

# House dust mite (Derp 10) and shellfish allergic patients may be at risk when consuming food containing mealworm proteins

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## SCOPE

Due to the imminent growth of the world population, shortage of protein sources for human consumption will arise in the near future. Alternative and sustainable protein sources (e.g. insects and algae) are now being explored for production of food and feed. In this project the safety of mealworm (*Tenebrio molitor* L.) proteins for human consumption was tested according to the European Food Safety Authority guidelines for allergenicity risk assessment of genetically modified organisms.

## METHODS

Different mealworm protein fractions were prepared, characterized and tested for cross-reactivity using IgE from patients with an inhalation or food allergy to related species (house dust mite (HDM) and shellfish (crustacean) and control serum using immunoblots and indirect basophil activation tests. Furthermore, the stability was investigated using an *in vitro* pepsin digestion test.

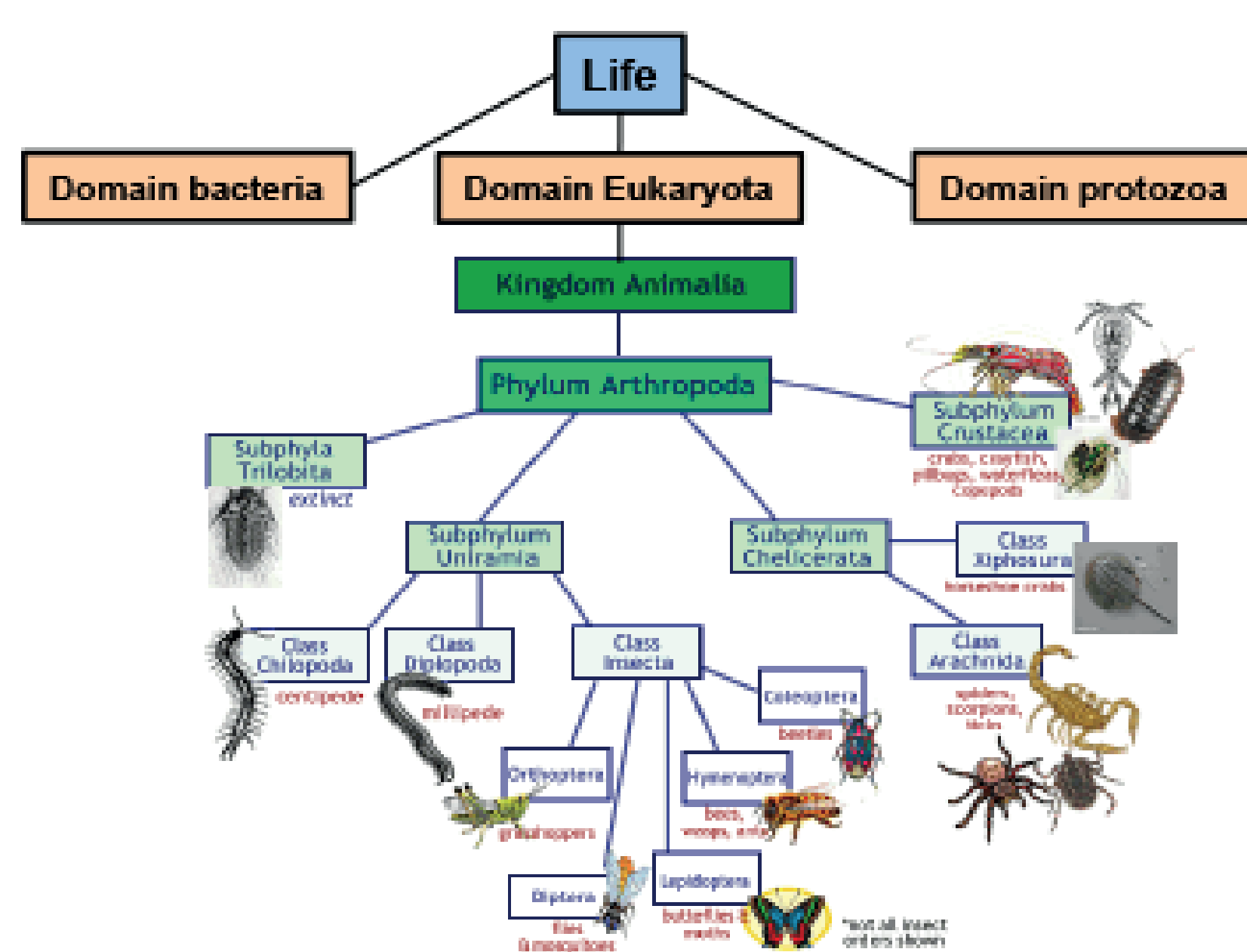


Fig. 1: Phylogenetic tree showing the phylum Arthropoda.

## MEALWORMS

The mealworm is the larval stage of the darkling beetle, *Tenebrio molitor*. According to the phylogenetic tree (Fig. 1) it belongs to the Arthropods and is related to shrimp, lobster, and HDM.

## MEALWORM EXTRACTS

Mealworms were extracted with 20 mM Tris buffer pH 7.6 (soluble fraction SRN1), dialysed (SRN2), and the insoluble matter was dissolved in 6M Urea (SRN3). In SRN1 and 2 comparable proteins were detected and in SRN3 proteins involved in muscle contraction were found (e.g. tropomyosin, actin, troponin) (Table 1).

