

## Investigating Novel Food Sensitization: A Real-Life Prevalence Study of Cricket, Locust, and Mealworm IgE-Reactivity in Naïve allergic Individuals

**Running Title:** Insect allergy in Naïve Individuals

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## Abstract

**Background** | With the global population on the rise, edible insects are considered a potential solution to food security, although concerns about risks such as anaphylaxis exist.

**Methods** | 2,014 participants underwent testing with the Allergy Explorer-ALEX-2 including extracts of three novel foods: *Acheta Domesticus* (Ad), *Locusta migratoria* (Lm), and *Tenebrio molitor* (Tm). The IgE-mediated sensitization status was investigated in participants who had never knowingly consumed these insects. Data was recorded using an electronic database.

**Results** | 195 individuals (9.7% of all participants) were sensitized to insects. Tropomyosin was co-recognized by 34%, and 18.5% were positive for arginine kinases. Reactivity to Sarcoplasmic-CB, Troponin-C, Paramyosin, or Myosin-light-chain was found in less than 5% of the population, whereas 108 individuals (55.4%) did not show any reactivity to invertebrate panallergens. Additionally, 33 individuals (16.9%) exhibited monosensitization exclusively to insects. Multivariate analysis revealed an inverse association between arachnid reactivity and sensitization to insect allergens, while *Mollusca*, *Blattoidea*, and tropomyosin reactivity displayed a direct relationship. Furthermore, Myosin-light-chain reactivity correlated with Ad and Lm, and Troponin-C with Ad and Tm sensitization.

**Conclusion** | Edible insect extract IgE sensitization was observed in individuals without prior exposure to such foods. Mites showed a low likelihood of being primary sensitizers due to their inverse association with insect reactivity. Conversely, the direct association of insect sensitization with mollusk and cockroach extract reactivity suggests their potential as primary sensitizers in these participants. Tropomyosin consistently exhibited a positive association with reactivity to all studied insects, supporting its role as a primary sensitizer.

**Key words:** Edible insects. Novel foods. *Acheta Domesticus*. *Locusta migratoria*. *Tenebrio molitor*. IgE-mediated sensitization. Tropomyosin. Arginine kinases.

## RESUMEN

**Antecedentes** | Con el aumento de la población mundial, los insectos comestibles se consideran una solución potencial para la seguridad alimentaria, aunque existe preocupación por riesgos como la anafilaxia.

**Métodos** | 2.014 participantes se sometieron a pruebas con el Allergy Explorer-ALEX-2 incluyendo extractos de tres nuevos alimentos: Acheta Domesticus (Ad), Locusta migratoria (Lm) y Tenebrio molitor (Tm). Se investigó la sensibilización mediada por IgE en participantes que no eran conscientes de haber consumido previamente estos insectos. Los datos se registraron en una base de datos electrónica.

**Resultados** | 195 individuos (9,7% de todos los participantes) estaban sensibilizados a los insectos. La tropomiosina fue co-reconocida por el 34%, y el 18,5% fueron positivos para las arginina quininas. La reactividad a proteína sarcoplasmica ligadora de calcio (Sarcoplasmic-CB), Troponina-C, Paramyosina, o y la cadena ligera de miosina se encontró en menos del 5% de la población, mientras que 108 individuos (55,4%) no mostraron ninguna reactividad a panalergenos de invertebrados. Además, 33 individuos (16,9%) mostraron monosensibilización exclusivamente a insectos. El análisis multivariante reveló una asociación inversa entre la reactividad a arácnidos y la sensibilización a alérgenos de insectos, mientras que la reactividad a Mollusca, Blattoidea y tropomiosina mostró una relación directa. Además, la reactividad a la cadena ligera de miosina se correlacionó con la sensibilización a Ad y Lm, y la troponina-C con la sensibilización a Ad y Tm.

