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Trichological analysis of guard hairs of *Mus musculus*, *Rattus rattus* and *Rattus norvegicus* (Rodentia: Muridae) applied to research and identification in food

Análise tricológica de pelos-guarda de *Mus musculus*, *Rattus rattus* e *Rattus norvegicus* (Rodentia: Muridae) aplicada à pesquisa e à identificação em alimentos

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ABSTRACT

Introduction: Rodents are among the most important pests in the world and when these individuals or their fur are found in food, they are considered foreign matter indicative of health risk. On the other hand, the presence of human and other mammalian hair is considered indicative of failures in good practices. Thus, the characterization of the hair of synanthropic rodents and its differentiation from other mammal species are relevant and necessary. **Objective:** To characterize the microstructural patterns of guard hairs of the three main species of rodents that infest food storage environments and to present a proposal for a protocol for the trichological analysis of isolated hairs. **Method:** Hair samples were plucked from collected specimens of the rodent species *Mus musculus*, *Rattus rattus* and *Rattus norvegicus*. Intact guard hairs were selected for the preparation of slides for observation of the microstructure. In total, 20 guard hairs were analyzed for the characterization of medullar patterns and 91 guard hair cuticular impressions were examined for the characterization of cuticular patterns. **Results:** It was observed that *M. musculus* presented alveolar medulla and losangic cuticle with variations in the shape and size of the scales. *R. rattus* and *R. norvegicus* presented reticulated medulla and losangic cuticle, also with variations. A protocol with an identification flowchart was presented for the analysis of the studied hairs. **Conclusions:** The hairs of the studied synanthropic rodent species can be differentiated from other mammalian species of health interest by the presence of alveolar and reticulated medullar patterns in the guard hair shield. For the studied species, only the medullar pattern of the guard hair shield confers a diagnostic character.

KEYWORDS: Food Inspection; Foreign Matter; Light Filth; Health Risk; Synanthropic Rodents

RESUMO

Introdução: Roedores estão entre as mais importantes pragas do mundo e, quando estes indivíduos ou seus pelos são encontrados nos alimentos, são considerados matérias estranhas indicativas de risco à saúde. Por outro lado, a presença de pelos humanos e dos demais mamíferos é considerada indicativa de falhas das boas práticas. Sendo assim, a caracterização dos pelos dos roedores sinantrópicos e a diferenciação dos pelos das demais espécies de mamíferos mostram-se relevantes e necessárias. **Objetivo:** Caracterizar os padrões microestruturais dos pelos-guarda das três principais espécies de roedores que infestam ambientes de armazenamento de alimentos e apresentar uma proposta de protocolo para análise tricológica de pelos isolados. **Método:** Amostras de pelos de roedores das espécies *Mus musculus*, *Rattus rattus* e *Rattus norvegicus* foram coletadas de espécimes colecionados e pelos-guarda íntegros foram selecionados para a preparação de lâminas para observação da microestrutura. No total, 20 pelos-guarda foram analisados para caracterização dos padrões medulares e 91 impressões cuticulares de pelos-guarda foram examinadas para caracterização de padrões cuticulares. **Resultados:** Observou-se que *M. musculus* apresentou medula alveolar e cutícula losângica com variações na forma e tamanho das escamas. *R. rattus* e *R. norvegicus* apresentaram medula reticulada e cutícula losângica, também com variações. Um protocolo com fluxograma de identificação foi apresentado para a análise dos pelos estudados. **Conclusões:** Os pelos das espécies de roedores sinantrópicos estudados podem ser diferenciados das demais espécies de mamíferos de interesse sanitário pela presença dos padrões medulares alveolar e reticulado no escudo de pelos-guarda. Para as espécies estudadas, somente o padrão medular do escudo dos pelos-guarda confere caráter diagnóstico.

PALAVRAS-CHAVE: Controle Sanitário de Alimentos; Matérias Estranhas; Sujidades Leves; Risco à Saúde Humana; Roedores Sinantrópicos

INTRODUCTION

Rodents are among the most important pests in the world[1,2,3]. These mammals not only cause physical damage, but also contaminate products with allergenic substances[4], pathogens[5,6], toxigenic fungi[7] and physical contaminants such as hair, urine, and feces[8]. It is public health knowledge that rodent urine and feces can contain parasites, pathogenic bacteria and viruses, such as: *Toxoplasma gondii*, *Salmonella* spp., *Staphylococcus aureus*, *Enterococcus* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia* sp., *Proteus* sp. and *Hantavirus* spp.[9]. Historically, rodents have been responsible for more diseases and deaths in humans than any other group of mammals[10].

