

House dust mite fauna characterization in the city of Rio de Janeiro and its importance in allergy diagnosis

Caracterização da fauna dos ácaros de poeira na cidade do Rio de Janeiro e sua importância em diagnósticos de alergias

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ABSTRACT

Introduction: The home environment is one of the most favorable spaces for the development of mites because of its low light, humidity, and temperature. Thus, it contributes to the growing cases of allergies among atopic individuals. **Objective:** To investigate the faunal profile of house dust mites in the city of Rio de Janeiro and the allergenic potential in this region. **Methods:** Thirty dust samples were collected from homes in the city of Rio de Janeiro, and the species found were classified according to their morphology, family, and genus by classification key. For the collection region, the total protein level was assessed by the Lowry method and electrophoresis under denaturing conditions (SDS-PAGE). **Results:** There was a predominance of *Pyroglyphidae* mites, accounting for 84.9% of samples; *Tyrophagus putrescentiae* accounted for 8%, *Blomia tropicalis* for 6%, *Cheyletus malaccensis* for 1%, and *Acarus siro* for 0.1%. The allergen protein content of the samples was the following: group 1 – 25 kDa (Der 1, Der p 1, and Blo t 1), group 2 – 15 kDa (Der f 2, Der 2, Tyr p 2, and Blo t 2), and group 3 – 29-30 kDa (Der f 3 and Blo t 3), which indicates that people in this region are susceptible to sensitization to these mites. **Conclusion:** Knowledge of the mite fauna in the region under study allows the guidance of health care professionals to perform skin tests for specific mites and conduct treatment according to the pool of mite extracts containing antigens, making immunotherapy more effective.

Keywords: Mites, identification, allergens.

RESUMO

Introdução: O ambiente domiciliar é um dos espaços favoráveis para o desenvolvimento de ácaros, tendo em vista a baixa luminosidade, umidade e temperatura, o que contribui para os crescentes casos de alergias em indivíduos atópicos. **Objetivo:** Investigar o perfil faunístico dos ácaros na cidade do Rio de Janeiro e o potencial alergêncio para essa região. **Métodos:** Foram coletadas 30 amostras de poeira em residências na cidade do Rio de Janeiro, e as espécies encontradas foram classificadas quanto à morfologia, família e o gênero por chave de classificação. Para as regiões das coletas, a carga total de proteínas contendo os alérgenos foi determinada pelo método de Lowry e eletroforese em condições desnaturantes (SDS-PAGE). **Resultados:** Os resultados mostram a predominância de 84,9% de ácaros da família *Pyroglyphidae*; para os demais ácaros o percentual corresponde a 8% *Tyrophagus putrescentiae*, 6% *Blomia tropicalis*, 1% *Cheyletus malaccensis*, e 0,1% de *Acarus siro*. O conteúdo proteico alergêncio constituinte das amostras foram, grupo 1: 25 kDa (Der 1, Der p 1 e Blo t 1); grupo 2: 15 kDa (Der f 2, Der 2, Tyr p 2 e Blo t 2); e para o grupo 3: 29-30 kDa (Der f 3 e Blo t 3), o que indica uma região passível à sensibilização de indivíduos por estes ácaros. **Conclusão:** O conhecimento da acarofauna nas regiões em estudo permite orientar a comunidade médica quanto à realização de testes cutâneos, além da terapêutica a partir do pool de extratos de ácaros contendo os antígenos, a fim de tornar a imunoterapia mais eficaz.

Descritores: Ácaros, identificação, alérgenos.

