

Psocoptera spp. (book louse): a new major household allergen in Mumbai

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Background: Exposure and sensitization to indoor allergens of house dust mites and insects is well established. However, there are no reports on the allergenicity of *Psocoptera* spp. (book louse). These insects are known to inhabit various terrestrial habitats such as tree bark (bark lice), old papers, books, stored food products, etc and gain entry into human dwellings through these modes. They have the potential to sensitize individuals with an occupational exposure to them.

Objective: Our objective is to use clinical and immunologic techniques to evaluate the extent of sensitization to *Psocoptera* in patients with nasobronchial allergy. We also have the objective to determine the extent of *Psocoptera* infestation in houses in Mumbai (Bombay).

Methods: An entomologic analysis of dust samples in houses in Mumbai was carried out and *Psocoptera* found in these samples was mass-cultured. Two hundred patients were checked for allergy to the *Psocoptera* extracts using skin test. Total IgE and specific IgE to *Psocoptera* in sera from *Psocoptera*-sensitive patients' sera were estimated by enzyme immunoassay. IgE-binding proteins of *Psocoptera* were determined by immunoblotting experiments.

Results: Among the 75 house dust samples from Mumbai analyzed for the presence of *Psocoptera*, 25% were found to be infested by *Psocoptera*. Strong skin sensitivity to *Psocoptera* was found in 20% of patients. Total IgE levels >150 IU/mL were found in these patients and high levels of specific IgE to *Psocoptera* were detected by ELISA. Immunoblot analysis revealed 67, 59, 43, and 27 kD proteins as major allergens of *Psocoptera*.

Conclusions: *Psocoptera* is an important allergen in Mumbai as it causes IgE mediated responses in 20% of the allergic population studied.

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INTRODUCTION

Indoor allergens contributed by several entities such as dust mites, insects, animal dander, pollen, and fungi are known to induce IgE-mediated responses in exposed and sensitized individuals. House dust mites and insects belong to the common phylum Arthropoda. Allergens of house dust mites and insects are the major agents of sensitization, which can cause allergic respiratory symptoms in a number

of cases. Changes in lifestyles of people throughout the world have further encouraged the growth of these arthropods and have increased allergen exposure. Allergenicity of dust mites of the Pyroglyphidae family, storage mites of Glycyphagidae family, and common indoor insects such as cockroaches has been extensively studied.^{1–3} An interesting insect known to infest houses is *Psocoptera*, or book louse. This insect is known to infest papers, books, and stored food products.^{4,5} Newspaper vendors, bookstall workers, printing press workers, and library staff also have an increased exposure to *Psocoptera*. Early studies on *Psocoptera* dealt mainly with the morphologic and genetic aspects of the insect.^{6–8} This is the first study in which 200 patients with nasobronchial

allergy were evaluated for possible *Psocoptera* sensitization using clinical and immunochemical techniques such as skin prick test, ELISA, and immunoblotting.

Description of *Psocoptera*

Psocoptera or Corrodentia insects are found in various terrestrial habitats such as tree bark (bark lice), decaying vegetable matter, bird nests, and several of the wingless species occur in human habitations, particularly in old papers and books; hence the name book lice. *Psocoptera* range in length from 1.5 to 5 mm. Most of these insects have a protective coloration. This group is distributed worldwide with nearly 2,000 species. *Psocoptera* possess the ability to digest cellulose. Therefore, they feed on moldy foods and bookbindings, which supply them with required starch or fungus mycelium. Sometimes they can become abundant in stored grains, leading to considerable wastage. They cause damage to libraries by consuming the starch in the bindings of the books, along the edges of papers, and defacing titles, thus making rebinding and repairing necessary. Some other species of *Psocoptera* are known to destroy preserved museum specimens.^{4,5}

Taxonomic position of the *Psocoptera* species

Phylum	: Arthropoda
Class	: Insecta
Sub-class	: Pterygota
Division	: Exopterygota
Order	: Psocoptera or Corrodentia
Family	: Liposcelididae
Genus	: Liposcelis
Species	: <i>Liposcelis divinatorius</i> ^{4,5,9}

