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LETTER

Commercial shellfish skin prick test extracts show critical variability in allergen repertoire

To the Editor,

Crustacean and mollusc (shellfish) allergy affects up to 3% of the general population, is usually lifelong and commonly triggers anaphylaxis.[1] Allergen repertoire diversity among hundreds of edible shellfish species worldwide is poorly reflected in available in vivo and in vitro diagnostic tools for shellfish allergy. Skin prick testing (SPT) is often the preferred first-line diagnostic approach. However, widely utilized commercial SPT extracts are generally not standardized, limiting the diagnostic value of results.[2] Asero et al. reported a heterogeneous abundance of three shellfish allergens in five commercial crustacean SPT extracts, resulting in 32 clinical profiles among 157 shrimp-allergic patients.[3] In 2019, we demonstrated considerable variability in allergen repertoire and IgE-binding for 27 commercial fish SPT extracts.[4] We now report an even greater, critical variability for 11 commercial crustacean and five mollusc SPT extracts, utilizing biochemical and immunological methods and mass spectrometry (see Appendix S1 for methodology and Table S1 for allergen extract details).

The total protein content varied up to 14-fold (0.1–1.4 mg/mL) in five shrimp (at least three different species), four crab, two lobster, two oyster and three clam/scallop extracts from six different manufacturers denoted by 'Species'-1 to −6 (Figure 1A). In the SDS-PAGE profiles, 1–15 distinct bands were visible (Figure 1B). Applying semi-quantitative immunoblotting using shrimp allergen-specific antibodies, up to four important allergens were detected in all extracts except Shrimp-5 and Oyster-2. Multiple bands of the major allergen tropomyosin were recognized with varying intensity in all except these two extracts (Figure 1C). The strongest signals were observed to extracts from manufacturer 1 and Shrimp-6, which also contained the highest total protein content. Heat-stable sarcoplasmic calcium-binding protein was detected strongly in 4/5 shrimp extracts and very weakly in Lobster-2 but in no mollusc extract due to antibody specificity to crustacean (Figure 1D). Heat-labile arginine kinase was detected in only three shrimp and three clam/scallop extracts (Figure 1E). Hemocyanin, which is most abundant in the haemolymph, was detected in 5/11 crustacean but no mollusc extract (Figure 1F).

Quantitative mass spectrometric analyses confirmed the observed patterns in allergen repertoires and revealed high variations in the relative abundance of all 12 shellfish allergens registered with the IUIS/WHO (www.allergen.org), accounting for 29–90% of all proteins (Figure 2A). However, heat-labile arginine kinase was detected in only two shrimp extracts by immunoblotting but in all shrimp extracts by mass spectrometry (3–11%), suggesting that some proteins may have been degraded into smaller fragments in some extracts. Heat-stable tropomyosin or sarcoplasmic calcium-binding protein was the most abundant allergen in all but one extract (collectively 15–84%), indicating possible heat treatment with subsequent removal of insoluble proteins. In Shrimp-5, hemocyanin was the most abundant protein at 14%.

Overall, extracts from manufacturer 1 (Greer USA) contained the highest total protein content and the most comprehensive allergen repertoire. Utilizing serum from five shellfish-allergic subjects, IgE-binding patterns to 14/16 extracts underlined high variance in anticipated in vitro and in vivo potency (Figure 2B, see Table S2 for subject details). IgE binding was observed in bands of all molecular weights and most prominently, tropomyosin bands. The strongest signals were to Shrimp-6 and Crab-1 followed by Shrimp-1 and Lobster-1. Critically, no IgE binding to Shrimp-5 and Clam-2 was observed, likely because of low protein and allergen content (Figure S1), suggesting a high risk of false-negative SPT results with these extracts. Signals to mollusc extracts were weaker as compared to crustacean extracts, which could be a reflection of the sensitization patterns of the subjects.

In conclusion, some commercial crustacean and mollusc SPT extracts lack sufficient amount and diversity of important shellfish allergens, hampering their utility for in vivo diagnosis. Clinicians currently cannot distinguish reliable extracts and may require SPT with fresh foods or in-house extracts to reflect regional species diversity, which can increase the risk of inducing reactions during testing.[5] Standardization of allergen extracts is urgently needed to improve the accuracy and reliability of SPT. Moreover, the development of region-specific recombinant allergen extracts with known quantities of clinically well-characterized allergen components, as suggested by Valenta et al., is likely necessary to achieve considerable improvements.[6] However, optimized in vitro component-resolved diagnostic tools might be beneficial and enable better predictions regarding cross-sensitisation, especially for individuals sensitized to only one shellfish allergen.[1]