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Introduction

The city of Rio de Janeiro has the second largest population in Brazil, about 6.747 million inhabitants in 2021,¹ and has a tropical, hot and humid climate,² with local variations due to differences in altitude, vegetable life and proximity to the ocean. These factors can influence the existence and development of several species of dust mites and increase the proliferation of mites in the domestic environment.³ Other contributions, such as evolutionary ecological and stochastic factors,⁴ can also contribute to this differentiation.⁵ The presence of these microorganisms in house dust particles exposes the population to aeroallergens constituted of mites' bodies and feces.^{6,7} These antigens can sensitize atopic individuals, considering their genetic susceptibility, thus triggering respiratory allergies such as rhinitis, bronchitis and asthma.¹⁻³ This study aims to identify the faunal composition of mites in the region of the city of Rio de Janeiro, in order to provide information on the predominance of existing species, and consequently, the main allergens inserted in the household perimeter in potential sensitizers. This paper aims to identify the faunal composition of mites in the region of the city of Rio de Janeiro, to provide information on the predominance of existing species, and consequently, the main allergens inserted in the household perimeter in potential sensitizers. The theme promotes data that can help professionals in the field of medical allergology, as it presents information on the predominance of species in different regions, and therefore, the specific allergens to be included in the therapies offered to the population.

Material and methods

Dust mites collection and culture

Thirty dust samples (beds, sofas, rugs and surfaces) from homes were collected in the counties of Rio de Janeiro, Brazil. The collection was carried out with the aid of vacuum cleaners and the material was stored in pots and identified for further analysis. In a container, dust samples and a nutritional material (1:15), composed of rabbit feed, wheat bran and wheat germ in a 1:1:1 ratio were mixed. The container was kept in a dome containing a 5.0 mol/l sodium chloride solution, so that the mites, upon leaving the cultivation system, were collected in a 400-meche

sieve. The mites were identified in compliance with the taxonomic classification according to the literature;^{11,12} and separated for the cultivation of a pure culture. For the negative control, the same type of container received only the feed without a sample. The pure culture for the different species was kept for 90 to 180 days at a temperature of 30 °C, with a relative humidity between 70%-80%.

Clarification with Lactophenol

For better morphological visualization, the mites were clarified according to the procedure presented by Flechtmann,¹³ with modifications. For the mass of mites, a solution of lactic acid (Sigma-Aldrich), phenol (Sigma-Aldrich), distilled water and methylene blue (Sigma-Aldrich) was applied in the proportions of 2:1:1:1, respectively, for a period of 48 h.

Preparation of protein extracts from mites

The preparation of protein extracts followed procedures described by Sánchez-Ramos et al.,¹⁴ with modifications. 10% (w/v) of the mite mass was added in 0.1 mol/l saline buffer (Ultrasonic, frequency 20 kHz) for cell disruption for 30 minutes, under an ice bath. The pH of the extract was adjusted with a 2.0 mol/l sodium hydroxide solution to pH 8.5. After homogenization, the extract was left to rest at 8 °C for 48 h and then centrifuged at 1500 x g for 30 minutes at 25 °C. The supernatant was filtered on 0.22 µm pore membrane (Filtrile) and added with 40% glycerol (v/v) (Sigma-Aldrich).

Protein content - Lowry method

The protein contents of the extracts were determined using the Lowry method 15. Briefly, an analytical curve was constructed from a standard solution of BSA protein (bovine serum albumin - Sigma-Aldrich), in the range of 5 µg/mL to 100 µg/mL. The procedure was performed by adding in a test tube 3.0 mL of sodium carbonate (Sigma-Aldrich) 1% (w/v), 0.5 mL of copper sulfate (Sigma-Aldrich) 0.1% (w/v). After homogenization, the tubes were left to rest for 10 minutes at 25 °C and 500 µL of Folin-Ciocalteu reagent (Sigma-Aldrich) (1:10) were added. Optical density (O.D.) was performed in a UV-Visible spectrophotometer (Spectrophotometer SP 1102 - Bel photonics - Brasil) at 750 nm.

Characterization by electrophoresis under denaturing conditions (SDS-PAGE)

The protein content of the mite extracts was obtained from the electrophoretic run of 20 µL of the homogenate samples in a reducing buffer solution containing TEMED (N,N,N',N'-tetramethylethylenediamine) and 2-mercaptoethanol, 20% (v/v) and bromophenol blue. The samples were reduced and denatured at 90 °C in a water bath and Applied on a 12.5% (v/v) acrylamide/bis-acrylamide gel. The Dual Xtra (Bio-Rad) standard was used with ranges between the molecular mass range of 250 to 10 kDa, which was used to construct the calibration curve in the Gel Analyzer software.

