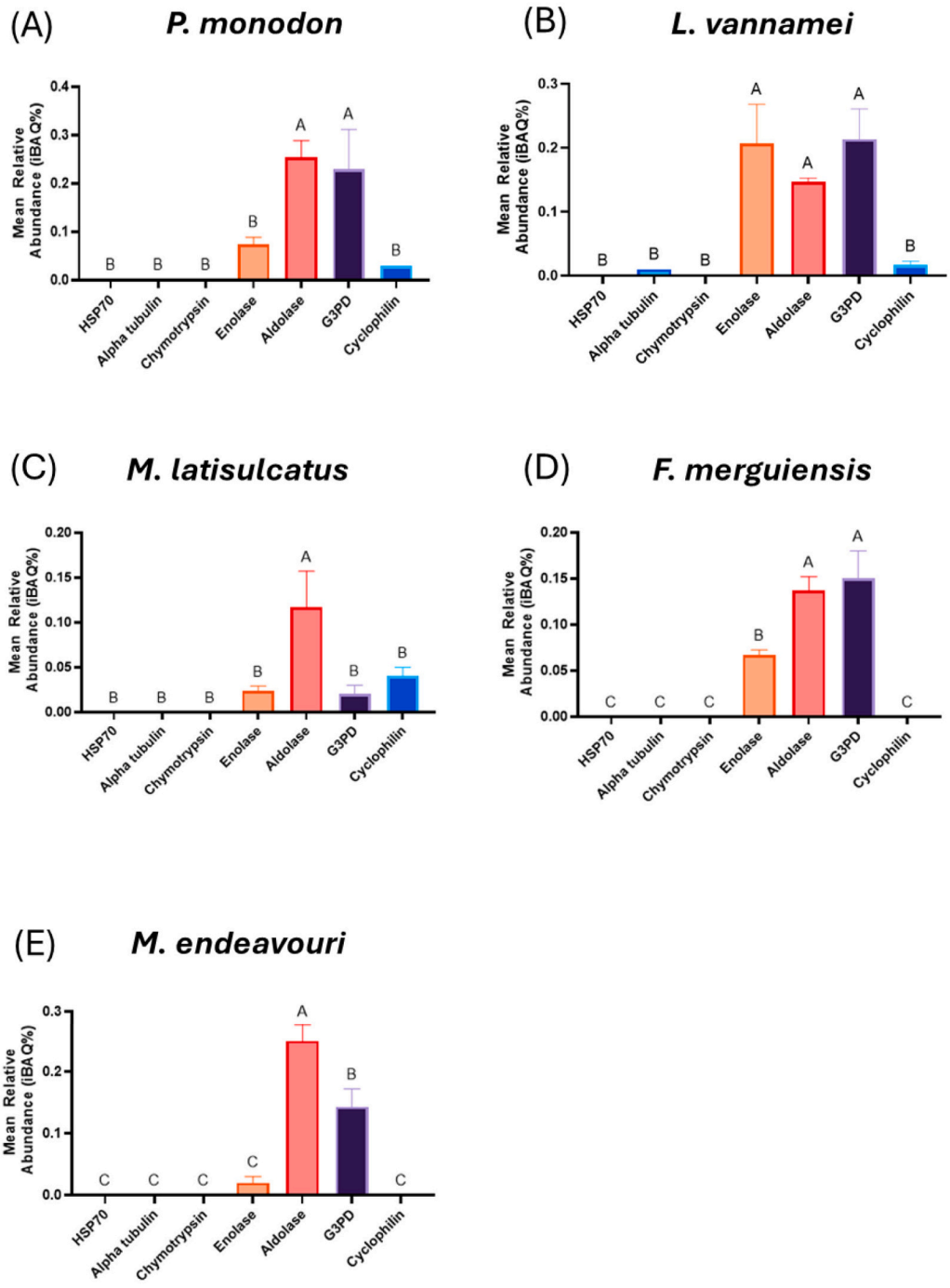

Fig. 7. Mean relative abundance (iBAQ %) of putative shrimp allergens in heat-treated protein extracts of the five shrimp species. (A) *P. monodon*, (B) *L. vannamei*, (C) *M. latisulcatus*, (D) *F. merguiensis*, (E) *M. endeavouri*. Statistical significance: different letters indicate $p < 0.05$.

allergen profiles, altering the relative dominance of specific proteins. Transcript (mRNA) abundance showed limited correlation with protein-level quantification, reinforcing the need for direct proteomic assessment. Finally, several previously identified putative allergens were confirmed at the protein level, supporting continued expansion of the shrimp allergome. Collectively, these findings have direct implications for allergen monitoring, food safety, and evidence-based risk assessment.

