

## Original article

# Differences in mite fauna between the continental and Mediterranean climates of Croatia: microscopy and Dustscreen™ test findings

**Background:** The aim of this study was to evaluate the difference between the mite fauna in the inland and coastal Croatia.

**Methods:** Ninety-nine floor house dust samples were collected: 28 from the coastal area, 31 from the inland rural and 40 from the inland urban area. Data on basic household characteristics were collected for 81 of 99 households. The mites were separated from the dust using the floatation method and then microscopically identified. The levels of *Dermatophagoides pteronyssinus* (Der p) 1, *Dermatophagoides farinae* (Der f) 1 and Der 2 were measured using the Dustscreen™ test. Ordinal descriptive statistics and nonparametric tests were used for data analysis, assuming statistical significance at  $P < 0.05$ .

**Results:** More than 70% of mites identified in all areas were pyroglyphids. Nonpyroglyphid mites accounted for 20–25% of all identified mites in the coastal areas (*Blomia*, *Lepidoglyphus* and *Glycyphagus*) and with 15% in the inland areas (*Lepidoglyphus* and *Acarus*). The highest Der p 1 median levels were found in the coastal area, with significantly lower levels in the inland rural and inland urban areas (4.5; 2; 0.85 µg/g of dust;  $P = 0.0001$ ). The highest Der f 1 median levels were found in the inland urban area, with significantly lower levels in the inland rural and coastal areas (0.88; 0.75; 0 µg/g of dust;  $P = 0.0013$ ). The levels of Der f 1 were significantly higher in samples taken from households with central heating than from those with traditional heating (1; 0.25 µg/g of dust;  $P < 0.01$ ).

**Conclusions:** Separate diagnostic dust mite lists for the coastal and inland areas because of climatic and dwelling differences required to be implemented. Compared with traditional heating, central heating significantly increases the risk of exposure to Der f 1 levels  $> 2$  µg/g of dust (odds ratio, 7.35; 95% confidence interval, 1.43–37.87;  $P = 0.01$ ).

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Key words: central heating; continental climate; Der f 1; Der p 1; domestic dust samples; house dust mites; indoor environment; immunodot; Mediterranean climate; nonpyroglyphid mites.

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Accepted for publication 5 March 2003

Dust mites are generally acknowledged as the most important sources of indoor allergens all over the world (1, 2). Mite fauna in Croatia has not yet been investigated. As Croatia has several climates (continental, Mediterranean and mountain), the inter-regional differences in mite populations can be expected. Moreover, our recent study showed a similar frequency of sensitization to pyroglyphid and nonpyroglyphid mites in urban populations of Croatia (3), corroborating the findings of similar studies in other countries (4–6). It is not clear whether these findings are the result of cross-reactivity between mite families (7) or are the consequence of exposure to nonpyroglyphid mites in urban indoor environment.

The aim of this study was to analyse if there is a difference in mite populations between the inland and coastal Croatia and to determine the levels of

pyroglyphid mite allergens in house dust samples from the areas with different climates and different heating.

## Material and methods

### Geographical and meteorological data

Zagreb and its rural surrounding situated in the north-west of the inland Croatia has a continental climate. Zadar and its rural surrounding situated on the Adriatic coast has Mediterranean climate. The 10-year data (1991–2000) obtained from the Croatian Meteorological and Hydrological Service, revealed that the monthly average relative humidity in both areas were between 70 and 90% throughout the year, and average air temperature was 11.3°C in Zagreb and 15.4°C in Zadar, with the most prominent differences between the two areas occurring in winter (November–February).

### Dust sample collection

House dust samples were collected from the floors of bedrooms and living rooms in the coastal (Zadar) and inland (Zagreb) areas of Croatia in February and March 2001, using the Dustscreen™ (Heska AG, Frieburg, Switzerland) vacuum cleaner adapter and filter. The dust was vacuumed until the filters were full. Filters were kept frozen at  $-18^{\circ}\text{C}$  until analysis carried out (8, 9). Dust samples from 99 houses were collected: 14 from the urban coastal area, 14 from the rural coastal area, 31 from the inland rural area and 40 from the inland urban area. We used a simple questionnaire to collect data from 81 of 99 sampled households. It included questions on the type and age of the building, heating system, furniture, mattresses and pillows. Heating was categorized as either traditional (using wood/coal, oil, electric power or gas stoves) or central. All heating systems in the coastal area ( $N = 24$ ) were traditional. Twenty-three and 34 heating systems in inland areas were traditional and central, respectively.