Characterization by Optical Microscopy and Scanning Electron Microscopy

For observation and identification of the mites, they were mounted on slides/coverslip in the presence of glutaraldehyde and then observed under an optical microscope (OPTON – TIM-208T). Also, the mite morphology was analyzed in a Scanning Electron Microscope (SEM) by a Jeol 7100FT Field Emission Cannon at 1 kV (LABNANO/CBBP), and a working distance of 8 mm. All samples were fixed to the surface of a metal blank with carbon tape. (Sputtercoater

BAL-TEC, SDC 005). The samples were observed in low magnification and protected with a thin layer of Au, whose deposition was made using a very low amperage to cause minimal damage to the external structures of the mites.

Results and discussion

Dust mites characterization

Six species of mites were found in house dust: *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farinae* (DF), *Blomia tropicalis* (BT), *Tyrophagus putrescentiae* (Tp), *Cheyletus malaccensis* and *Acarus siro*. The predominance was for the genus *Dermatophagoides* of the *Pyroglyphidae* family, as shown in Figure 1. The mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are cited in the literature as the main representatives regarding their allergenic potential, triggering respiratory allergies worldwide.^{16,17} Other studies also report their abundance and prevalence in domestic dusts.¹⁸⁻²⁰ These results are in agreement with Silva et al.,¹⁹ the authors report that, for the city of Londrina (State of Paraná), southern Brazil, percentages of 82% for the *Pyroglyphidae* family, 9.4% for the *Glycyphagidae* family were found in house dust and 0.9% and for the

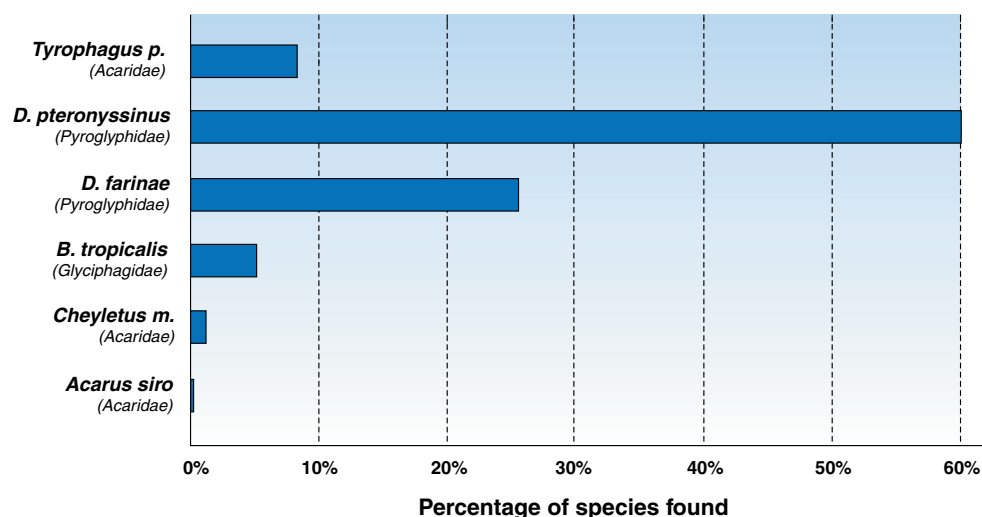


Figure 1

Faunistic profile and taxonomic classification for mites found in 30 house hold dust samples in the city of Rio de Janeiro

Acaridae family. It is important to consider that the other mites present also have significant relevance, as the literature reports the preparation of a mixture of these mites for immunotherapeutic desensitization purposes.²¹

The abundance of species found in the dust samples (Figure 1) is a factor that may be correlated with variable abiotic conditions present in the state of Rio de Janeiro, but these factors are unfavorable for the development of the species *Blomia tropicalis*, *Cheyletus malaccensis* and *Acarus siro*, for example, are found with less incidence in the samples and difficult to grow. However, opposite percentages to these were found by Serravalle et al.²² in regions of the State of Bahia-BA (Brazil), in the percentages for *Dermatophagoides pteronyssinus* of 70%, *Cheyletus malaccensis* 50%, *Blomia tropicalis* 30%, *Dermatophagoides farinae* 8% and *Tyrophagus putrescentiae* 6%. This may be related to the abiotic conditions in this region. Baqueiro and collaborators,²³ report that for the city of Salvador - BA there is a prevalence of *Blomia tropicalis* (89%), compared to *Dermatophagoides* mites (31.6%) in the rainy season.