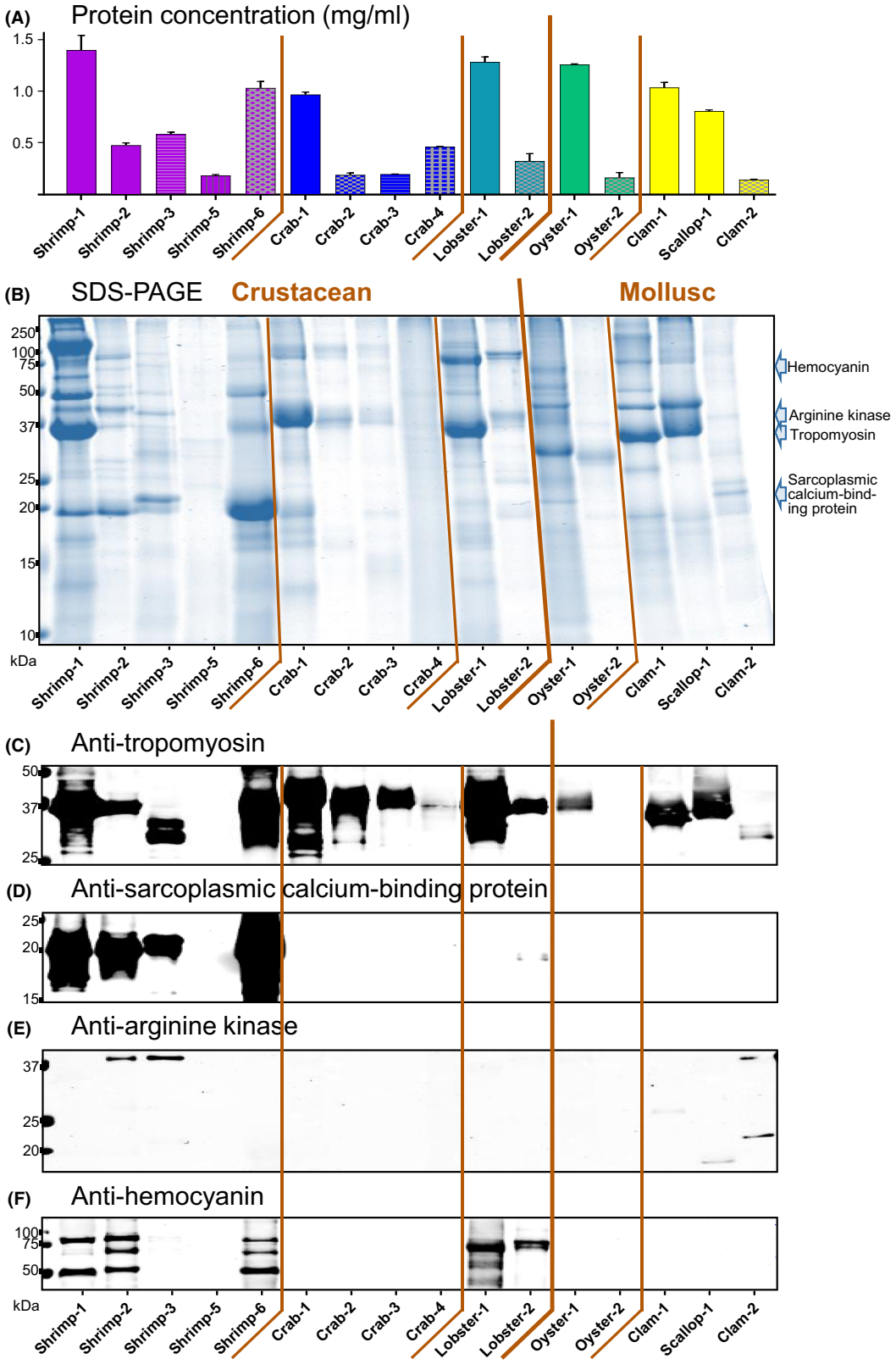

FIGURE 1 Protein concentration, SDS-PAGE profile and shellfish allergen-specific antibody reactivity of 11 crustacean and five mollusc SPT extracts from six different manufacturers. Protein concentrations were determined and the mean values from three replicates with the corresponding standard deviation are shown (A). The extracts were further analysed by SDS-PAGE (B) and immunoblots using antibodies raised against shellfish allergens tropomyosin (C), sarcoplasmic calcium-binding protein (D), arginine kinase (E) and hemocyanin (F) from shrimp. The expected molecular weight of these four allergens is indicated in A based on.[1] Different manufacturers are denoted by −1 to −6 and listed in Table S1, along with further species details.