### Dust sample analysis

Each dust sample was divided into two, weighing  $100 \pm 1$  mg. One was used for the microscopic identification of mite species and the other for Dustscreen™ test measurements.

Mites were identified by light microscopy in 44 dust samples: seven from the coastal urban area, 11 from the coastal rural area, eight from the inland urban area and 18 from the inland rural area. The mites were separated from the dust using a modified floatation method by Hart & Fain (10). Microscopic preparations were made with Hoyer's medium and mite species were identified by comparing them with the pictorial identification keys for the domestic mites (11) and with the permanent preparations of mite cultures (C.B.F.LETI, S.A., Madrid, Spain).

The Dustscreen™ test was used to measure the levels of *Dermatophagoides pteronyssinus* (Der p) 1, *Dermatophagoides farinae* (Der f) 1 and Der 2 (equal mixture of Der p 2 and Der f 2) in all 99 dust samples applying the standard procedure described elsewhere (12, 13). Optical density was read in arbitrary units using an original densitometer (FAG VIPDENS 111, FAG Lausanne, Switzerland). Corresponding concentrations were obtained using a standard curve for each allergen. According to the quantity of dust sample and the volume of extraction buffer, the results were expressed as  $\mu\text{g/g}$  of dust.

### Statistical analysis

Statistica for Windows, Kernel release 5.5A was used for data analysis. As the values were not normally distributed, we had to use the methods of ordinal descriptive statistics and nonparametric tests for testing differences (chi-square test, Kruskal–Wallis ANOVA and

Kolmogorov–Smirnov test). Odds ratio was calculated by using the logistic regression. The statistical significance was assumed at  $P < 0.05$  (14).

### Results

A total of 460 mites were identified (see Table 1). Pyroglyphid mites dominated in all areas accounting for  $>70\%$  of all identified mites. *Dermatophagoides pteronyssinus* was the most common mite in all areas ( $>50\%$  of all identified mites). *Dermatophagoides farinae* was more common in the inland urban than in the coastal rural area (13%, 1.9%;  $P = 0.0002$ ) and in the inland rural than in the coastal either urban (17.6%, 5.6%;  $P = 0.0043$ ) or rural (17.6%, 1.9%;  $P = 0.0043$ ) areas. *Euroglyphus maynei* was more common in the urban than in the rural areas (inland: 19.4%, 7.4%;  $P = 0.005$ ; coastal: 8.3%, 0.9%;  $P = 0.0234$ ). It was also more common in the inland than in the coastal areas (urban: 19.4%, 8.3%;  $P = 0.018$ ; rural: 7.4%, 0.9%;  $P = 0.036$ ). Nonpyroglyphid mites were the most common in the coastal areas, accounted for 25% of all identified mites in the rural and 20.4% in the urban coastal area. In the coastal region, the most frequent nonpyroglyphid mite genera were *Blomia*, *Lepidoglyphus* and *Glycyphagus* (about 10%). Inland, the most common nonpyroglyphid mite genera were *Tarsonemus*, *Lepidoglyphus* and *Acarus* (about 5%).

Der p 1 had the highest levels (median) in dust samples from the coastal area, and significantly lower levels in samples from the inland rural and the inland urban areas (4.5; 2; 0.85  $\mu\text{g/g}$  of dust;  $P = 0.0001$ ). The levels of Der f 1 were the highest in dust samples from the urban inland area, and significantly lower in samples from the inland rural and coastal areas (0.88; 0.75; 0  $\mu\text{g/g}$  of dust;  $P = 0.0013$ ). The levels of Der 2 showed the same dynamic as Der p 1 levels, with the highest values in dust samples from the coast, and significantly lower values in dust samples from the inland rural and the inland urban areas (0.8; 0.5; 0.2  $\mu\text{g/g}$  of dust;  $P = 0.0002$ ) (Table 2).