**Conclusión** | Se observó sensibilización IgE a extractos de insectos comestibles en individuos sin exposición previa a los mismos como alimentos. Los ácaros mostraron una baja probabilidad de ser sensibilizadores primarios debido a su asociación inversa con la reactividad a insectos. Por el contrario, la asociación directa de la sensibilización a insectos con la reactividad a extractos de moluscos y cucarachas sugiere su potencial como sensibilizadores primarios en estos participantes. La tropomiosina mostró sistemáticamente una asociación positiva con la reactividad a todos los insectos estudiados, lo que respalda su papel como sensibilizador primario.

**Palabras clave:** Insectos comestibles, Nuevos alimentos, Acheta domesticus, Locusta migratoria, Tenebrio molitor, Sensibilización mediada por IgE, Tropomiosina, Arginina quinasa.

**Summary box** | A study of 2,014 participants using Allergy Explorer-ALEX-2 investigated IgE sensitization to edible insects. 9.7% were sensitized to at least one insect, with 34% recognizing Tropomyosin and 18.5% positive for arginine kinases. Notably, 55.4% showed no reactivity to invertebrate panallergens. Mites had an inverse relationship with insect reactivity, while mollusc and cockroach extracts had a direct association. Tropomyosin could act as a primary sensitizer.

## 1 | Introduction

### 1.1 | Background/rationale

With the world's population projected to reach 9-11 billion by 2050, the challenge of feeding the growing populace becomes increasingly daunting[1]. As traditional food resources face scarcity and difficulties in production, alternative and sustainable food sources are gaining attention. Edible insects, a rich source of protein and essential nutrients, have emerged as a potential solution to address global food security concerns[2].

Currently, more than 2 billion people around the world include insects as a regular part of their diet, consuming over 2,000 different edible insect species[3]. Edible insects have garnered recognition for their significant nutritional value, minimal environmental impact, and highly efficient production process. In comparison to traditional livestock, they demand fewer resources like water and land, making them sustainable and promising food sources for the future[3].

In Europe, insects are considered novel food because their historical consumption within the European Union has been limited. [4] However, many people are unaware that insects are already a part of the European diet. It is estimated that approximately half a kilogram of insects is inadvertently consumed per person annually, as they are integrated during the production of various foods such as cereals and tomatoes[5]. Certain EU countries, like the Netherlands and Belgium, have recognized the potential of insect-based products and have been selling them for

several years[6]. Recently, Italy has also joined this trend by introducing products made from proteins derived from *Tenebrio molitor* (Tm) into the market.

Despite the numerous benefits of edible insects, their consumption is not without risks. Literature reports include case studies[7][8] and systematic reviews[9][10] that document possible adverse reactions, including anaphylaxis[11][12][13], triggered by the ingestion of insects. Given the expanding interest in insect-based diets, understanding the prevalence and nature of such reactions becomes crucial.

Many of the allergenic components identified to date in the meat of edible insects are represented by panallergens already described in arthropods (mites and crustaceans), molluscs, and nematodes, such as tropomyosin, arginine kinase, troponin C, sarcoplasmic calcium binding, Myosin light chain, Triosephosphate isomerase, and paramyosin[14][9]. However, additional molecules specific to insects have been identified, such as chemosensory proteins (CSP), odorant or pheromone-binding proteins (OBP), and hexamerin[15][16]. This suggests that such allergenic sources may represent a significant cause of adverse reactions, even severe ones, eventually labeled as idiopathic in the Western population, which is not accustomed to consuming such protein sources.

## 1.2 | Objectives

This study surveyed the IgE-mediated sensitization status of three insects currently available for in vitro IgE reactivity detection, namely, cricket[17], locust, and mealworm[18], among subjects that never knowingly consumed edible insects before.

## 2 | Methods

### 2.1 | Study design

We conducted a single-centre cross-sectional observational clinical survey on crickets (*Acheta domesticus* - *Ad*), migratory locusts (*Locusta migratoria* - *Lm*), or mealworms (*Tenebrio molitor* – *Tm*) sensitization in a population that had never consciously consumed insect proteins in their diet before.