In addition to agricultural *commodities*, rodents also contaminate processed food. After invading a new location, such as warehouses or supermarkets, rats and mice inevitably start gnawing on food and packaging, contaminating the environment with their fur and excretions[11]. The main rodent species that infest food storage environments are the introduced exotic species: *Mus musculus* Linnaeus, 1758 (house mouse), *Rattus rattus* Linnaeus, 1758 (black rat) and *Rattus norvegicus* Berkenhout, 1769 (brown rat)[3].

Rodents ingest a daily amount of food equivalent to 10% of their weight[12] and contaminate much more than this through their feces, hair and urine, making the food unfit for human consumption[13]. For this reason, the Resolution of the Collegiate Board (RDC) of the Brazilian National Health Surveillance Agency (Anvisa) nº 623, of March 9, 2022, which provides for the tolerance limits for foreign matter in food, the general principles for their establishment and the methods of analysis for conformity assessment purposes, considers that rodents (black rat, brown rat and house mouse) and bats, whole or in parts, are foreign matter indicative of a health risk because they carry pathogens into food (art. 3, point IX, subparagraphs b and c). In addition, the presence of human hair and that of other mammals is only considered indicative of failures in good practices (art. 3, point X, subparagraph c)14. Therefore, when investigating foreign matter in food, it is essential to characterize the hairs of the most common synanthropic rodents, namely mice (*Mus musculus*) and rats (*Rattus* spp.), in order to differentiate them from the hairs of other species of health interest according to the aforementioned resolution (i.e. humans and other mammal species).

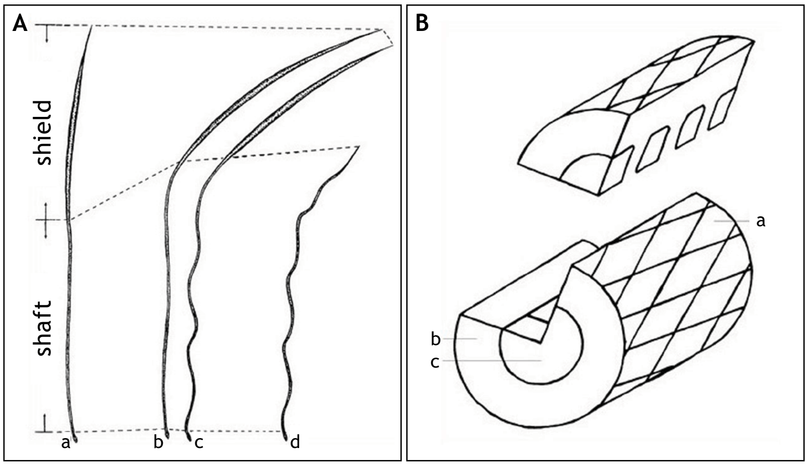
Among the different types of mammalian hair (i.e. vibrissae, overhairs, underhairs, guard hairs), the primary and secondary guard hairs are the most useful for microscopic identification because they have the most diagnostic microstructural characteristics for the taxa. The guard hairs present, from the base to the tip: the bulb, the shaft and the shield (Figure 1A). In the majority of mammal species, the guard hairs are made up of three concentric layers of cells, from the inside out: the medulla, the cortex and the cuticle (Figure 1B). The main diagnostic features are the cuticular patterns observed on the shaft and the medullar patterns observed on the shield of the guard hairs[15]. The microscopic identification of mammalian hairs is a useful technique for identifying hairs in different contexts, such as the quality control of commercial animal fibres[16,17,18,19,20], forensic investigations[21,22,23] and food quality control[23,24,25]. Except for the efforts of the latter authors, who treat rodents at the taxonomic level of the family (i.e. Muridae), no illustrated publications were found. Additionally, identification protocols aimed at helping the expert in laboratory analyses of food contaminant hairs, in order to characterize the hairs of the most common synanthropic rodents and classify foreign matter as indicative of a health risk or not, as recommended by RDC Anvisa nº 623/2022, are not available.

