

In vivo and in vitro allergenicity of the domestic mite *Chortoglyphus arcuatus*

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Background: *Chortoglyphus arcuatus* is frequently identified in mattress dust samples in coastal areas of northern Spain.

Objectives: To establish the prevalence of positive skin test reactions to *C arcuatus* and to analyze its allergenicity and cross-reactivity.

Methods: One hundred thirty-eight consecutive patients entered the study. The main referred symptoms were rhinoconjunctivitis and asthma. Skin tests were performed with extracts of *Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *C arcuatus*, and *Glycyphagus domesticus*. Enzyme-linked immunosorbent assay inhibition experiments were conducted with the different mite species and immunoblots with serum samples from 31 sensitized patients. Conjunctival provocation tests were performed in 10 patients with *C arcuatus*-positive skin test reactions and in 9 with negative results; all the patients had positive skin test reactions to *D pteronyssinus*.

Results: The prevalence of positive skin test reactions to *D pteronyssinus* was 94.2% and to *C arcuatus* was 58%. There was a significant correlation between the number of mites to which patients were sensitized and the time of disease evolution ($P = .02$). Results of enzyme-linked immunosorbent assay inhibition experiments suggested minimal cross-reactivity between *C arcuatus* and *D pteronyssinus*. Immunoblot results confirmed specific IgE binding to several bands. Conjunctival test results were positive in 9 of 10 *C arcuatus*-positive individuals and slightly positive in 2 of 9 *C arcuatus*-negative, *D pteronyssinus*-positive individuals.

Conclusions: There is a high prevalence of sensitization to *C arcuatus* in northern Spain. Sensitization to this species should be considered of clinical significance. There is minimal cross-reactivity between *C arcuatus* and *D pteronyssinus*.

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INTRODUCTION

The mite species *Chortoglyphus arcuatus* is common in Galicia, Spain, where it has been described in 72.3% of the mattress dust samples collected in Pontevedra, in 45% in La Coruña, and in 38.6% in Lugo.¹ Phylogenetically, *C arcuatus* belongs to the order Astigmata, superfamily Glycyphagoidea, family Chortoglyphidae.² It was first described in 1879, and since then it has been widely cited in flour, floor dust of barns, mills, stables, granaries, stored food (wheat rye, oats, and seeds), poultry mixtures, and farm dust in England, France, Belgium, Germany, the Netherlands, Poland, Russia, New Zealand, and Korea.^{3,4} It has also been described in house dust in the United States,^{5–7} Chile,⁸ Peru,⁹ Brazil,¹⁰ and Colombia.¹¹ In Spain, it has been cited in several regions, including Valladolid,¹² Barcelona,¹³ Santiago de Compostela,¹⁴ and the Canary Islands.¹⁵

Sensitization to *C arcuatus* has also been described in the United States,^{16–18} Brazil,¹⁹ Italy,²⁰ Switzerland,²¹ Germany,²² Argentina,²³ Chile,²⁴ and Colombia.^{25,26} A high prevalence of sensitization to this mite species has also been detected in children evaluated for severe asthma in Florida.²⁷ In vivo sensitization to this mite species has been described in several

Latin American countries in patients with asthma,²⁸ ranging from 33% in Mexico to 76% in asthmatic children in Brazil. Although the allergenicity of this mite species has not been extensively evaluated, minimal cross-reactivity with Pyroglyphid mites, such as *Dermatophagoides pteronyssinus*,²⁵ has been suggested. However, *C arcuatus* seems to be more cross-reactive with other storage mites belonging to the families Acaridae, such as *Aleuroglyphus ovatus*,²⁵ and Glycyphagidae, such as *Blomia kulagini* and *Lepidoglyphus destructor*.²⁹

To our knowledge, the allergenicity of *C arcuatus* had not been studied using provocation tests. Based on the high prevalence of this mite species in house dust in Galicia, and its ubiquitous presence worldwide, we decided to undertake the present study to evaluate the allergenicity of *C arcuatus*. The objectives of this study were to establish the prevalence of positive skin test reactions to *C arcuatus* in allergic patients, to analyze its cross-reactivity with *D pteronyssinus* and other mite species, and to conduct conjunctival provocation tests with an extract of *C arcuatus* to evaluate the clinical significance of these sensitizations.