We also investigated the association of this reactivity with the presence of specific IgE antibodies against other insects, arachnids, crustaceans, or molluscs.

Finally, we assessed the presence of symptoms related to environmental or food allergies in these subjects.

### 2.2 | Setting

The study involved 2,014 unselected participants born in Central or Southern Italy, attending the outpatient Allergy Unit of IDI-IRCCS, Rome, due to a history of adverse reactions to foods, allergic rhinitis, bronchial asthma, and/or atopic eczema.

The IDI-IRCCS serves as a National Reference Center for Dermatological Diseases.

Data collection took place between January 2021 and August 2023. Demographic information and clinical data were recorded using a customized electronic database.

### 2.3 | Participants

The main eligibility criterion for the study was the presence of IgE-mediated reactivity that could be assessed by the array used to evaluate participants with a clinical history suggestive of allergy-mediated disease. Sensitization to at least one of the insect' extract spotted on the IgE microarray (*Ad*, *Lm*, or *Tm*) characterized the subgroup used for data analysis.

We included all participants who showed reactivity to molecules or extracts from invertebrates as a comparison and to identify potential molecular similarities, including Coleoptera, specifically Tm; Blattodea, specifically *Blattella germanica* and *Periplaneta americana*; Hymenoptera, specifically *Apis mellifera*, *Vespula vulgaris*, *Dolichovespula maculata*, *Polistes dominulus*, and *Solenopsis richteri*; Ixodida, specifically *Argas reflexus*; Orthoptera, including Gryllidea, specifically Ad, and Acrididea, specifically Lm; Crustacea (i.e. *Chionoecetes* spp., *Crangon crangon*, *Homarus gammarus*, *Litopaenaeus setiferus*, *Pandalus borealis*, and *Penaeus monodon*); Mollusca (i.e. *Loligo* spp., *Mytilus edulis*, *Ostrea edulis*, *Pecten* spp, and *Ruditapes* spp.); Chelicerata (i.e. Astigmata including *Acarus siro*, *Blomia tropicalis*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Glycyphagus domesticus*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentia*); and Nematoda (*Anisakis simplex*).

## 2.4 | Variables

The first variables taken into consideration were age and sex. Additionally, we classified the observed reactivities based on the recognition of pan-allergens available for IgE evaluation, whose homologous components had already been demonstrated in insect meats (i.e., Tropomyosin, Myosin light chain, Arginine Kinase, Troponin C, and Paramyosin). Finally, we assessed the cross-reactivity in participants with reactivity to the examined insects, to molecules or extracts derived from other insects, nematodes, crustaceans, or arachnids.

Given the observational nature of the study, no randomization procedure was implemented during enrollment.

## 2.5 | Data sources/ measurement

Serum IgE reactivity was analyzed using the Allergen ExplorerALEX® version 2 (Macroarray Diagnostics, Vienna, Austria), where different allergens and extracts are spotted onto a nitrocellulose membrane in a cartridge chip, including crickets (AD), migratory locusts (LM), or mealworms (TM) extracts.

The chip was incubated with 0.5 mL of a 1:5 dilution of the patient's serum, containing a CCD inhibitor under agitation. After two hours of incubation, the chips are washed three times, and a pre-titred dilution of anti-human IgE labelled with alkaline phosphatase is added and incubated for 30 min. After another cycle of extensive washing, the enzyme substrate is added, and after eight minutes, the reaction is stopped by the addition of 100 µL of ALEX Stop Solution. The membranes are dried, and a charge-coupled device camera measures the intensity of the colour reaction for each allergen spot. The dedicated software digitalizes the images and prepares a report that lists the allergens and components and their score in kUA/L. Finally, systematic variations in signal levels between lots were normalized by heterologous calibration against an IgE reference curve. A curve fit was calculated, and the resulting equation was applied to transform arbitrary intensity units into quantitative units. Lot-specific calibration parameters are encoded in the barcode. The measuring range of ALEX-specific IgE is 0.3-50kUA/L, according to the guidelines of the manufacturer.