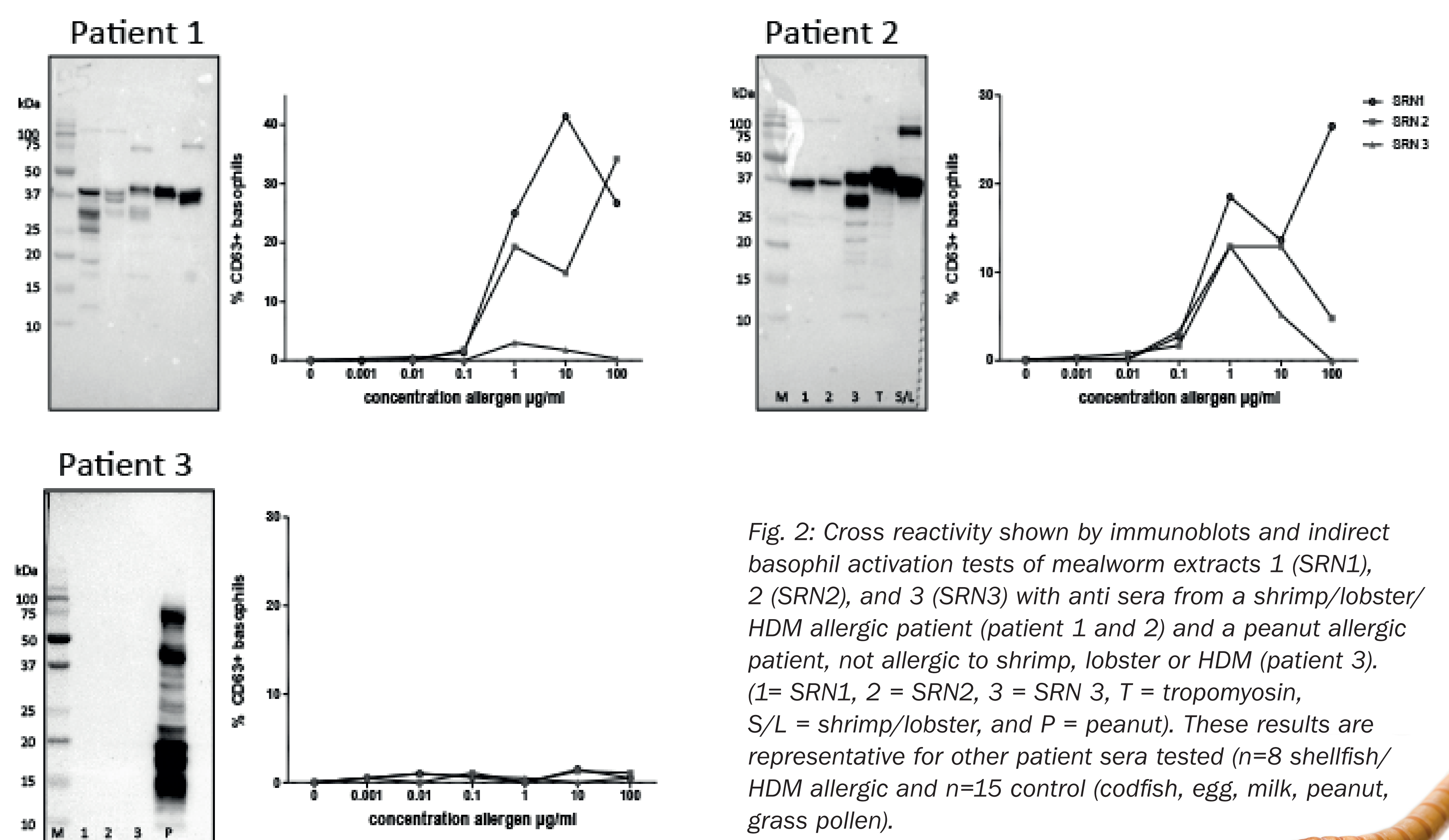


Fig. 2: Cross reactivity shown by immunoblots and indirect basophil activation tests of mealworm extracts 1 (SRN1), 2 (SRN2), and 3 (SRN3) with anti sera from a shrimp/lobster/HDM allergic patient (patient 1 and 2) and a peanut allergic patient, not allergic to shrimp, lobster or HDM (patient 3). (1= SRN1, 2 = SRN2, 3 = SRN 3, T = tropomyosin, S/L = shrimp/lobster, and P = peanut). These results are representative for other patient sera tested (n=8 shellfish/HDM allergic and n=15 control (codfish, egg, milk, peanut, grass pollen).

## CROSS-REACTIVITY

IgE from both HDM and shellfish allergic patients cross reacts with proteins in mealworm (Fig 2). This cross-reactivity was functional, as shown by the induction of basophil activation. Cross-reactive proteins were identified as tropomyosin in SRN3 and arginine kinase in SRN1 and 2 using LC-MS-MS. These proteins are well known allergens. Control serum from a peanut allergic patient did not react.

## PROTEIN STABILITY

Mealworm extracts were tested in a static pepsin digestion model and the cross-reactive proteins were not completely digested within 60 min. Compared to other allergens, Ara h2 which is still present in an intact form after 60 min and Ara h1 which is digested within 15 seconds, this is mildly stable (Fig. 3).