A key outcome of this study was the marked interspecies variability observed in both known and putative shrimp allergen abundance. Although the five species examined are all widely consumed shrimp, their allergen profiles were not equivalent. Such variation is highly relevant to food systems, where shrimp ingredients are often labelled generically despite the use of different species depending on geography, seasonality, supply chain availability, or product cost [8]. From a risk assessment perspective, these findings suggest that allergen exposure may vary according to species composition, particularly in products containing mixed crustacean ingredients. Current approaches that assume interchangeability between shrimp species may therefore oversimplify the true risk of shrimp allergy.

Tropomyosin is widely recognised as the major shrimp allergen and remains a clinically important pan-allergen due to its strong IgE

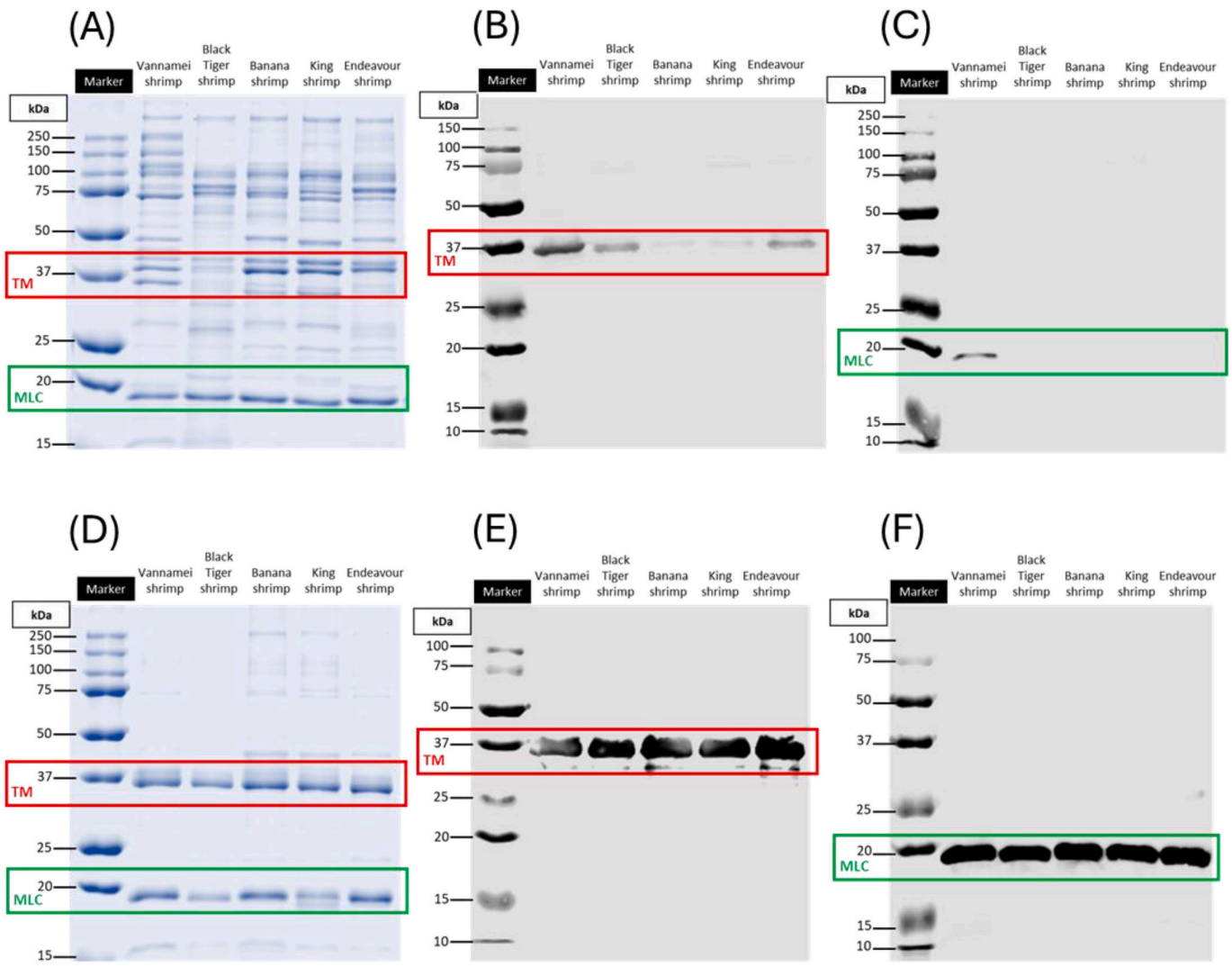

Fig. 8. SDS-PAGE and immunoblot using anti-Tropomyosin antibody and anti-MLC2 antibody on five shrimp species; (A-C) Raw extracts, (A) SDS-PAGE, (B) anti-tropomyosin (TM) antibody, (C) anti-MLC2 antibody; (D-F) Heated extracts, (D) SDS-PAGE, (E) anti-TM antibody, (F) anti-MLC2 antibody. TM bands are boxed in red, and MLC2 bands are boxed in green.

reactivity and cross-reactivity with other invertebrates, including mites and insects [4,13,39–44]. However, the present proteomic data demonstrated that tropomyosin was not consistently the most abundant allergen in raw extracts. Instead, AK represented the dominant allergenic protein fraction in several species, while proteins such as MLC2 and SCP also contributed substantially depending on species. These findings emphasise an important distinction between clinical relevance and abundance. Certain proteins may be highly immunologically significant despite low abundance, while highly abundant proteins may also contribute significantly to allergen load and sensitisation risk [45,46]. Therefore, focusing analytical detection solely on tropomyosin may not fully capture the broader allergen composition of shrimp products.