The molecular content of the insect extracts under study was indirectly assessed in vitro using extracts of *Blattella germanica* and *Dermatophagoides pteronyssinus* (both obtained from ROXALL ARISTEGUI ITALIA) ELISA inhibition tests using sera from 10 well-characterized participants allergic to *Acheta domestica*, *Locusta migratoria*, and *Tenebrio molitor*.

The specific IgE levels in these sera were re-evaluated using the commercially available Allergy Explorer-ALEX®-2 (Macro-ArrayDX Wien, Austria), a CE-certified platform that includes various allergen extracts and individual components spotted onto a nitrocellulose membrane in a cartridge chip. In brief, 100 µl of each serum were incubated overnight with an equal volume of PBS (control) or both *Blattella germanica*, or *Dermatophagoides pteronyssinus*, respectively. Subsequently, the "inhibited" samples were processed as previously described.

## 2.6 | Bias

The diagnosis of food allergy was not confirmed through blinded or open oral food challenges.



## 2.7 | Quantitative variables

Quantitative measurements of specific IgE towards insects under study were compared among the various clinical subsets of participants, between males and females, and across different age groups.

## 2.9 | Statistical methods

All data were analyzed using the SPSS/PC + statistical package for statistical evaluation (IBM SPSS, version 29, Chicago, IL). The TD-Synergy Laboratory Information System was used to search and collect demographic (age and gender), clinical, and laboratory data for participants who attended the outpatient Allergy clinic and underwent specific IgE testing. In univariate analysis, the non-parametric Mann-Whitney U-test (two groups) was first used to compare continuous IgE values in males, females, and subjects with or without a given clinical involvement. Subsequently, each variable of interest was dichotomized as negative or positive to examine the proportion of subjects with symptoms in the two resulting groups.

Pearson's  $\chi^2$  test or Fisher's exact test (used for two-by-two contingency tables with less than 50 cases) were used to assess if paired observations on two variables expressed in a contingency table, were independent of each other.

We performed multiple logistic regression for the clinical variables with dichotomous scores (present, absent) to see whether the association between clinical symptoms and different allergens reactivity was present after simultaneously adjusting for the other variables of interest.

To provide a visual representation of the distribution of the different molecules in panallergen families, we have produced Venn diagrams using the VennMaster 0.38 package [19].

## 2.10 | Ethical issues

The study was approved by the Ethical Committee of IDI-IRCCS (IDI-IRCCS CE | 495-17). Data collection was conducted anonymously, utilizing only information obtained from

routine specialist surveys. Recruited participants provided informed consent for the utilization of their clinical data in an anonymous format.

### 3 | Results

#### 3.1 | Participants

Among 2,014 consecutive individuals with various allergic conditions, including respiratory issues, food-related allergies, and atopic dermatitis, who underwent proteomic evaluation using the ALEX-2 test, 195 participants were reactive to at least one of the extracts derived from house crickets (*Acheta domesticus*, Ad = 161, 83%), migratory locusts (*Locusta migratoria*, Lm = 100, 51%), or mealworms (*Tenebrio molitor*, Tm = 154, 79%).

The distribution of the examined population is provided in the accompanying Table 1.

The Venn diagram in Figure 1 illustrates the co-recognition relationships of the three examined extracts. Forty-six per cent of participants showed reactivity to all three extracts, 15% exhibited mono-reactivity to Tm, 3% were sensitized only to Lm, and 18% were sensitized to Ad in the absence of IgE recognition to the other two extracts.