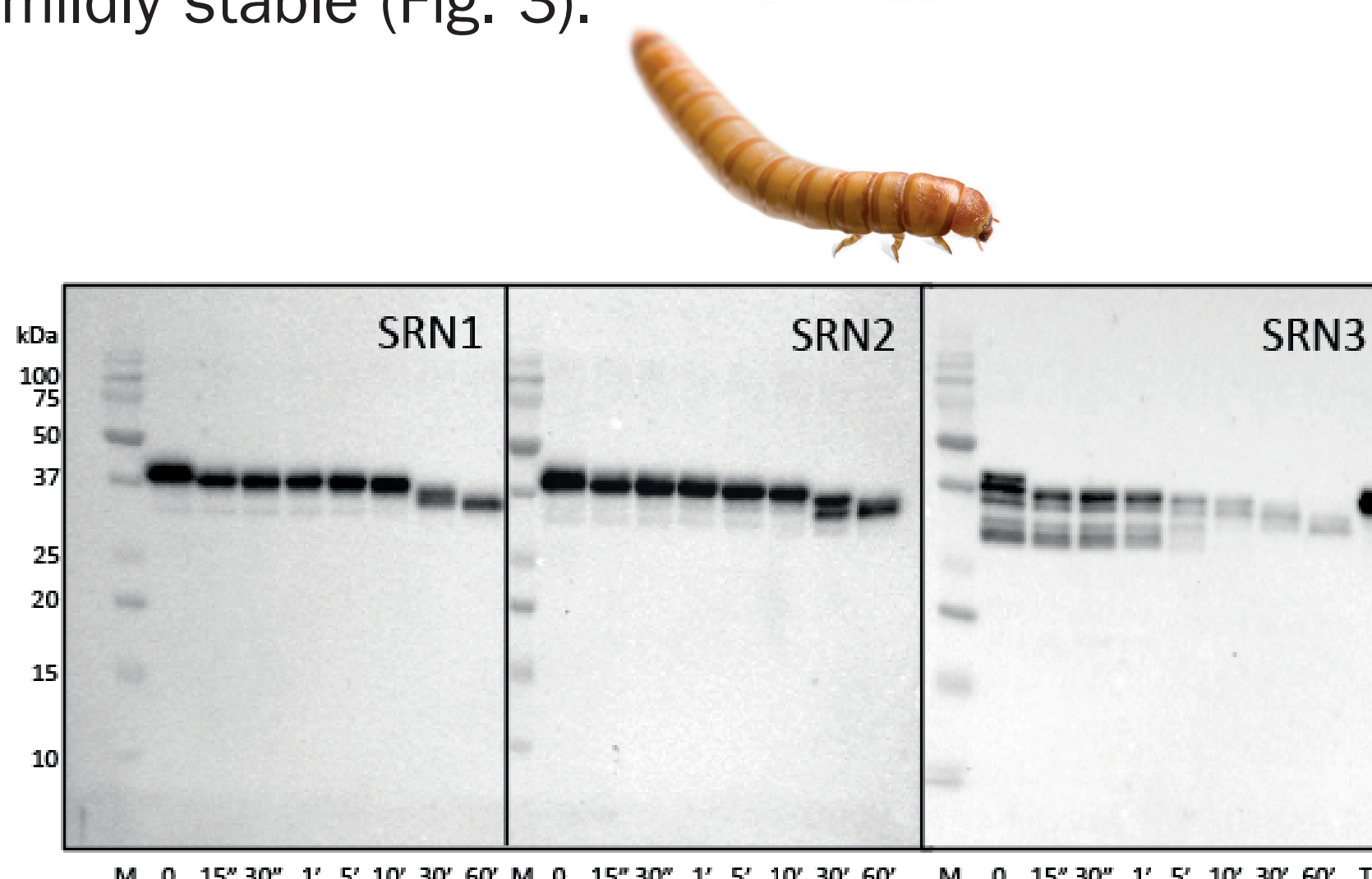


Fig. 3: Immunoblots of the digestion kinetics of mealworm extract SRN1, 2, and 3 in a static pepsin digestion model. Samples were taken after 0, 15, and 30 seconds and 1, 5, 10, 30, and 60 min. (M = marker and T = tropomyosin). Serum from a typical shellfish/HDM allergic patient was used.

Table 1: Most abundant proteins identified in extract SRN1+2 and SRN 3 using LC-MS-MS

SRN1 and SRN 2	SRN3
Calcium-transporting ATPase sarcoplasmic	Myosin heavy chain
<b>Arginine kinase</b>	Actin
POTE ankyrin domain family member F	Late histone H2A
Tubulin alpha-1	<b>Tropomyosin-1</b>
Catalase	Pupal cuticle protein G1A
<b>Alpha-amylase</b>	Ovalbumin
Muscle-specific protein 20	<b>Tropomyosin-2</b>
Glyceraldehyde-3-phosphate dehydrogenase	Myosin-2
Ovalbumin-like	ATP synthase subunit beta OS
Tubulin beta chain (Fragment)	Fructose-1,6-bisphosphatase class 3
Elongation factor 2	Troponin T
V-type proton ATPase catalytic subunit A	
<b>Sarcoplasmic Ca binding protein</b>	
40S ribosomal protein S5a	

**Bold** printed proteins are known allergens.

## CONCLUSIONS

HDM and shellfish allergic patients may be at risk when consuming food containing mealworm proteins. Some patients will react more to proteins in the soluble fraction (e.g. arginine kinase) and others to the insoluble fraction (e.g. tropomyosin).