Tropomyosin and AK are established cross-reactive invertebrate allergens with relevance beyond food exposure alone [4,13,40,41,47,48]. Shrimp tropomyosin shares strong homology with aeroallergens such as house dust mite Der p 10 and cockroach Bla g 7, while shrimp AK cross-reacts with mite Der p 20 [49–51]. This relationship is clinically significant, as individuals sensitised to house dust mite may experience allergic reactions upon first shrimp exposure through pre-existing IgE cross-recognition. In this context, the species-dependent variation in tropomyosin and AK abundance observed in the present study suggests that the magnitude of clinical risk may also vary between shrimp species and processing states. These findings further indicate that shrimp allergen composition may influence sensitisation patterns and complicate the interpretation of diagnostic IgE testing in polysensitised individuals.

Thermal processing produced pronounced changes in allergen composition, demonstrating that allergen profiles in cooked shrimp differ substantially from those of their raw material. A consistent trend was the relative reduction of AK following heating, whereas tropomyosin and MLC2 became proportionally enriched in heated extracts. This likely reflects differences in protein physicochemical properties, including thermal denaturation, aggregation, solubility, and proteolytic susceptibility. Structural muscle-associated proteins, such as tropomyosin, are known to possess considerable heat stability, whereas enzymatic proteins may be more vulnerable to structural disruption during cooking, as observed in several studies [18,52,53]. From a food science perspective, this finding is highly relevant because most shrimp is consumed after boiling, steaming, frying or roasting, or as an ingredient in processed foods.

These processing-induced changes have important implications for allergen detection technologies. Many commercially available immunoassays are developed using selected reference materials or purified proteins, often centred on tropomyosin [10]. While such strategies may perform well for heated products enriched in heat-stable allergens, they