With this in mind, the aim of this study was to characterize the cuticular and medullar patterns of the guard hairs of the three rodent species that most commonly contaminate food: *Mus musculus* (house mouse), *Rattus rattus* (black rat) and *Rattus norvegicus* (brown rat) and to present a protocol to aid in the trichological analysis of isolated hairs.

METHOD

Tufts of hair from taxidermized rodents of the species *Mus musculus* (two individuals), *Rattus rattus* (one individual) and *Rattus norvegicus* (two individuals) were collected manually from the back of specimens collected at the Cytogenetics and Conservation Genetics Laboratory of the Genetics Department of the Federal University of Paraná (UFPR). The hair samples were taken to the Microscopy and Morphology Laboratory of the Litoral Sector of UFPR, where whole guard hairs (with bulb and apex) were selected using a Bioval® stereoscopic microscope.

The slides were prepared containing three to eight guard hairs selected for observation of the medulla and cuticle according to the protocol of Quadros and Monteiro-Filho[27] (Appendix I, p. 278). In total, 20 guard hairs were analyzed to characterize



Source: Quadros[26].

Figure 1. Schematic drawing (A) of the types of hair present in mammalian fur according to Teerink[15], as follows: (a) primary guard hair; (b) secondary guard hair with a straight shaft; (c) secondary guard hair with a wavy shaft; (d) underhair. (B) layers that make up the hair of most mammal species, from the outermost to the innermost: (a) cuticle; (b) cortex; (c) medulla.

Table. Number of slides made and guard hairs used to observe the medulla and cuticle of *Mus musculus*, *Rattus rattus* and *Rattus norvegicus* hairs.

Rodent species	Number of slides for medulla observation	Number of guard hairs analyzed	Number of slides for obse rving the cuticle	Number of cuticular impressions of guard hair examined
Mus musculus	1	7	6	34
Rattus rattus	1	5	5	22
Rattus norvegicus	1	8	8	35

Source: Prepared by the authors, 2021.

medullar patterns and 91 cuticular impressions of guard hairs were examined to characterize cuticular patterns (Table).

Both the study of the microstructure of the guard hairs and the photomicrographs were made using a Leica DM 2500 optical microscope (Microsystems, Wetzlar, Germany), at 100, 200 and 400 times magnification, at the Food Microscopy Laboratory of the Chemical and Bromatological Sciences Center of the Regional Laboratory Center of the Adolfo Lutz Institute of Ribeirão Preto VI. The microstructure of the rodent hairs was analyzed and described according to Teerink[15] and Quadros and Monteiro-Filho[28]. The nomenclature used for the medullar and cuticular patterns follows that proposed by Quadros and Monteiro-Filho[28]. The English or French names proposed by other authors and mentioned in this study, as well as their correspondence with the medullar and cuticular patterns observed in this study, can be found in Quadros and Monteiro-Filho[8] (Tables III and IV, p. 287). Previous studies used to understand the hair morphology of the three rodent species here addressed were carried out by Teerink[15], Brunner and Coman[29], Keogh[30] and Keller[31].

RESULTS AND DISCUSSION

The results presented describe the cuticular and medullar patterns of the three synanthropic rodent species studied, focusing on their differentiation from other mammal species of health interest. They also highlight differences and inconsistencies in relation to the literature, the causes of which are discussed. In addition, a trichological analysis protocol is suggested for hair isolates found in food.

With the techniques employed, it was possible to observe that the guard hairs of the three species are flattened dorso-ventrally, with the concave and convex faces showing up in the preparation of the slides with the whole hairs and in the cuticular impressions. Brunner and Coman[29] based their diagnosis mainly on the characteristics of the cross-sections, which means that comparisons with this study are limited. However, the perception that the hairs analyzed here are reniform or concave-convex is in line with the descriptions by Brunner and Coman[29] and Teerink[15], as detailed in the species descriptions below.