Gender was found to be associated with reactivity to insects, particularly Ad and Lm. Males demonstrated notably higher average values and a higher frequency of positive responses: 21.4% vs. 14.4% in females for the cricket, and 14.4% vs. 8.1% for the locust ( $P < 0.01$ ).

Interestingly, none of the individuals who tested positive for insects had knowingly consumed edible insects in the past. While some had experienced allergic reactions to molluscs (21.3% reported a moderate reaction, and 3.2% experienced a severe reaction) or crustaceans (9.6% and 3.2% had moderate and severe reactions, respectively), 41% of the tested individuals had not reported any adverse reactions after consuming these foods.

Additional investigations into the reactivity profiles of arthropod-derived molecules showed that approximately 40% to 60% of the population demonstrated sensitivity to allergenic components found in crustaceans, molluscs, and nematodes. These components included

Tropomyosin, Arginine kinase (AK), myosin light chain (MLC), Troponin C (TnC), and sarcoplasmic Ca<sup>++</sup>-binding protein (SCB) (Table 2).

When we further examined all participants reactive to tropomyosin (104 individuals, 52 F) or arginine kinase (68 participants, 39 F), as reported in Table 3, only a portion of them also showed reactivity to insect extracts, with significant variability in molecular recognition from patient to patient. Generally, the fraction of individuals sensitized to insects was higher among tropomyosin reactors (ranging from 48% to 88%) compared to arginine kinase (AK) reactors, where those positive to AK also showed reactivity to insects in 40% to 53% of cases.

Remarkably, more than half (108, 55.4%) of the insect-reactive individuals displayed no sensitization to any of the molecules included in the panel tested (Table 2).

To delve deeper into this subset of participants, we analyzed their recognition profile of molecular components and extracts from other invertebrates assessed in the test, including *Astigmata* (mite), *Blattodea*, *Crustacea*, *Mollusca*, and *Hymenoptera*.

Participants who tested negative for Tropomyosin, AK, MLC, TnC, SCB, and paramyosin exhibited reactivity to mite-derived molecules in more than 50% of cases, and 20% to 30% showed co-sensitization to Hymenoptera. Interestingly, about 30% per cent of individuals reactive to cricket and mealworm, and less than 20% of those reactive to locusts were not co-sensitized to any of the extracts or molecules from mites, cockroaches, or other invertebrates. As a result, 33 (16.9%) participants were sensitized only to the 3 categories of insects evaluated in this study (Figure 2).

The IgE-mediated reactivity to arachnids showed an inverse association with the presence of IgE recognition for at least one insect among the three examined (OR=0.54; 95%CI 0.3-0.8, P=0.001). Conversely, the recognition of extracts or molecules from molluscs, crustaceans, cockroaches, and Hymenoptera consistently demonstrated a direct association with reactivity to crickets, locusts, and mealworms (Table 4, left-hand side).

Using multiple logistic regression analysis, we included all examined allergenic extracts or molecules, along with age and sex, in the model. The results confirmed an inverse relationship between *Astigmata* (mite) reactivity and sensitization to Ad or Tm. Moreover, even after adjusting for sex and age, a direct association persisted for *Mollusca*, *Blattodea*, and

tropomyosin. Additionally, MLC showed a direct association with Ad and Lm, Tnc with Ad and Tm, and Hymenoptera with Lm (Table 4, right-hand side).

Inhibition experiments carried out with 10 selected sera adsorbed with cockroach and *Dermatophagoides pteronyssinus* extracts showed that the mite extract was able to completely inhibit IgE reactivity to insects in 6 cases and nearly completely (with a single exception) in the remaining samples (supplementary table). The *Blattella germanica* extract exhibited lower efficacy compared to mites, especially in the case of *Tenebrio molitor*.