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MATERIALS AND METHODS

Patients

Two hundred consecutive patients attending two different asthma and allergy clinics in Mumbai (Bombay) were recruited for this study. Case history of patients was obtained with the help of a standard questionnaire. All the patients had symptoms of asthma as well as rhinitis. Physical examination of these patients with nasobronchial symptoms was followed by pulmonary function testing (spirometer, Med Science, St. Louis, MO) and modified skin prick test.¹⁰ The control group consisted of 15 nonatopic, healthy volunteers and 10 atopic patients showing negative skin reaction to Psocoptera extract but positive reaction to one or more of the allergens tested. Informed consent was obtained from each patient before his enrollment in the study (Informed consent approved by review board of Medical Research Society, Sir. H. N. Hospital).

Dust Samples Analysis

Dust samples were collected from houses of patients having a history of dust allergy. Samples were collected from the floor as well as from mattresses and/or carpets. Samples were transported in small sterile glass bottles fitted with tight plugs of nonabsorbent cotton. Each sample was filtered through a 1-mm mesh sieve to remove the large particles and fibers from the dust. This filtered dust was spread evenly on the center of a Petri dish and kept undisturbed for 5 to 10 minutes. Psocoptera were found to have a tendency to migrate toward the periphery of the Petri dish. Since their bodies are heavy compared with mites, they are unable to climb the walls of the Petri dish and they can be picked up from the Petri dish with the help of a camel brush. However, since the insects move with a great speed, the task of isolating them is difficult. Permanent mounts of Psocoptera were prepared for entomologic identification. They were first cleared in 70% ethanol and mounted in Hoyer's media.¹¹

Antigen extracts

The *Psocoptera* spp., which were selectively isolated from the dust samples, were cultured in 100-mL conical flasks containing supplemented yeast powder sterilized at 70° C as cultured medium. After 3 weeks, the insects isolated from the food medium with an in-house modified Tullgren apparatus¹¹ were killed by deep freezing for 24 hours. They were vacuum-desiccated for another 24 hours. The insect bodies were defatted for 4 hours using diethyl ether in a Soxhlet apparatus (Borosil Co, Calcutta, India) and traces of ether were removed by drying. Psocoptera proteins were extracted (1:20 wt/vol) in 0.2 mol/L of ammonium bicarbonate for 24 hours at 4° C followed by centrifugation at 18,000 rpm (23,940 g). The supernatant was dialyzed overnight at 4° C against deionized water. The dialyzed extract was recentrifuged, filtered with 0.22 µm Millipore filter (Millipore Corp., Bedford, MA) and stored at -20° C until further use. For skin-testing, fat-free Psocoptera bodies were extracted in the same buffer containing 50% glycerol.

Skin Testing

All the 200 patients included in the study were skin-tested by modified skin prick method using a standard battery of allergens (E-Merck, Indore, India) and the Psocoptera extract. Histamine (1 mg/mL) was used as positive control and glycerinated saline as negative control. Skin test reactions were

read after 15 minutes of testing. Wheal sizes of 3 to 6 mm, were graded as 3+ reactions and wheal >6 mm or pseudopod formation were graded as 4+ reactions. Sera of these skin test-positive patients and of the control volunteers were collected for further in vitro investigations.¹⁰

IN VITRO INVESTIGATIONS

Sodium Dodecyl Sulfate

Polyacrylamide Gel Electrophoresis and Immunoblotting

Total protein content of the extract of Psocoptera was determined by the method of Bradford.¹² Laemmli's sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 12.5% gel) was used for the separation of Psocoptera proteins.¹³ Electroblood transfer of these proteins and immunoblot analysis was performed as described by Towbin et al.¹⁴ Horse radish peroxidase (HRP)-conjugated goat antihuman IgE (Sigma, St. Louis, MO) was used as a secondary antibody and the IgE-binding components were visualized using diaminobenzidine substrate with hydrogen peroxide in phosphate buffer saline (PBS), pH7.4.