MATERIALS AND METHODS

Patient Population

One hundred thirty-eight consecutive patients (mean $\pm$ SD age, 23.9 $\pm$ 13 years; mean $\pm$ SD age when symptoms began, 15.8 $\pm$ 11.7 years; and mean $\pm$ SD time of evolution, 7.4 $\pm$ 5.2 years) entered the study. The main referred symp-

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toms were rhinoconjunctivitis and asthma. Skin tests were performed with mite extracts belonging to the families Pyroglyphidae (*D pteronyssinus*), Glycyphagidae (*L destructor* and *Glycyphagus domesticus*), Acaridae (*Tyrophagus putrescentiae*), and Chortoglyphidae (*C arcuatus*). *Chortoglyphus arcuatus* and *G domesticus* extracts contained 2 mg of freeze-dried material; the remaining extracts were standardized (Laboratorios LETI S.L.). The number of mite species to which the patients were sensitized and the time of evolution of the disease were correlated using Spearman analysis. After written consent was obtained, blood samples were obtained from 32 individuals, and the serum samples were stored frozen at -20°C until further analysis.

Allergen Extract

One hundred grams of a full-grown culture of *C arcuatus* was extracted (1:10 wt/vol) in 0.01M phosphate-buffered saline (PBS) (pH 7.4) for 4 hours at 4°C under continuous magnetic stirring. After homogenization, the extract was centrifuged at 16,000g at 4°C for 30 minutes, and the supernatant was collected. The remaining raw material was again reconstituted in 1:10 (wt/vol) 0.01M PBS and extracted overnight at 4°C under continuous magnetic stirring. Afterward, the extract was centrifuged and the supernatant was separated and mixed with the first extraction, sterile filtered, and dialyzed against bidistilled water in 3.5-kDa dialysis membranes (Cellu Sep; Membrane Filtration Products Inc, Seguin, TX), filtered, frozen, and freeze-dried.

Conjunctival Provocation Tests

After written consent was obtained, double-blinded conjunctival provocation tests were performed in 10 patients who had positive skin test reactions to *C arcuatus* and *D pteronyssinus* (group 1) and in 9 patients who had positive skin test reactions to *D pteronyssinus* and negative reactions to *C arcuatus* (group 2). All the patients had rhinoconjunctivitis with or without asthma. One physician performed the test and another evaluated the results. The maximum concentration used was 1 drop of a sterile filtered *C arcuatus* extract, reconstituted at 1 mg/mL. Three 1:10 dilutions were prepared and applied at 30-minute intervals until the highest concentration was applied or the patient experienced a positive reaction. The drops were instilled into the lower conjunctival sac of an eye. Isotonic sodium chloride solution was applied in the contralateral eye as a control and was evaluated according to the method described by Bonini et al³⁰ with minor modifications. All the reactions were evaluated by the same physician 20 minutes after the test was conducted. Redness, tearing, itching, and edema (inferior eyelid) were evaluated. Itching was determined by the patient using a graded analog scale from 0 to 4. Each of these symptoms received a maximum score of 4, and the total possible maximum score was 16. All the reactions resolved spontaneously after a saline wash, without any complications or medication use. A reaction was considered positive when the patient developed erythema and pruritus of the conjunctiva with a minimum score of 4 from

baseline. Eight nonallergic individuals were used as controls. The results are expressed as maximum symptom scores per patient. Nasal symptoms were also considered positive if the patient had signs and symptoms of rhinorrhea, nasal itching, or sneezing.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis was used to determine the antigenic profile of the *C arcuatus* extract. Samples were run in SDS-PAGE gels with 2.67% C, 15% T acrylamide as described by Laemmli.³¹ The samples were denatured at 100°C for 10 minutes and were centrifuged for 1 minute at 16,000g. Fifty micrograms of lyophilized material was loaded onto the gels, which were stained with Coomassie brilliant blue R-250 (BioRad Laboratories, Hercules, CA). The antigenic profile was studied using a scanner (JX-330; Sharp Corp, Tokyo, Japan) and was analyzed using Image Master 1-D Elite v 4.00 (Pharmacia Biotech, Uppsala, Sweden).