Characterization of the guard hairs of the species studied with a view to identifying unknown hair samples

Mus musculus

The medullar pattern on the shield of the guard hairs is of the alveolar type, showing four alveoli with well-defined outlines across the width of the guard hairs (Figures 2A and 2B), which corroborates Teerink's description[15] for the species. According to the nomenclature used by this author, this pattern is called isolated. The illustrations presented by Brunner and Coman[29] for the *Mus musculus* medulla show the reticular pattern for the species, which the author calls wide aeriform lattice. This differs from what was observed here for *Mus musculus*.

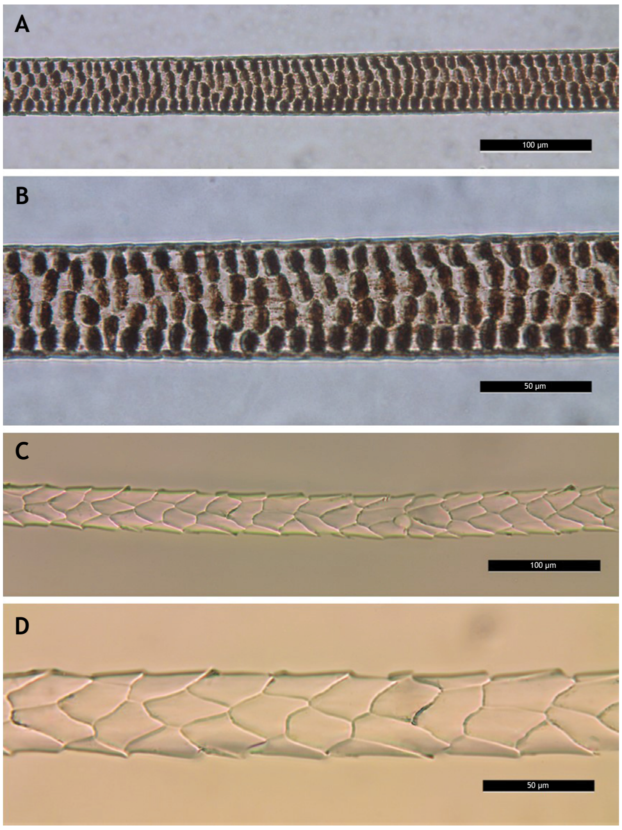
The cuticular pattern on the shaft is rhombic[28], but, as also described by Teerink[15], it was observed that there can be variations in the shape and size of the scales between the concave and convex faces of the guard hairs (Figures 2C and 2D). Keogh[30] describes the presence of wide scales with smooth margins on the cuticle of *Mus musculus*, as observed in this study, but calls the pattern *petal*.

The average length of the primary guard hairs of *M. musculus* is 12 mm, according to Teerink[15]. It was not the subject of this study to measure the length of the hairs, but it was observed that for *Mus musculus* the hairs were always shorter than for *Rattus rattus* and *R. norvegicus*, which is in line with the author's measurement of between 15 and 25 mm for *Rattus* spp.

Rattus rattus

The medullar pattern of the shield is reticulated, with five to six spaces delimited in the reticule across the width of the hair. The boundaries of the lattice spaces are irregularly shaped and sometimes difficult to see (Figures 3A and 3B). The pattern observed is in line with Brunner and Coman[29] who call it a wide aeriform lattice. Teerink[15] does not differentiate between the alveolar and reticulated patterns, as Quadros and Monteiro-Filho[28], and Keller[31] describes the reticulated pattern for rodents as a lattice and calls it *en treillis*.

The cuticular pattern on the concave face of the guard hair shaft has scales with a double oblique orientation in relation to the longitudinal axis of the hair, as described by Quadros and Monteiro-Filho[28]. The scales in the groove are as wide as they are long and conform to the rhombic pattern (Figure 3C). Similarly, on the convex face, the cuticular pattern is similar to the rhombic pattern described by Quadros and Monteiro-Filho[28], but the scales vary in shape and size (Figure 3D). This is reported by Teerink[15], who refers to this pattern as irregular (irregular diamond petal). For Keogh[30], the cuticular pattern of *R. rattus* is called mosaic. Although the name of the pattern used by Keogh[30] is different, the illustrations presented by this author show a rhombic pattern according to the nomenclature used in this study, which coincides with that observed here in the groove of the concave face and the convex face.



Source: Prepared by the authors, 2021.