Total and Allergen-Specific IgE Estimation

Total IgE in the patients' sera was estimated in terms of IU/mL by the Quorium enzyme immunoassay method (Quorium Diagnostics, Glaxo, India) with standards supplied by the manufacturers. Polystyrene microtitration wells (Nunc, Roskilde, Denmark) were coated



Figure 1. Psocoptera species, *Liposcelis divinatorius* (L.d.).

Table 1. Skin Sensitization to *D. farinae*, *B. tropicalis*, Cockroach, and Psocoptera

Total patients	<i>D. farinae</i>	<i>B. tropicalis</i>	Cockroach	Psocoptera
200	57 (29%)	45 (23%)	62 (31%)	39 (20%)

with Psocoptera allergen extract (5 µg) in sodium carbonate buffer, pH 9.6. ELISA for allergen specific IgE estimation was performed by Voller and Bidwell's method.¹⁵ HRP-conjugated goat antihuman IgE (Sigma) was used as secondary antibody and the substrate was o-phenylenediamine with hydrogen peroxide. Absorbance was measured at 492 nm (Nunc ELISA reader).

RESULTS

Analysis of dust samples from houses of 75 patients with a history of dust allergy, revealed presence of the insect Psocoptera (book louse) species, *Li-poscelis divinatorius* (L.d.) in 19 (25%) houses in the city (Fig 1). These insects were observed to be wingless, light-brown colored, and fast-moving. Four (5%) of the samples were heavily infested with these insects. *Dermatophagoides* mites were found in highest abundance, ie, 36 (48%) houses and *B. tropicalis* was found in 30 (40%) houses.

Two hundred consecutive subjects with nasobronchial allergy were skin-tested and 39 (20%) subjects were observed with strong positive skin reactions to Psocoptera. The prevalence of positive skin test reactions to *Dermatophagoides* species, *D. farinae* (Df) was 57 (29%), to cockroach was 62 (31%), and to *B. tropicalis* was 45 (23%; Table 1). Of the 39 patients showing strong sensitivity to Psocoptera, 25 (62.5%) also reacted concomitantly to Df, 15 (38.5%) to cockroach, and 13 (32.5%) reacted to paper dust. Among all the mites and insects tested, exclusive skin sensitivity to Psocoptera was observed in 17 (9%) subjects. The 15 nonatopic control subjects had negative skin test reactions to all the aeroallergens tested. The skin test results of 39 Psocoptera-sensitive patients are presented in Table 2.

The protein content of L.d. extract was found to be 2.5 mg/mL. SDS-

PAGE of L.d. revealed 13 bands with a molecular weight range of 90 to 15 kD (Fig 2). Immunoblots identified 8 IgE-binding protein bands on probing with HRP-conjugated goat antihuman IgE followed by addition of substrate and color reagent. The strongest reactions

were to components at 67, 59, 43, and 27 kD. Minor allergenic components at 85, 32, and 30 were also identified in 10 sera. No IgE binding components were observed when control sera were used (Fig 3).

Total serum IgE was significantly higher in asthmatic patients as compared with the negative controls. Thirty-seven of 39 patients had elevated total IgE levels >150 IU/mL. Similarly, 8 of 10 atopic controls showed high levels of total IgE, whereas very

Table 2. Data Showing Skin Sensitivity Pattern in the Psocoptera-Sensitive Patients' Sera

Sr. No.	Patient's name	<i>D. farinae</i>	Paper dust	Cockroach
1	M.J.	3+	—	—
2	S.J.	3+	3+	—
3	S.S.	—	—	—
4	S.B.	3+	3+	—
5	M.K.	—	—	—
6	S.L.	—	3+	—
7	M.I.	3+	3+	3+
8	A.K.	3+	—	—
9	H.P.	3+	3+	3+
10	P.G.	4+	—	—
11	S.M.	2+	—	—
12	K.K.	—	—	3+
13	S.S.	3+	—	3+
14	C.S.	3+	—	3+
15	D.D.	3+	—	—
16	P.V.	3+	3+	—
17	G.S.	4+	3+	3+
18	A.R.	3+	3+	3+
19	K.M.	3+	—	—
20	R.K.	3+	3+	3+
21	D.C.	—	—	3+
22	A.U.	—	—	3+
23	U.R.	—	—	—
24	P.J.	3+	—	—
25	B.K.	3+	—	3+
26	A.B.	3+	—	3+
27	S.J.	3+	3+	3+
28	P.K.	—	—	3+
29	G.C.	3+	—	—
30	C.S.	3+	—	—
31	K.S.	2+	3+	—
32	S.J.	—	—	—
33	A.B.	2+	2+	—
34	U.G.	—	—	3+
35	A.J.	3+	2+	—
36	A.K.	3+	2+	—
37	R.P.	—	—	—
38	T.S.	3+	3+	—
39	S.B.	3+	3+	—