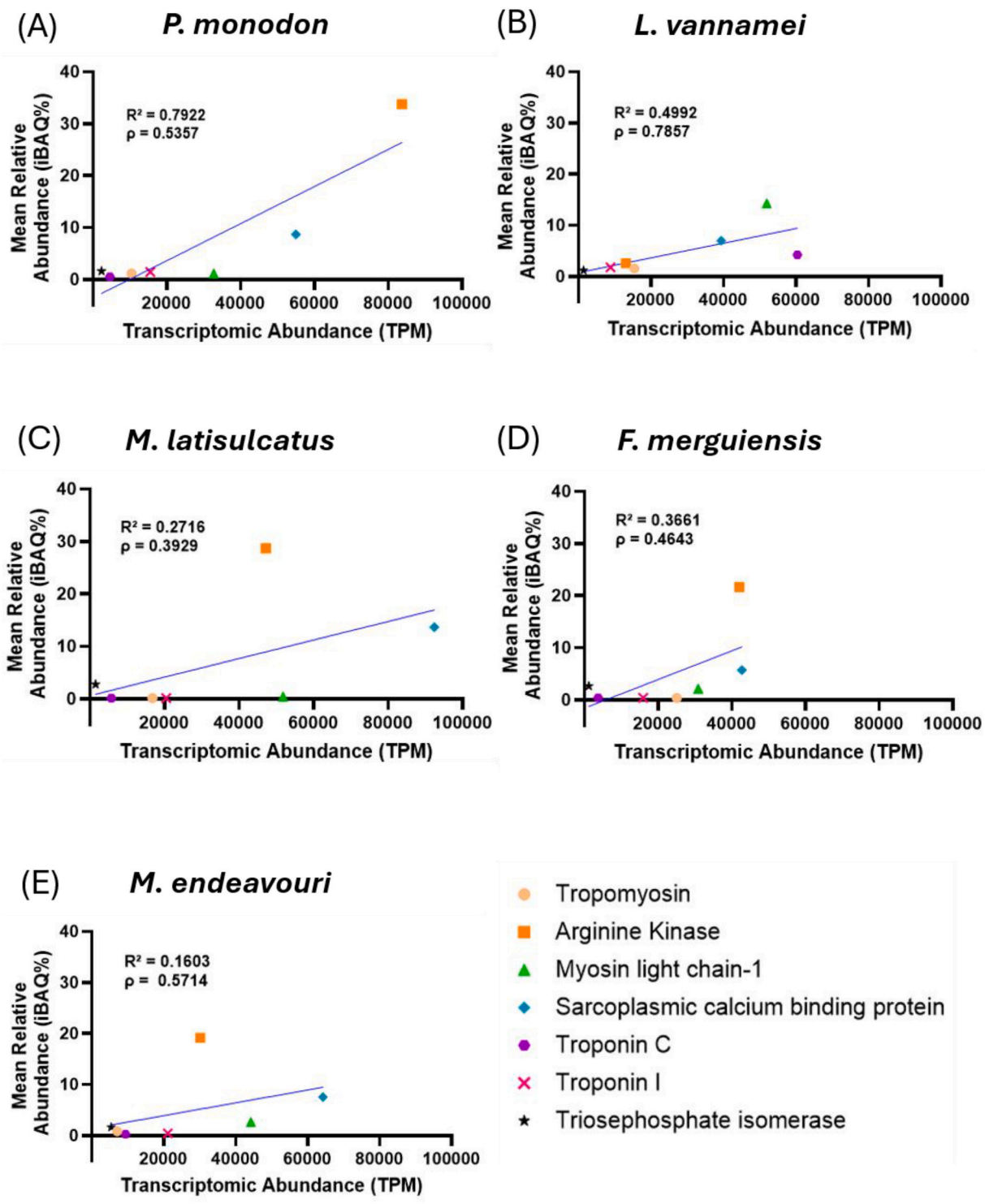

Fig. 9. Correlation between mean relative abundance (iBAQ%) and transcripts per million (TPM) for seven of the eleven known allergens across the five shrimp species. (A) *P. monodon*, (B) *L. vannamei*, (C) *M. latisulcatus*, (D) *F. merguiensis*, (E) *M. endeavouri*. Statistical analysis used the Pearson correlation coefficient to determine R-squared values and Spearman's rank correlation coefficient (ρ) to account for the non-normal, compositional nature of the data. Note: Only 7 allergens with corresponding transcriptomic and proteomic abundance data available across all analysed species were included in the correlation analysis.

may underestimate total allergen burden in raw or minimally processed products where other proteins, such as AK, are more prominent. Conversely, assays targeting heat-labile proteins may underperform in cooked foods. This highlights the need for analytical validation across multiple species and processing states rather than reliance on a single allergen component, such as tropomyosin.

Immunoblot analysis using anti-tropomyosin and anti-MLC2 antibodies broadly supported the proteomic findings. In raw extracts, a stronger MLC2 signal in *Litopenaeus vannamei* was consistent with its elevated quantitative abundance, while a lower tropomyosin signal in *Melicertus latisulcatus* aligned with proteomic measurements. Following heat treatment, stronger and more consistent tropomyosin and MLC2

detection across species reflected their relative enrichment in the proteomic dataset. This has previously been observed in several studies which examined the effects of heat treatment on allergen profile [9,18,34,54]. However, band intensity was not always proportional to measured proteomic abundance, likely due to biological or technical differences, including epitope accessibility, protein conformation, transfer efficiency, and extraction buffer effects [35,55–57]. These results indicate that antibody-based methods remain valuable for targeted screening but lack the multiplexed quantitative resolution achievable by mass spectrometry.