Immunoblot

Electrophoretically separated proteins were transferred to P-Immobilon membranes (Millipore, Bedford, MA). After transfer, the membranes were dried at room temperature for 4 hours. The membranes were then incubated overnight with the individual serum samples diluted in 0.01M PBS–Tween 2%. Specific IgE binding was detected by chemiluminescence with peroxidase-conjugated monoclonal anti-human IgE (Ingenasa, Madrid, Spain) for 2 hours.

Enzyme-Linked Immunosorbent Assay Inhibition

A serum pool was prepared with aliquots of 15 serum samples from *C arcuatus*– and *D pteronyssinus*–sensitized patients; the mean $\pm$ SD optical density of specific IgE for *C arcuatus* was 0.7 ± 0.7 and for *D pteronyssinus* was 2.6 ± 0.9 . Plastic microtiter plates (Immulon IV; Dynex Technologies, Chantilly, VA) were coated with a *D pteronyssinus* or *C arcuatus* extract (10 μg of protein/per milliliter) overnight. Several dilutions were made from the 6 analyzed mite species. Each dilution was incubated with the serum pool for 2 hours at room temperature. Positive and negative controls were also included. Afterward, the dilutions were transferred to the *D pteronyssinus*– or *C arcuatus*–coated plates and were incubated overnight. After washing, 100 μL of anti-human IgE labeled with peroxidase was added and let to stand for 30 minutes at room temperature. After washing, the plates were developed for 30 minutes, and the process was stopped with 1 N of sulfuric acid.

RESULTS

Skin Prick Testing

The prevalence of positive skin test reactions to *D pteronyssinus* was 94.2%, to *G domesticus* was 89.6%, to *L destructor* was 89.1%, to *T putrescentiae* was 87% (1 patient was exclusively sensitized to this species), and to *C arcuatus* was 58% (2 patients were exclusively sensitized to this species).

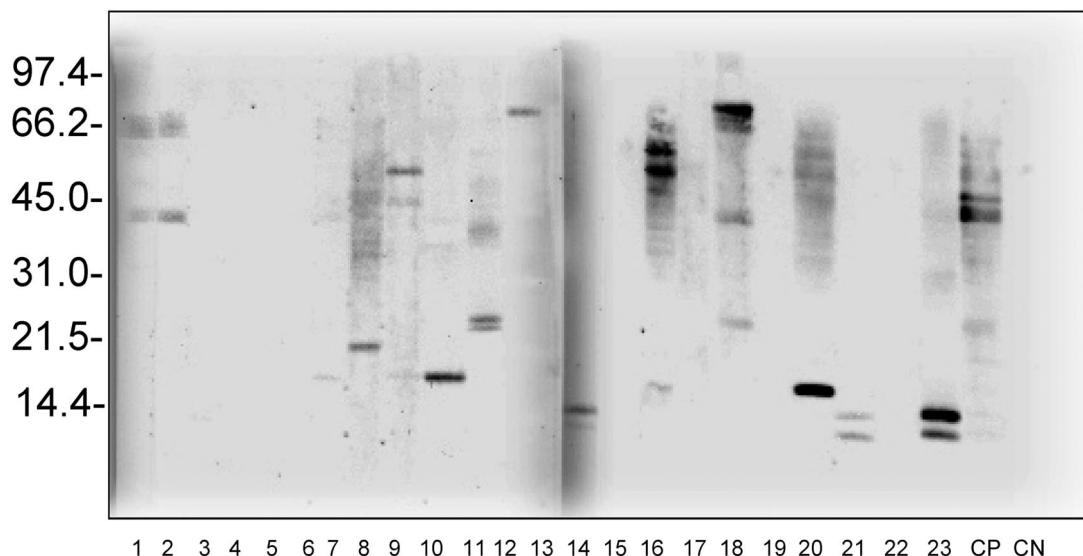


Figure 1. Individual immunoblots with 23 serum samples from patients with *Chortoglyphus arcuatus*-positive skin test reactions. CP indicates positive control; CN, negative control.