Wheal size diameter: 3–6mm, 3+ reaction; > 6mm with pseudopod formation, 4+ reaction. *D. farinae*, paper dust and cockroach antigen supplied by E-Merck, Indore, India.

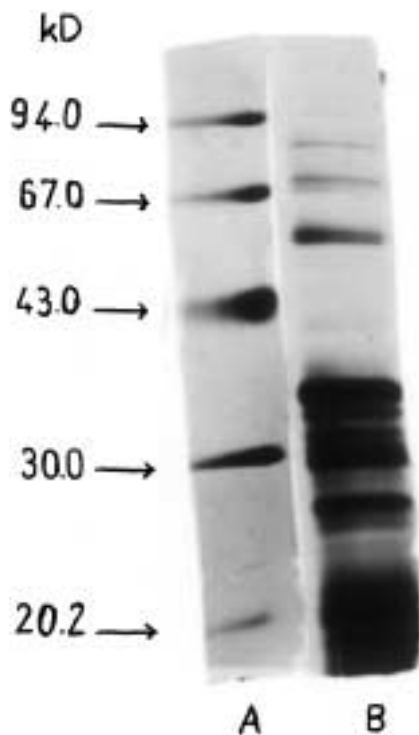


Figure 2. *Psocoptera* sp. L.d. proteins separated by SDS-PAGE on a 12.5% gel and silver stained. Lane A: Molecular weight marker (Pharmacia) and Lane B: *Psocoptera* proteins.

low levels (<150 IU/mL) were found in nonatopic controls. High levels of specific IgE to *Psocoptera* were found in the patient's sera as compared with the control sera. Undetectable levels of specific IgE to *Psocoptera* were seen in the control group (Fig 4).

DISCUSSION

House dust is a conglomeration of dust mites, storage mites, and insects such as cockroaches, which generate allergens. This study has highlighted the importance of the dust entity *Psocoptera* (book lice) for the first time. The infestation of houses in Mumbai by *Psocoptera* can be attributed to various factors. Books and papers harboring these insects could serve as vehicles for *Psocoptera* to enter the dwellings. Many Indians generally make an annual stock of food-grains during summer. To prevent the infestation or growth of insects during this storage period, the food-grains are dried at

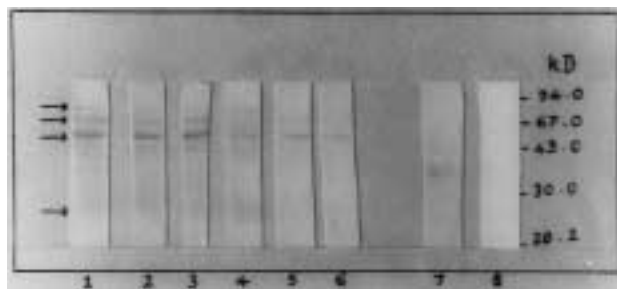


Figure 3. Immunoblotting to detect specific IgE in patients sera against *Psocoptera* sp. (L.d.) proteins. Lanes 1 to 6, patient's sera; Lane 7 atopic, L.d. negative; and Lane 8, nonatopic control sera.

least two to three times in strong sunlight, sieved, and stored in airtight containers. However, the food-grains are not immediately heat-dried after they are brought in the houses. Hence, insects such as *Psocoptera*, if present in the food-grains, can easily gain entry into homes during this time. Housing conditions can also play an important role in *Psocoptera* infestation. Houses where books and papers are not stored in shelves or where high humidity conditions encourage mold growth could provide an environment conducive to the proliferation of these insects.