However, in those three participants non-reactive to any panallergen present in the array, the signal was entirely abolished, as observed with the inhibition performed by *Dermatophagoides pteronyssinus*. Interestingly, focusing on the 5 samples reactive to tropomyosin and arginine kinase, once again, the mite extract demonstrated a greater inhibitory capacity for tropomyosin compared to the cockroach extract. The *Blattella germanica* extract inhibited the signal of Ani s 3 from Anisakis in 4 out of 5 samples but did not show effective inhibition of the other studied molecules. In the case of AK, only Der p 20 was effectively inhibited in one instance, while in all other experiments, both mite and cockroach extract exhibited very limited inhibition of the signal.

## 4 | Discussion

### 4.1 | Key results

Recently, there has been a growing number of reports discussing the negative effects caused by consuming or merely coming into contact with edible insects [7, 12]. A study focusing on Chinese literature revealed that 16% of cases between 1980 and 2007 experienced severe reactions, such as anaphylaxis, after being exposed to grasshoppers, locusts, or larvae[13]. Presently, this matter has limited significance for the European population. Nevertheless, it is reasonable to consider that it might gain more importance and urgency, particularly considering recent decisions that allow the use of insect-derived proteins as food sources within the European Union[4].

In our research, we have observed sensitization to extracts of edible insects, with a higher prevalence among males. The origin of this sensitization, in the absence of prior

exposure, might be attributed to the inadvertent ingestion of insect proteins found in various foods[5,20]. Furthermore, the inverse correlation between sensitization to mites (both extracts and specific molecules) and sensitization to certain edible insects (Ad and Lm) suggests that mites are unlikely to be the primary sensitizers in these cases.

On the contrary, the positive correlation between insect sensitization and the IgE recognition of molecular components from molluscs, cockroaches, and even Hymenoptera (in the case of reactivity to locusts) suggests that these allergenic sources may share common molecular elements with the insects studied in our investigation. In this context, potential allergenic proteins present in Tm were already assessed in two separate groups: one comprising individuals allergic to crustaceans, and the other consisting of a cohort allergic to dust mites[18]. The Authors not only confirmed the presence of known proteins like tropomyosin or AK (arginine kinase) but also identified other specific insect proteins, such as apolipoprotein-III, larval cuticular protein, and the 12 kDa hemolymph protein. Additionally, a bioinformatic and proteomic analysis driven by the same group allowed for the characterization of further potential allergens found in insects. Some of these allergens are highly prevalent in molluscs and are also shared with *Blattoidea*, such as the Cockroach allergen-like protein[8,15].

An important observation is that only a subset of participants (ranging from 40% to 60%, depending on the specific insect under consideration) exhibited co-sensitization to molecules that are already known and can be tested in vitro, such as tropomyosin, AK (arginine kinase), Tnc (troponin C), MLC (myosin light chain), or SBP (serum binding protein). Consequently, there was a group of participants who reacted to insect extracts, indicating their ability to recognize molecular components that are currently not detectable in vitro with the existing resources. This highlights the continued significance of allergenic extracts in modern allergy diagnostics. Despite the advancements in knowledge and the availability of molecules for in vitro evaluation of allergic participants, the use of allergenic extracts is still relevant and far from outdated. When dealing with new sources of sensitization, the initial step to identify and subsequently study or classify participants is through the use of allergenic extracts.

Tropomyosin consistently exhibited a positive association with reactivity to Ad, Lm, and Tm. It should be considered that not all subjects reactive to tropomyosins were simultaneously positive to insect extracts, and sometimes they were positive to certain insects but not to others. This indicates significant variability in antigenic recognition among participants. However, it

should be noted that in the case of certain edible insect species, there is demonstrated variability in the cross-reactivity of these molecules [21]. Regarding Tm, our findings revealed a significant association with Troponin C, which aligns with previous literature [8]. Limited cross-reactivity between Tm and Ad arginine kinases was also evident [22]. Nevertheless, it is crucial to avoid solely relying on structural identity to predict cross-reactivity, as confirmation through further tests, including food challenges, is essential [17].