A significant correlation was detected among sensitizations to *D pteronyssinus*, *G domesticus*, *T putrescentiae*, and *L destructor* ($P = .009$). However, there was no significant correlation between sensitization to *C arcuatus* and the remaining mites. There was a significant correlation between the number of mites to which patients were sensitized and the time of evolution of the disease ($P = .02$).

SDS-PAGE and Immunoblot Experiments

The SDS-PAGE from *C arcuatus* extract showed several proteins with molecular weights of 8 to 97 kDa. The allergenic profile of the extract was studied by immunoblot using serum samples from 31 individuals with *C arcuatus*-positive skin test results. Most patients had specific IgE that recognized proteins with molecular weights of 8 to 90 kDa, with great interpatient variability (Fig 1). Figure 2 shows the immunoblot results of 8 individuals who had a positive conjunctival test reaction with the *C arcuatus* extract. The most predominant bands were identified between below 14 and 25, between 30 and 45, and between 46 and 65 kDa. Patient 4 did not show specific IgE binding bands on the immunoblot but experienced a positive conjunctival test reaction at the final dilution used, with a total score of 8.

Conjunctival Provocation Tests With *C arcuatus*

Two groups of patients were challenged. In group 1, conjunctival test results were positive in 9 of 10 individuals, and in group 2, in 2 of 9 (contingency table analysis: $P = .006$). Mean $\pm$ SD symptom scores in group 1 were 7.1 ± 3.1 . Three of these patients were positive at 0.1 mg/mL and 6 at the highest dose administered (1 drop of a 1-mg/mL solution). In group 2, mean $\pm$ SD symptom scores were 1.4 ± 2.0 , and both individuals reacted at the maximum dose. Nasal

symptoms after the conjunctival provocation tests were recorded in 6 patients in group 1 and in 1 in group 2.

Enzyme-Linked Immunosorbent Assay Inhibition Experiments

Figure 3 shows the results of the enzyme-linked immunosorbent assay (ELISA) inhibition experiments to determine cross-reactivity between *C arcuatus* and the remaining mite species. The amount necessary to reach the 50% inhibition point using *C arcuatus* extract in the solid phase was 0.203

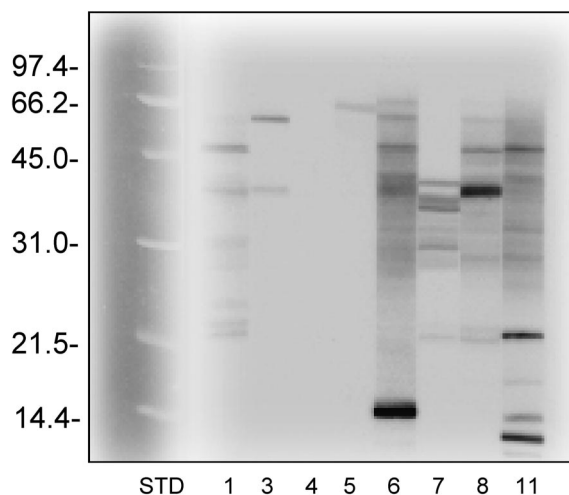


Figure 2. Immunoblots with individual serum samples from 8 patients who had positive conjunctival provocation test reactions with *Chortoglyphus arcuatus*. STD indicates molecular weight standards. No bands are seen in lane 4, which corresponds to a patient with a positive provocation challenge.