According to the type of heating, the level of Der f 1 was significantly higher in dust samples taken from households with central heating than from those with traditional heating (1; 0.25  $\mu\text{g/g}$  of dust;  $P < 0.01$ ). Such

Table 1. The results of microscopic mite identification

| Region          | Number of mites (%) | Pyroglyphid mites (%) |           |           |            | Nonpyroglyphid mites (%) |           |         |          |           |         |         |           |
|-----------------|---------------------|-----------------------|-----------|-----------|------------|--------------------------|-----------|---------|----------|-----------|---------|---------|-----------|
|                 |                     | DP                    | DF        | EM        | Total      | LD                       | Bs        | TP      | As       | Gs        | Ch      | Tar     | Total     |
| CU (7 samples)  | 108 (100%)          | 71 (65.7)             | 6 (5.6)   | 9 (8.3)   | 86 (79.6)  | 1 (0.9)                  | 6 (5.6)   | –       | –        | 11 (10.2) | 2 (1.9) | 2 (1.9) | 22 (20.4) |
| CR (11 samples) | 108 (100%)          | 78 (72.2)             | 2 (1.9)   | 1 (0.9)   | 81 (75.0)  | 11 (10.2)                | 11 (10.2) | 2 (1.9) | –        | –         | –       | 3 (2.8) | 27 (25.0) |
| IU (8 samples)  | 108 (100%)          | 57 (52.8)             | 14 (13.0) | 21 (19.4) | 92 (85.2)  | 5 (4.6)                  | –         | 1 (0.9) | –        | –         | 2 (1.9) | 8 (7.4) | 16 (14.8) |
| IR (18 samples) | 136 (100%)          | 80 (58.8)             | 24 (17.6) | 10 (7.4)  | 114 (83.8) | 5 (3.7)                  | –         | 1 (0.7) | 10 (7.4) | –         | –       | 6 (4.4) | 22 (16.2) |

CU: coastal-urban; CR: coastal-rural; IU: inland-urban; IR: inland-rural; DP: *Dermatophagoides pteronyssinus*; DF: *Dermatophagoides farinae*; EM: *Euroglyphus maynei*; LD: *Lepidoglyphus destructor*; Bs: *Blomia* species; TP: *Tyrophagus putrescentiae*; As: *Acarus* species; Gs: *Glycyphagus* species; Ch: *Cheilatus* species; Tar: Tarsonemidae.

Table 2. The levels of pyroglyphid mite allergens

| Region                    | Der p 1 ( $\mu\text{g/g}$ ) median<br>(interquartile range) [range] | Der f 1 ( $\mu\text{g/g}$ ) median<br>(interquartile range) [range] | Der 2 ( $\mu\text{g/g}$ ) median<br>(interquartile range) [range] |
|---------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------|
| Coastal ( $N = 28$ )      | 4.5 (1.5–8.5) [0–25]                                                | 0 (0–0.3) [0–20]                                                    | 0.8 (0.325–2.25) [0–4.2]                                          |
| Inland-urban ( $N = 40$ ) | 0.85 (0.175–2.45) [0–10]                                            | 0.88 (0.25–3.35) [0–6]                                              | 0.2 (0–0.375) [0–1.3]                                             |
| Inland-rural ( $N = 31$ ) | 2 (0–20) [0–20]                                                     | 0.75 (0.65–1) [0–12]                                                | 0.5 (0.1–0.9) [0–3.5]                                             |

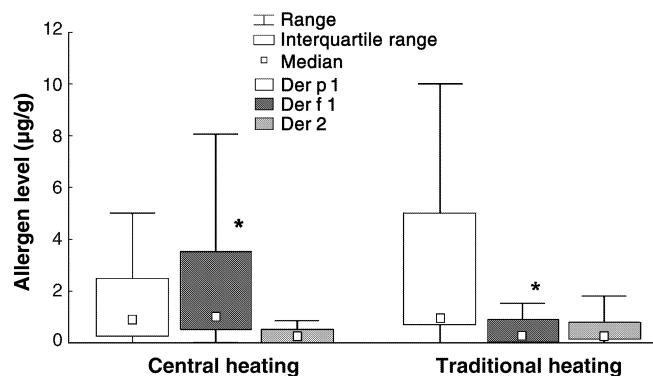


Figure 1. Pyroglyphid mite allergen levels according to the type of heating. \* $P < 0.01$ .

difference was not found for Der p 1 and Der 2 (Fig. 1). Central heating increases the risk of exposure to Der f 1 levels  $> 2 \mu\text{g/g}$  of dust compared with traditional heating (81 dust samples from both regions: odds ratio, 5.88; 95% confidence interval, 1.82–18.94;  $P = 0.003$ ; 57 dust samples from the inland area only: odds ratio, 7.35; 95% confidence interval, 1.43–37.87;  $P = 0.01$ ).