The Figure 2 shows the species found in the dust samples. In view of the small sampling of mites with lower incidences (*Acarus siro*, *Blomia tropicalis* and *Cheyletus malaccensis*) the images were obtained in an exceptional and scarce way.

In the collected powders, two species of mites of the Cheyletidae family were found: *Cheyletus malaccensis* and *Cheyletus bidentatus* (Figure 3). They were identified by the classification key of Fain et al.^{23,24} These species are known to control the culture of other mites (predator), and may be present in grains, stored cereals and birds.²⁵ However, the *Cheyletus* species, considering their diet, can concentrate allergens from other mites.²⁶

Dust Mites Allergen Extract

For the extracts of the mites under study, the protein content was 2.45 mg/mL for *Dermatophagoides farinae*; 4.12 mg/mL for *Dermatophagoides pteronyssinus*; 1.17 mg/mL for *Blomia tropicalis*; 2.83 mg/mL for *Tyrophagus putrescentiae* and 1.02 mg/mL for *Cheyletus malaccensis*. In the total protein content, the presence of protein fractions corresponding to allergenic antigens must be considered, which have already been identified in the literature with their respective equivalent molecular masses, in Kilodalton

(kDa).^{27-29,12} These pieces of information corroborate the data obtained by Soares et al.,³⁰ where the authors studied the sensitization profile to dust mite allergens in outpatients in the city of Rio de Janeiro. From skin tests, it was shown that 67.5% of individuals with rhinitis, with or without asthma, showed reactivity to the mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*.

These proteins were also identified by gel electrophoresis, as shown in Figure 4.

For the tropical *Dermatophagoides* and *Blomia* mites, the markings referring to the main allergens of group 1 are found in 25 kDa (Der 1, Der p 1 and Blo t 1), characterized as cysteine protease and group 2 in 15 kDa (Der f 2, Der 2, Tyr p 2 and Blo t 2) represented by lipid-bound protein. Der f 3 and Blo t 3 allergens were identified for 29-30 kDa, characterizing trypsinlinked to the enzyme serine protease.^{30,31} Cross-activity has already been observed between *Dermatophagoides pteronyssinus* (Der p 1), *Dermatophagoides farinae* (Der f 1) and *Blomia tropicalis* (Blo t 1), for example, a fact reported by Guillemainault et al.³² For the mite family of *Cheyletus malaccensis* proteins were identified at 20 kDa, 26 kDa, in agreement with Mihos et al.³³ Considering the low percentage in the samples of *Acarus siro*, the mass obtained in the culture was a limiting factor for the preparation of protein extracts for purposes of electrophoretic characterization. Based on these results, it is suggested the use of a mix of mite extracts (pool) as a more efficient immunotherapeutic treatment for the desensitization of atopic individuals.

Conclusion

It was possible to present a qualitative overview of the species found in the city of Rio de Janeiro, Basil, which indicates an indication of how susceptible individuals are in this region, regarding their exposure to allergens present in the residential environment. The *Dermatophagoides* species prevails over all other species, but there is the possibility of co-sensitization by other mites present in house dust, which makes this information relevant for the medical community and the body that manages public health.