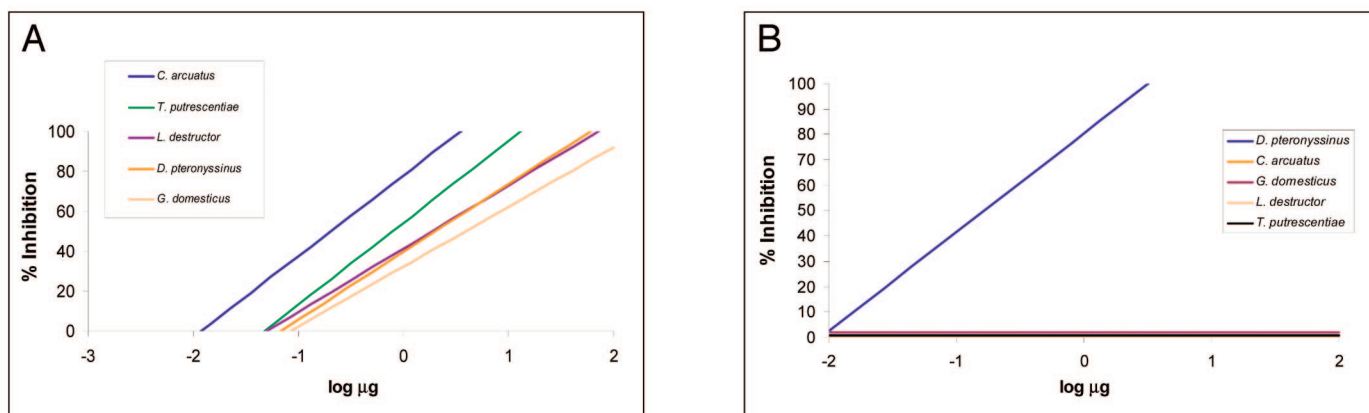


Figure 3. Enzyme-linked immunosorbent assay inhibition experiments in the solid phase using *Chortoglyphus arcuatus* (A) and *Dermatophagoides pteronyssinus* (B).

µg for *C arcuatus* and 2.022 µg for *D pteronyssinus*, suggesting minimal cross-reactivity between extracts. When *C arcuatus* was used on the solid phase, all the analyzed mite species produced inhibition, suggesting a certain degree of cross-reactivity. However, *T putrescentiae*, *L destructor*, *D pteronyssinus*, and *G domesticus* needed 3.87, 9.25, 9.96, and 19.21 times more allergen to produce 50% inhibition. All the curves remained within the limits of parallelism, suggesting a similar allergenic composition, although important differences were observed in the amounts of allergen needed to produce 50% inhibition. Based on these observations, *G domesticus* seems to have the least cross-reactivity with *C arcuatus*. When *D pteronyssinus* was used in the solid phase, *D pteronyssinus* needed 0.168 µg of freeze-dried extract to produce 50% inhibition, whereas the different mite species did not inhibit, suggesting a lack of cross-reactivity between *D pteronyssinus* and the remaining mite species investigated.

DISCUSSION

In the present study, we describe a high rate of sensitization to *C arcuatus* and other species in Galicia. Sensitization to different mite species has been the subject of several publications^{32–35} that have demonstrated a high prevalence of cutaneous sensitivity to different mite species, including storage mites, in this area. In the present study, the prevalence of positive skin test reactions to *D pteronyssinus* was 94.2%; to *G domesticus* was 89.6%, to *L destructor* was 89.1%, to *T putrescentiae* was 87% (1 patient was exclusively sensitized to this species), and to *C arcuatus* was 58% (2 patients were exclusively sensitized to this species). We tried to identify, before ELISA inhibition experiments were conducted, if by skin testing there was a significant association between skin test sensitivity to *C arcuatus* and to the remaining analyzed mite species. We did not detect a significant correlation between sensitization to *C arcuatus* and the remaining mites, suggesting the specificity of the reaction. This finding may have important implications when selecting mite species for diagnosis and immunotherapy.