Figure 2. Optical photomicrographs of the medullar and cuticular patterns of the guard hairs of *Mus musculus*. Medulla visualized under magnification of (A) 200x and (B) 400x; and cuticle visualized under magnification of (C) 200x on the concave side and (D) 400x on the convex side.

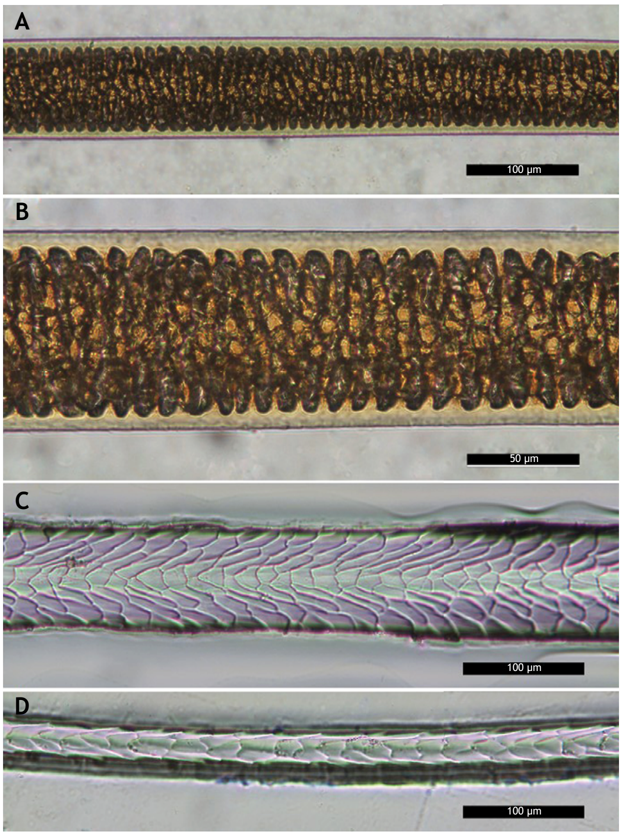


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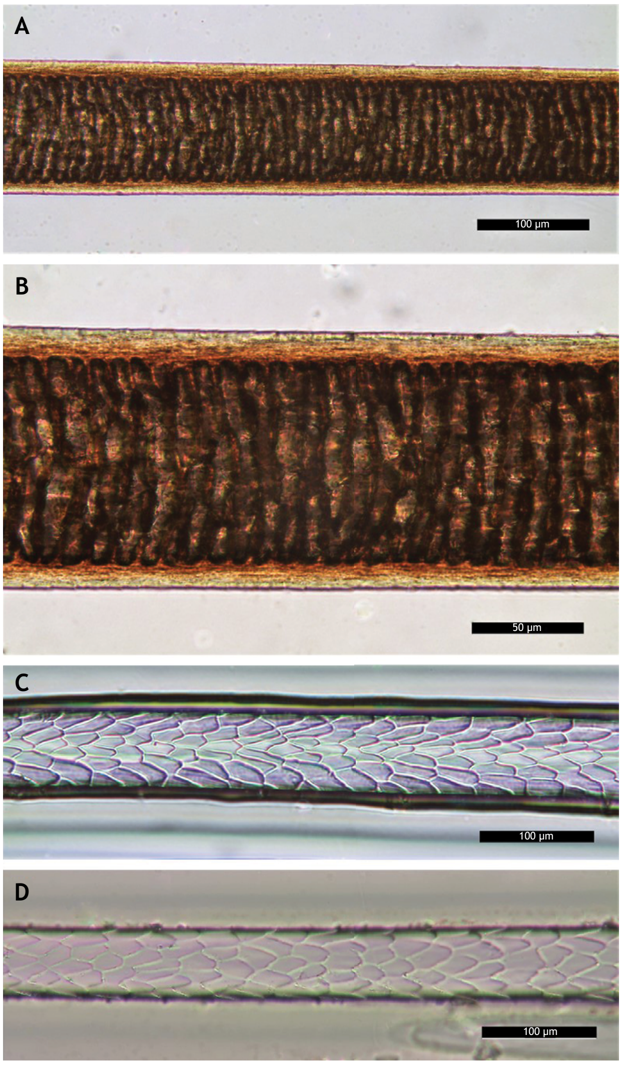
Figure 3. Optical photomicrographs of the medullar and cuticular patterns of the guard hairs of *Rattus rattus*. Medulla visualized under magnification of (A) 200x and (B) 400x; (C) concave side and (D) convex side of the cuticle visualized under 200x magnification.