Inhibition experiment indicate that both mites and cockroach contain molecules able to elicit an IgE reaction against insects, albeit with different efficiencies. Considering the molecular component of each extracts, tropomyosin, and in a lesser extent Arginine kinase, might be considered common mite allergens for insects' sensitization, while cockroach contain a larger fraction of unidentified constituents.

The clinical significance of these sensitizations is another important aspect to consider. Less than 50% of cases had previous reactive episodes, mostly related to molluscs, with crustaceans being involved in less than 10% of the subjects examined. A study involving 15 shrimp-allergic participants demonstrated that those who were co-sensitized to tropomyosin or AK reacted to double-blind placebo-controlled food challenges (DBPCFC) with Tm, leading to the development of moderate to severe symptoms [23]. Generally, individuals with allergies to crustaceans and/or molluscs should be made aware of the possibility of cross-reaction with edible insects [23](24). Similarly, participants who are allergic to dust mites and keep reptile pets at home, as these animals are often fed live insects such as grasshoppers, crickets, or locusts, should also be cautious [17].

It is important to emphasize the added value of using a technology that involves chelation, albeit imperfect, of the signal generated by CCD recognition, as many arthropod allergens are glycosylated and therefore the presence of IgE against CCDs can be a trivial cause of cross-reactivity.

## 4.2 | Limitations

The clinical significance of the insect-specific sensitization patterns in patient data was not evaluated via food challenges. This selective approach might not have impacted the subset

of participants who consistently tolerate foods containing panallergens like tropomyosin or arginine kinase.

### 4.3 | Generalizability

The studied population hails from a temperate, westernized country. Therefore, what has been observed should be understood as applicable to the climate, dietary habits, and food choices of this population and may not necessarily be equally applicable to other contexts and diverse settings.

### 4.4 | Conclusion

The findings presented in this study raise significant questions about the implications of sensitization to edible insects. Although the exact cause of sensitization remains uncertain, it is plausible to consider that inadvertent ingestion of insect proteins may be a contributing factor. The possibility of inhalant dust mite allergy being linked to the subsequent development of tropomyosin-mediated food allergy, as observed in pollen fruit syndrome, has been a subject of debate for years [24]. The shared molecular recognition between insects and certain allergenic sources highlights the need for further investigation to understand potential cross-reactivity. Additionally, the growing interest in edible insects as a sustainable protein source demands a thorough assessment of the risks associated with their allergenicity to safeguard public health[2,4,6,25]. It is worth noting that processing methods used for foods, such as enzymatic hydrolysis or high-temperature treatments, can modify the allergenicity of insect proteins, potentially reducing their allergenic potential [17]. As this issue gains prominence, future research and regulatory efforts should focus on establishing standardized testing methods to identify allergenic components in edible insects[4]. Proper labelling and consumer awareness campaigns can also play a crucial role in mitigating potential risks for sensitized individuals.

In conclusion, sensitization to edible insects poses a potential health concern that warrants further exploration. While the current prevalence is low, the increasing acceptance of insects as food sources necessitates proactive measures to address the allergenicity aspects associated with these novel dietary choices.

**Institutional Review Board Statement** | The research was conducted ethically following the World Medical Association Declaration of Helsinki and the study protocol was reviewed and approved by the IDI-IRCCS's committee, approval number IDI-IRCCS CE | 495-17.

**Data Availability Statement** | The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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**Conflict of interest** | Disclosure of potential conflict of interest: Enrico SCALA has received consultant arrangements and speakers' bureau participation from Stallergenes and Thermo Fisher Scientific., The rest of the authors declare that they have no relevant conflicts of interest.