We also identified a statistically significant correlation between the number of mite species to which patients were sensitized and the time of evolution of the disease. This seems to be a common feature of mite allergy in areas where mites are abundant, as previously suggested.³³ It could be possible that the longer patients are exposed to mite allergens, the more intense becomes the exposure and the greater the probability of becoming sensitized to more species. Therefore, cumulative exposure to mite allergens may be mediating the evolution of the disease. Immunotherapy given early enough to children monosensitized to house dust mites seems to prevent new sensitizations.³⁶

This study confirms the importance of *C arcuatus* as a source of relevant allergens in Galicia. We detected a high prevalence of sensitization to *C arcuatus* in Lugo, which is comparable with that detected in tropical or subtropical areas of the world. These results are in agreement with those obtained by Puerta et al^{25,26} analyzing patients residing in the tropical area of Colombia. The latter study demonstrated minimal cross-reactivity between *C arcuatus* and *Dermatophagoides* species as evaluated by ELISA inhibition experiments, which was confirmed in the present study. However, previous studies have not addressed provocation challenges with *C arcuatus*. The present study confirms the clinical significance of sensitization to *C arcuatus* in *C arcuatus*-sensitized individuals and suggests minimal cross-reactivity, as corroborated by the fact that most patients sensitized to *D pteronyssinus* but negative to *C arcuatus* did not experience a positive conjunctival challenge with *C arcuatus*. In the 2 patients in whom a reaction took place, the reactions were mild and weaker than in *C arcuatus*-positive individuals. We acknowledge that a positive conjunctival challenge result does not imply allergic respiratory disease. However, in the context it was produced, and considering similarities with other mite species, we cannot minimize the possibility that *C arcuatus* is an inducer of allergic rhinoconjunctivitis because 6 of the 9 patients who had a positive challenge reaction to *C*

arcuatus experienced nasal symptoms, to a variable degree, once challenged.

A similar study was conducted by Stanaland et al³⁷ in the United States. These researchers performed nasal provocation tests in 2 groups of mite-allergic patients. One group was positive to *D pteronyssinus* and *Blomia tropicalis* and the second group was positive to *D pteronyssinus* alone and negative to *B tropicalis*. The study confirmed the in vivo allergenicity of *B tropicalis*, a mite species that is now considered to be of allergologic importance in tropical and subtropical environments.³⁸ Because the distribution of *C arcuatus* is worldwide, it would not be surprising if reports on the allergenicity of *C arcuatus* increase with time.

Immunoblot analysis of *C arcuatus* extracts using 31 serum samples from *C arcuatus*-sensitized individuals revealed a variable pattern of allergen recognition. More than 10 allergens were identified, but the overall rate of recognition was low. Eight of the 10 patients who underwent conjunctival challenges provided a blood sample, which was used in the immunoblots. The IgE binding of these sera was similar to that of the remaining individuals who participated in the study.

This study confirms a high prevalence of cutaneous sensitivity to mites in Galicia. Sensitization to *C arcuatus* is common, and 2 monosensitized patients were identified. The clinical significance of sensitization to *C arcuatus* in *C arcuatus*-sensitized individuals with allergic rhinoconjunctivitis with or without asthma was confirmed using conjunctival challenge tests. Sensitization to *C arcuatus* should be considered clinically relevant in areas where this species is present. *Chortoglyphus arcuatus* fulfills Thommen's postulates modified for mites. It is present in the environment in large enough quantities to produce sensitization. Sensitization has been proved by in vivo and in vitro testing. The contact of *C arcuatus* allergens with a sensitized individual produces allergic symptoms, and, therefore, sensitization to this mite species should be considered of clinical significance owing to its lack of cross-reactivity with *D pteronyssinus*.