## Discussion and conclusions

This study showed that mite species and the levels of the main pyroglyphid mites allergens (Der p 1, Der f 1, Der 2) in household dust samples varied between two Croatian regions with different climate. Differences in air temperature between the coastal and inland Croatia seem to have affected the distribution of mite species and mite allergen levels in examined regions, regardless of nearly identical humidity in both regions.

Dust samples from the coastal region with mild winters and high relative humidity throughout the year showed the highest mean level of Der p 1 ( $4.5 \mu\text{g/g}$  of dust), which is higher than that recorded in literature for moderate climates ( $0.5$ – $1.5 \mu\text{g/g}$  of dust) (15–17) and lower than reported in subtropical and tropical climates ( $> 8 \mu\text{g/g}$  of dust) (18, 19). Nonpyroglyphid mites, dominantly *Glycyphagidae*, were significantly present in the urban and rural coastal household dust samples (20–25% of all identified mites). The most prominent genera *Blomia*, *Lepidoglyphus* and *Glycyphagus* should be included in the allergological diagnostic considerations of patients from this region.

Inland Croatia with continental climate has colder winters and the households are better insulated and heated more. Heating, particularly central heating, is often associated with higher indoor temperatures, lower air-exchange and maintained or lower relative humidity in winter (17). These differences in the indoor environment are most likely to spread *D. farinae* population in dust samples from inland areas of Croatia. Similar findings have also been recorded in other studies of households set in moderate and cold climates, with the mean levels of Der f 1 comparable with the present study results (about  $1 \mu\text{g/g}$  of dust) (16, 17). *Dermatophagoides farinae* seems to be less dependent on indoor humidity than *D. pteronyssinus*, because of its protonymph which can survive dry indoor conditions (20). It is worth noting that besides *Dermatophagoides* significant amounts of *E. maynei* were found in dust samples from the inland region (20% of all identified mites in urban inland dust samples) and that this mite must be considered in allergological diagnostics in patients from this region. Inland, nonpyroglyphid mites were represented dominantly by genera *Lepidoglyphus* and *Acarus* (about 5% of all identified mites). *Tyrophagus* species was present only sporadically in both regions and the exposure to this mite seems of little importance in Croatia. This confirms that the earlier findings about high prevalence of sensitization to *Tyrophagus putrescentiae* in urban inland population of Croatia (35.8% of skin prick test positive and 22.4% of IgE-sensitized subjects to this mite) were not the result of environmental exposure, but of cross-reactivity and low specificity of the allergen extract for skin testing (3, 21).

Climatic differences in Croatia are associated with different dwelling patterns between the two regions, which is particularly true for heating. Heating seems to have a significant impact on mite populations. Compared with the traditional heating, central heating involves seven times greater risk of exposure to the levels of Der f 1  $> 2 \mu\text{g/g}$  of dust, which is the threshold level for developing sensitization to *D. farinae* (8). Besides Der p 1 and Der f 1 levels, we also measured Der 2 levels (an equal mixture of Der p 2 and Der f 2) which followed only the pattern of Der p 1 levels. This finding may be important because some new tests for fast measurement of exposure to *Dermatophagoides* species, for example, the lateral flow tests (22), are based on Der group 2 measurement only. Our results suggest that Der 2 allergens mixture can only be useful in the evaluation of exposure to *D. pteronyssinus*, but not to *D. farinae*.

We can conclude that inter-regional climatic differences in Croatia involve different indoor dust mite exposure. This suggests that different diagnostic dust mite lists would be more appropriate for testing general population from the coastal and inland Croatia. The coastal list should include *D. pteronyssinus*, *Blomia*

*tropicalis* and *Lepidoglyphus destructor* and the inland list *D. pteronyssinus*, *D. farinae*, *E. maynei* and *L. destructor*. There are many countries with continental and Mediterranean climates and similar household characteristics and the results of this study may be applicable to them.

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