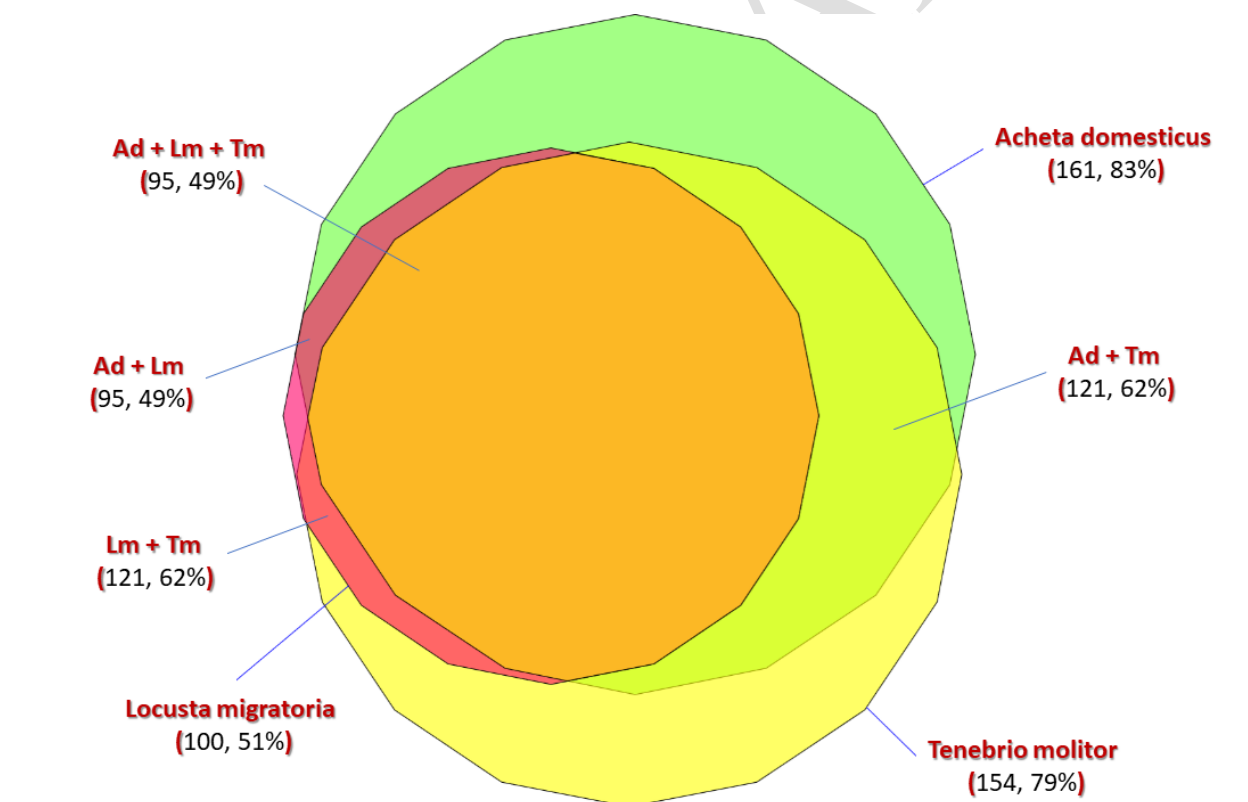
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## Figure Legend

**Figure 1** | The Venn diagram illustrates the co-recognition relationships of the three examined insect extracts, namely house crickets (*Acheta domestic us*, Ad = 161, 83%, pale green), migratory locusts (*Locusta migratoria*, Lm = 100, 51%, pale purple), or mealworms (*Tenebrio molitor*, Tm = 154, 79%, yellow). In addition, the Venn diagram shows the number and proportion of the respective population in the case of multiple reactivity to multiple insect species.



**Figure 2** | The figure shows the prevalence of co-recognition of molecules or extracts derived from other invertebrates in subjects IgE reactive for insects but negative for Tropomyosin, Arginine Kinase, Myosin Light chain, Troponin C, Sarcoplasmic Ca<sup>++</sup> BP, and paramyosin. The label 'none' indicates the fraction of participants only reactive to Ad, Lm, or Tm.