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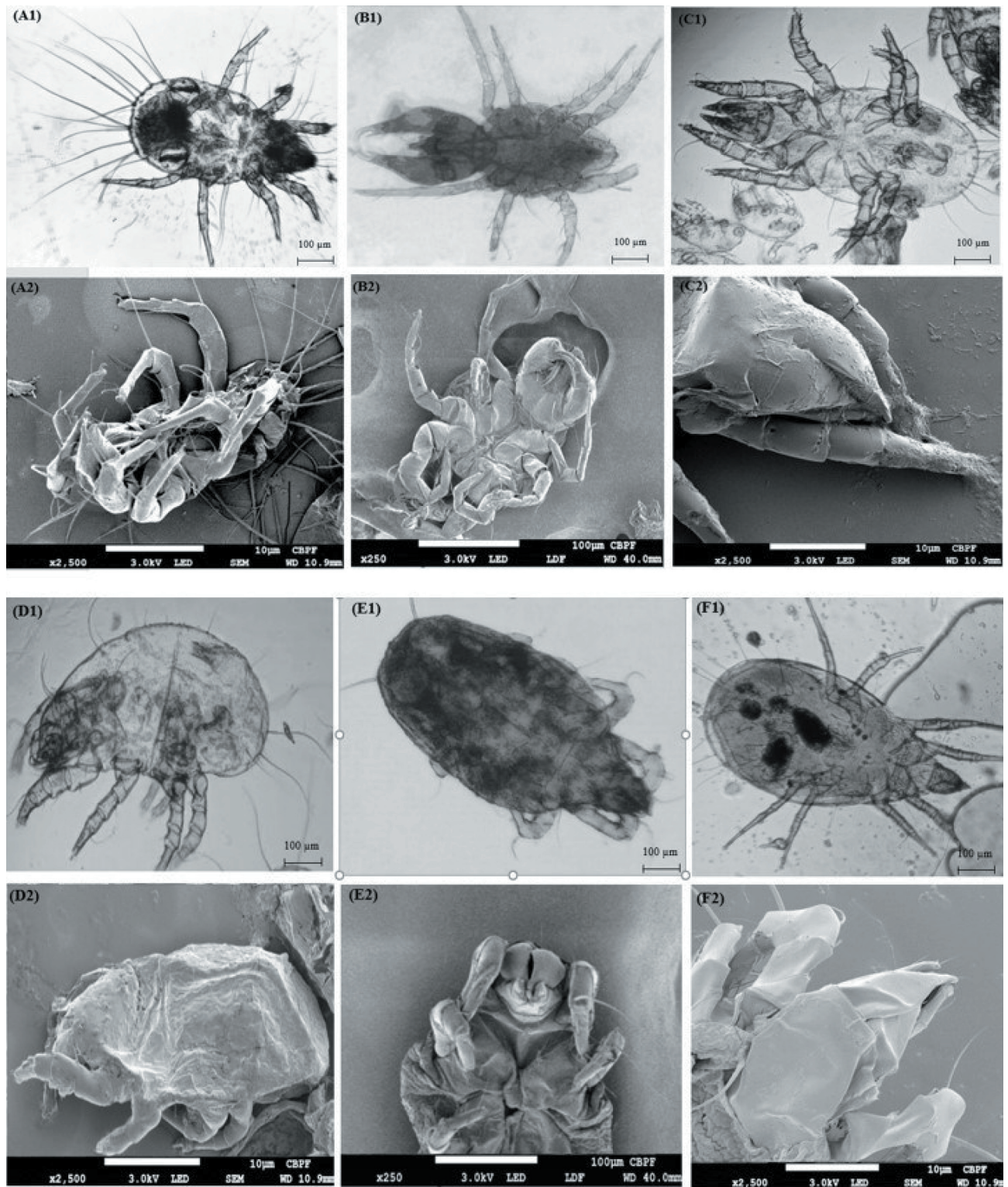


Figure 2

Opticalmicroscopy (OPTON–TIM-208T) and scanning electron micrographs (Joel, 1000 kv, LED) for mites found in the metropolitan region of the city of Rio de Janeiro - Brazil. (A1-A2): *Blomia tropicalis*, (B1-B2): *Cheyletus malaccensis*; (C1-C2 and D1-D2): *Dermatophagoides farina* and *pteronysinus*, respectively; (E1-E2): *Acarus siro* and (F1-F2): *Tyrophagus putrescentiae*