The book louse or *Psocoptera* can be considered an important dust constituent of houses in Mumbai for two reasons. Firstly, 25% of the residences of dust allergy patients from the city are infested by *Psocoptera*, and also 20%

of the allergic population studied are sensitized to the allergens of this insect. The probable role of *Psocoptera* in sensitizing school children and in childhood asthma also needs to be investigated. Further, newspaper vendors, book-stall workers, library workers, etc, are at risk of an occupational exposure to the *Psocoptera* allergens. Thus, sensitization to these allergens can prove doubly hazardous for certain individuals.

Biochemical analysis demonstrates multiple allergenic proteins of *Psocoptera* in the 30 to 80 kD molecular weight region. In addition to this, high levels of *Psocoptera*-specific IgE have been observed in the patient's sera. Thus, sensitization to the *Psocoptera* allergens can imply important consequences for individuals sensitized to

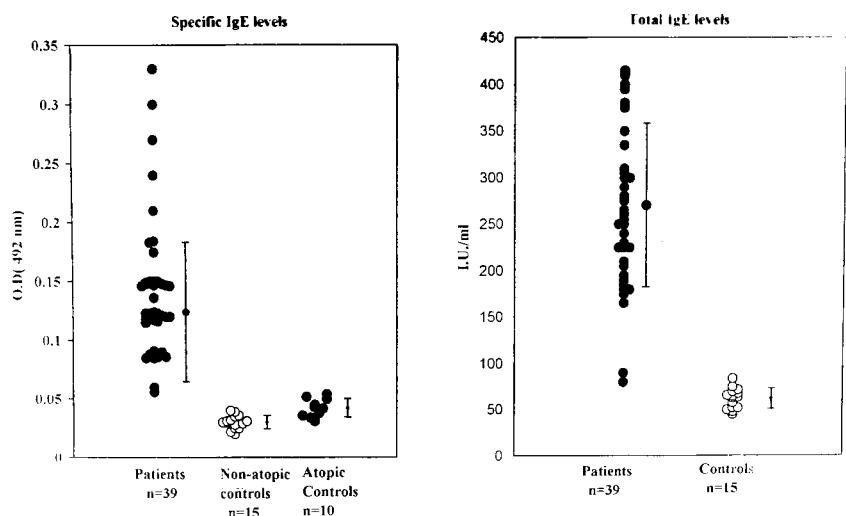


Figure 4. Levels of total IgE and specific IgE to *Psocoptera* sp. L.d. proteins in sera of patients. *Psocoptera*-sensitive patients, N = 39; nonatopic controls, N = 15; and atopic controls, N = 10.

these allergens. The entomologic, clinical, and immunochemical results of this study can be applicable to other regions in subtropics with similar geographic and living conditions.

CONCLUSION

The present study has established the hitherto unknown allergenic nature of the book louse, Psocoptera. Earlier data from our clinics in Mumbai suggested highest IgE-mediated responses in asthma and allergy population toward cockroach, followed by *Dermatophagoides* mites, then to storage mites like *B. tropicalis*, and pollen and fungi rated next. However, clinical and immunochemical results of the present study have established Psocoptera as an insect of major (20% sensitization) clinical significance rating as the fourth important sensitizer and being a more potent aeroallergen than pollen and fungi. Also, the immunoblotting and specific IgE responses to Psocoptera have further confirmed that Psocoptera is indeed a major cause of sensitization among nasobronchial allergy patients of Mumbai. Therefore, it is proposed that Psocoptera needs to be included in the panel of allergens in countries such as India. The 12.5 million population of Mumbai is mainly cosmopolitan with people coming to reside from different regions in India and, therefore, the findings of this study can be possibly extrapolated to the Indian population in general. In view of the newly discovered allergenic potential of Psocoptera, studies of the benefits of immunotherapy against these organisms are indicated. Control of these organisms by insecti-

cide or environment manipulation should also be investigated.

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