An additional important finding was the limited concordance between transcriptomic and proteomic protein abundance. Although previous transcriptomic analyses were highly effective for identifying known and candidate allergens [15], mRNA levels did not consistently predict protein abundance across species. This finding is consistent with several other studies in the literature [31,58,59]. The postulated reason is that the translation efficiency, protein turnover, post-translational modification, intracellular compartmentalisation, and extraction behaviour all influence measured protein levels independently of transcript abundance [29,30,58,59]. These data reinforce that transcriptomics is highly valuable for allergen discovery and allergen repertoire mapping, but it should not be interpreted as a substitute for food-level allergen quantification.

The present study also confirmed several putative allergens at the protein level, particularly aldolase and enolase, which were detected at significant abundance in multiple species. Putative allergens were defined based on prior transcriptomic identification rather than in silico structural prediction, providing an orthogonal validation approach. A subset of these proteins has also been implicated as allergens in other invertebrate or food sources and may represent clinically relevant but under-recognised shrimp allergens [4,40,60,61]. Their consistent presence supports continued investigation using patient IgE binding studies, basophil activation assays, and component-resolved diagnostic platforms. Expansion of shrimp allergen panels beyond historically dominant allergens may improve diagnostic precision for sensitised individuals with heterogeneous profiles of IgE antibodies.

This study has several strengths, including the comparative analysis of five commercially relevant shrimp species, assessment of both raw and heat-treated extracts, and integration of proteomic, transcriptomic, and immunological datasets. Limitations include the use of relative rather than absolute quantification, the absence of direct patient IgE reactivity testing in the current workflow, and the use of a standardised heat-treatment model (95 °C for 10 min in PBS). While this approach provides a reproducible framework for assessing thermal effects on allergen composition, it does not fully replicate the diversity of domestic and industrial processing conditions, including variations in cooking method, duration, temperature, food matrix, and water activity. Consequently, the observed changes in allergen abundance should be interpreted within the context of this experimental model. Future studies investigating additional processing conditions and food matrices would further improve understanding of allergen behaviour in real-world food systems; and incorporating targeted absolute quantification and clinical sera validation would further strengthen translational relevance.

5. Conclusion

In conclusion, quantitative proteomic analysis demonstrated that shrimp allergen composition varies substantially between species and is significantly altered by heat processing. While all eleven known shrimp allergens were detected across the five analysed species, their relative abundance differed markedly, with AK dominating raw extracts and heat-stable allergens such as tropomyosin and MLC2 becoming relatively enriched following thermal treatment. Several putative allergens previously identified through transcriptomic analyses, including aldolase and enolase, have now been confirmed at the protein level. Furthermore, the limited concordance demonstrated between transcriptomic and proteomic abundance highlights the importance of direct protein-level assessment for accurate allergen characterisation. Collectively, these findings demonstrate that shrimp allergen profiles cannot be adequately represented by a single species or allergen marker and emphasise the value of quantitative proteomics for comprehensive allergen monitoring, improved detection strategies, and evidence-based risk assessment of shrimp-containing foods.

CRediT authorship contribution statement

Shaymaviswanathan Karnaneedi: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thomson Poly:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Sahel Heidari:** Writing – review & editing, Formal analysis. **Emily Jerry:** Writing – review & editing, Formal analysis. **Janitha Iddagoda:** Writing – review & editing, Validation, Methodology. **Vachiranee Limviphuvadh:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Sebastian Maurer-Stroh:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation. **Thimo Ruethers:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roni Nugraha:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andreas Ludwig Lopata:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

ChatGPT 5.5 (OpenAI) and Claude Opus 4.7 were used solely to improve the language, grammar, and readability of the manuscript. These tools were not used for study design, data analysis, statistical testing, data interpretation, figure preparation or scientific decision-making. Interpretations of all quantitative results were made and independently verified by the authors, who reviewed and edited all content and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2026.153942.

Data availability

The raw LC-MS/MS data for all three replicates of the five shrimp species, under both raw and heat-processed conditions, are openly available in Zenodo at https://doi.org/10.5281/zenodo.21451942.

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