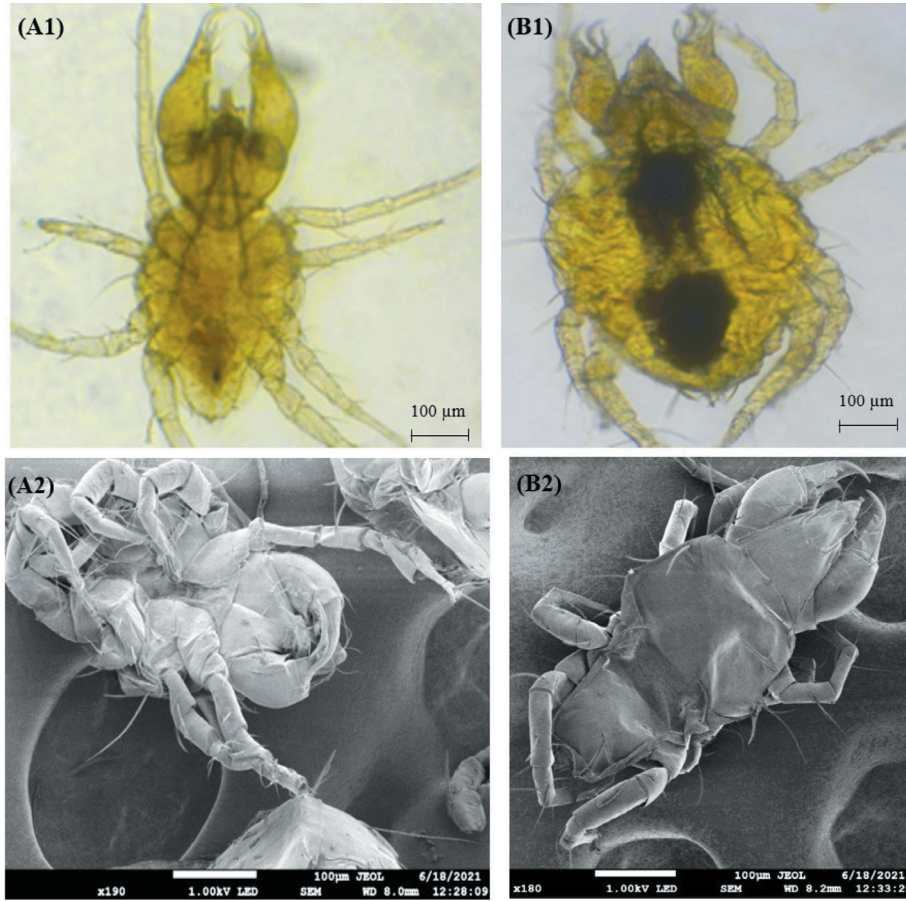


Figure 3
Optical microscopy (OPTON –TIM-208T) and scanning electron micrographs (Joel, 1000 kv, LED) for the mite species: (A1-A2) *Cheyletus malaccensis* and (B1-B2) *Cheyletus bidentatus*, found in the samples of household dust in the city of Rio de Janeiro - Brazil

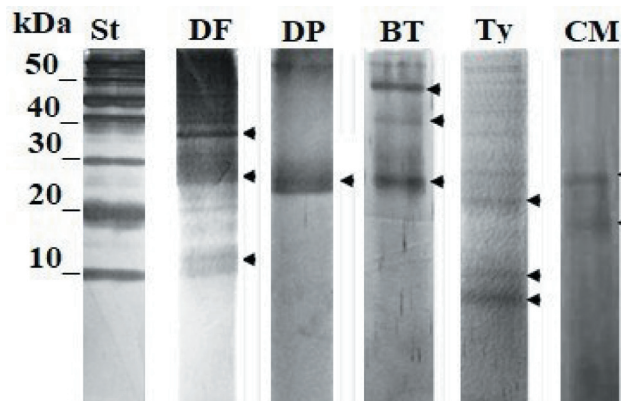


Figure 4
SDS-PAGE, 12.5% acrylamide/bis-acrylamide gel for the electrophoretic run of mite extracts present in household dust samples collected in the city of Rio de Janeiro - Brazil. (St) Standard marker proteins, (DF) *Dermatophagoides farinae*, (DP) *Dermatophagoides pteronyssinus*, (BT) *Blomia tropicalis*, (Ty) *Tyrophagus putrescentiae*, (CM) *Cheyletus malaccensis*

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