REFERENCES

- Boquete M, Iraola V, Fernández-Caldas E, et al. House dust mite species and allergen levels in Galicia, Spain: a cross-sectional, multicenter, comparative study. *J Invest Allergol Clin Immunol*. 2006. In press.
- O'Connor BM. Acari: astigmata. In: Parker S, ed. *Synopsis and Classification of Living Organisms*. Vol 2. New York, NY: McGraw-Hill; 1982:146–169.
- Hughes AM. *The Mites of Stored Food and Houses*. London, England: Ministry of Agriculture, Fisheries, and Food; 1977: 185–187. Technical Bulletin 9.
- Franz JT, Masuch G, Musken H, Bergmann KC. Mite fauna of German farms. *Allergy*. 1997;52:1233–1237.
- Fernández-Caldas E, Fox RW, Trudeau WL, Bucholtz GA, Ledford DK, Lockey RF. House dust mite allergy in Florida: mite survey in homes of mite allergic individuals. *Allergy Proc*. 1990;11:263–267.
- Nelson HS, Fernández-Caldas E. Prevalence of house dust mites in the Rocky Mountain states. *Ann Allergy Asthma Immunol*. 1995;75:337–339.
- Ronald NC, Enns WR. A preliminary survey of mites in poultry litter in Missouri. *J Kansas Entomol Soc*. 1971;44:397–399.
- Franjola R, Malonnek M. Housedust mites in the city of Valdivia, Chile [in Spanish]. *Bol Chil Parasitol*. 1995;50: 16–20.
- Croce M, Costa-Manso E, Baggio D, Croce J. House dust mites in the city of Lima, Peru. *J Invest Allergol Clin Immunol*. 2000;10:286–288.
- da Silva Ezequiel O, Gazeta GS, Amorim M, Serra-Freire NM. Evaluation of the acarofauna of the domiciliary ecosystem in Juiz de Fora, State of Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz*. 2001;96:911–916.
- Fernández-Caldas E, Puerta L, Mercado D, Lockey RF, Caraballo LR. Mite fauna, Der p I, Der f I and *Blomia tropicalis* allergen levels in a tropical environment. *Clin Exp Allergy*. 1993;23:292–297.
- Armentia A, Martínez A, Castrodeza R, et al. Occupational allergic disease in cereal workers by stored grain pests. *J Asthma*. 1997;34:369–378.
- Blasco Sabio C, Gallego J, Portús M. Estudio de la acarofauna del polvo doméstico de Barcelona y poblaciones circundantes. *Allergol Immunopathol*. 1975;III:403–418.
- Vidal C, Chomon B, Perez-Carral C, Gonzalez-Quintela A. Sensitization to *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Acarus siro* in patients allergic to house dust mites (*Dermatophagoides* spp.). *J Allergy Clin Immunol*. 1997;100: 716–718.
- Sánchez-Covisa A, Rodríguez-Rodríguez JA, de la Torre F, García R. Fauna d'acariens de la pousière domestique dans l'île de Tenerife. *Acarologia*. 1999;XL:55–58.
- Marx JJ Jr, Twiggs JT, Ault BJ, Merchant JA, Fernández-Caldas E. Inhaled aeroallergen and storage mite reactivity in a Wisconsin farmer nested case-control study. *Am Rev Respir Dis*. 1993;147:354–358.
- Yadav A, Elder BL, Morgan MS, Vyszynski-Moher DL, Arlian LG. Prevalence of serum IgE to storage mites in a southwestern Ohio population. *Ann Allergy Asthma Immunol*. 2006;96: 356–362.
- Trudeau W, Fernández-Caldas E, May J, Pratt D, Lockey R. Allergenicity of the mite *Chortoglyphus arcuatus*. *J Allergy Clin Immunol*. 1990;85:183.
- Rizzo MC, Fernández-Caldas E, Sole D, Nasipitz CK. IgE antibodies to aeroallergens in allergic children in Sao Paulo, Brazil. *J Invest Allergol Clin Immunol*. 1997;7:242–248.
- Di Berardino L, Angrisano A, Gorli L, Cattaneo M, Lodi A. Allergy to house dust and storage mites in children: epidemiologic observations. *Ann Allergy*. 1987;59:104–106.
- Mumcuoglu Y. Immunologic investigations of house-dust and house-dust mites, I: Schultz-Dale test (author's transl) [in German]. *Allerg Immunol (Leipz)*. 1977;23:107–110.
- Musken H, Franz JT, Wahl R, et al. Sensitization to different mite species in German farmers: clinical aspects. *J Invest Allergol Clin Immunol*. 2000;10:346–351.
- Neffen HE, Fernández-Caldas E, Predolini N, Trudeau WL, Sanchez-Guerra ME, Lockey RF. Mite sensitivity and exposure in the city of Santa Fe, Argentina. *J Invest Allergol Clin Immunol*. 1996;6:278–282.
- Calvo M, Fernández-Caldas E, Arellano P, Marin F, Carnés J, Hormaechea A. Mite allergen exposure, sensitisation and clin-