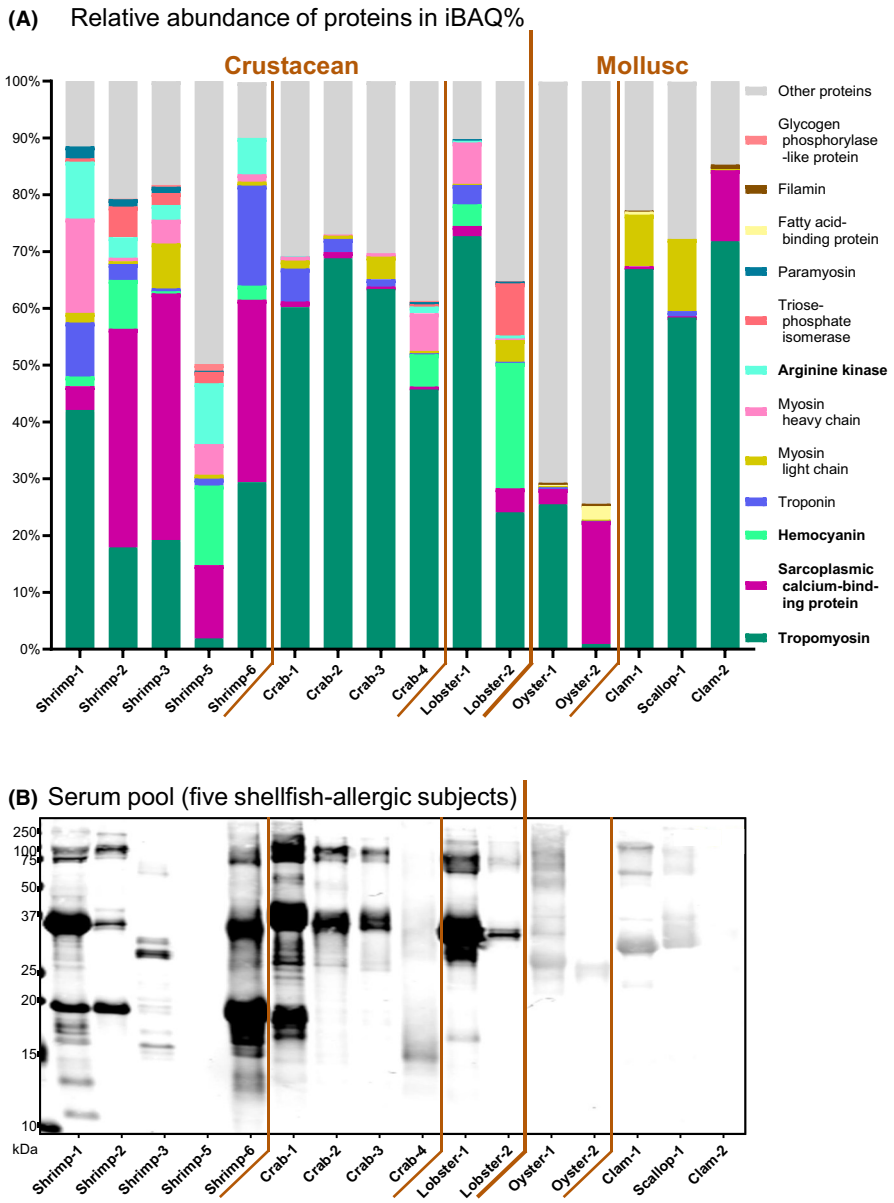

FIGURE 2 Relative protein abundance and IgE-binding patterns in 11 crustacean and five mollusc SPT extracts from six different manufacturers (−1 to −6). The iBAQ% value indicates relative abundance of each protein including several isoforms and is determined with MaxQuant after tryptic digestion and mass spectrometry (A). The 12 shellfish allergens are defined by the IUIS/WHO (www.allergen.org) and listed in ascending overall abundance. Allergens in bold were also analysed with allergen-specific antibodies (Figures 1C–F). IgE-binding was investigated by immunoblotting using a serum pool from five shellfish-allergic subjects (B). Refer to Table S1 for details on extracts and Table S2 for clinical characteristics of subjects.

KEYWORDS

allergy diagnosis, non-standardized allergen extracts, seafood allergens, shellfish allergy, tropomyosin

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest regarding the work presented in this manuscript.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.