- ical symptoms in Valdivia, Chile. *J Investig Allergol Clin Immunol*. 2005;15:189–196.
25. Puerta L, Fernández-Caldas E, Lockey RF, Caraballo LR. Sensitization to *Chortoglyphus arcuatus* and *Aleuroglyphus ovatus* in *Dermatophagoides* spp. allergic individuals. *Clin Exp Allergy*. 1993;23:117–123.
26. Puerta L, Fernández-Caldas E, Lockey RF, Caraballo LR. Mite allergy in the tropics: sensitization to 6 domestic mite species in Cartagena, Colombia. *J Investig Allergol Clin Immunol*. 1993;3:198–204.
27. Nelson RP Jr, DiNicolo R, Fernández-Caldas E, Seleznick MJ, Lockey RF, Good RA. Allergen-specific IgE levels and mite allergen exposure in children with acute asthma first seen in an emergency department and in nonasthmatic control subjects. *J Allergy Clin Immunol*. 1996;98:258–263.
28. Fernández-Caldas E, Baena-Cagnani CE, Lopez M, et al. Cutaneous sensitivity to six mite species in asthmatic patients from five Latin American countries. *J Investig Allergol Clin Immunol*. 1993;3:245–249.
29. García-Robaina JC, Eraso E, De la Torre F, et al. Extracts from various mite species contain cross-reactive and noncross-reactive IgE epitopes: a RAST inhibition study. *J Investig Allergol Clin Immunol*. 1998;8:285–289.
30. Bonini S, Berruto A, Tomassini M, Carlesimo S, Bucci MG, Balsano F. Conjunctival provocation test as a model for the study of allergy and inflammation in humans. *Int Arch Allergy Appl Immunol*. 1989;88:144–148.
31. Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature*. 1970;227:680–685.
32. Vidal C, Boquete O, Gude F, et al. High prevalence of storage mite sensitization in a general adult population. *Allergy*. 2004;59:401–405.
33. Boquete M, Carballada F, Armisen M, et al. Factors influencing the clinical picture and the differential sensitization to house dust mites and storage mites. *J Investig Allergol Clin Immunol*. 2000;10:229–234.
34. Marcos Bravo C, Luna Ortiz I, Outon A, González Vázquez SR. Allergy to storage mites. *Allergy*. 1999;54:769–770.
35. González de la Cuesta C. Estudio retrospectivo de sensibilización a ácaros en pacientes con alergia respiratoria en Orense. *Rev Esp Alergol Inmunol Clin*. 1998;13:15–18.
36. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children mono-sensitized to house dust mite by specific immunotherapy: a six-year follow-up study. *Clin Exp Allergy*. 2001;31:1392–1397.
37. Stanaland BE, Fernández-Caldas E, Jacinto CM, Trudeau WL, Lockey RF. Positive nasal challenge responses to *Blomia tropicalis*. *J Allergy Clin Immunol*. 1996;97:1045–1049.
38. Fernández-Caldas E, Lockey RF. *Blomia tropicalis*, a mite whose time has come. *Allergy*. 2004;59:1161–1164.

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