Rattus norvegicus

The medulla of the shield is reticulated, as also described by Brunner and Coman[29], and has five to seven reticular spaces across the width of the guard hairs. However, unlike in *R. rattus*, the delimitation of the spaces is inconspicuous and they are arranged transversely, often fused, with the formation of transverse stripes arranged along the shield of the guard hairs (Figures 4A and 4B). Quadros and Monteiro-Filho[28] present the formation of the striped pattern for sigmodontine rodents (e.g. *Akodon* sp.), however, for these authors, the striped medullar pattern is the result of the arrangement and transverse fusion of alveoli of the alveolar pattern, a transition from alveolar to striped. Silveira et al.[32], studying nine species of the genus *Akodon*, corroborate the existence of this modification of the alveolar pattern into striped, which they call "mixed alveolar and striped medullar pattern". In this study, in the case of *R. norvegicus, it* was decided not to call the pattern striped despite the appearance of the stripes, because the medullar pattern of *R. norvegicus* comes from the fusion of reticular spaces of the reticular pattern, and not from the alveolar pattern, as originally described by Quadros and Monteiro-Filho[28] for the striped pattern. As reported for *R. rattus*, Teerink[15] does not differentiate the reticulated pattern from the alveolar pattern, so, for this author, the medulla of *R. norvegicus* is *isolated*.

The cuticular pattern observed on the central portion of the guard hair shaft is rhombic, as described by Teerink[15]. On the concave face, the scales vary more in shape and size and are discreetly arranged in a double oblique pattern in relation to the longitudinal axis of the guard hairs (in a "V" shape) when compared to *R. rattus*. On the convex side, the rhombic pattern shows greater regularity in the shape and size of the scales (Figures 4C and 4D). In the proximal region of the shaft there is a mosaic cuticular pattern that makes the transition between the bulb and the rhombic cuticular pattern, characteristic of the guard hair shaft of this species. This was also observed by Teerink[15]. For Keogh[30], what diagnoses *R. norvegicus* is the presence of this transitional mosaic pattern on the shaft (waved mosaic).

Silveira et al.[23] reported on the cuticular and medullar patterns of rodent hair at the taxonomic level of the family (i.e. Muridae), but did so on the basis of the three species studied here (i.e. *M. musculus*, *R. rattus*, R. *norvegicus*). According to the authors, the cuticle of these species is foliaceous, contrary to what was observed in this study. As Quadros and Monteiro-Filho[33] pointed out when analyzing feline hair, "the differences in foliaceous and rhombic cuticular patterns are subtle and difficult to observe". Also in this regard, Silveira et al.[23] found that the medulla is alveolar in *Mus musculus*, as reported in this study, but also in *Rattus* spp., in disagreement to the present study, which identified the reticulated pattern. Some authors do not differentiate between these patterns (alveolar and reticulated)[15,31], suggesting that they may be the same pattern visualized in two different ways for reasons not explored in the studies. Also along these lines, Brunner and



Source: Prepared by the authors, 2021.

Figure 4. Optical photomicrographs of the medullar and cuticular patterns of the guard hairs of *Rattus norvegicus*. Medulla visualized under (A) 200x and (B) 400x magnification; (C) concave side and (D) convex side of cuticle visualized under 200x magnification.

Coman[29] consider the reticulated pattern of Quadros and Monteiro-Filho[28] as lattice and the alveolar pattern as aeriform lattice, i.e. for the authors the two patterns are close and have the appearance of a lattice. In the aeriform lattice pattern, air spaces appear as a net or lattice[29].

Trichological analysis protocol for hair found in food

The rhombic cuticular pattern, which recurs with slight variations in the synanthropic rodent species studied, is present in sigmodontine wild rodents and in several species of other mammal orders such as Didelphimorpha and Carnivora[33], including domestic cats[23]. Thus, the identification of hair fragments formed only by the shaft, in which the cuticle is a diagnostic character, leads to an inconclusive result in the sense of classification in item IX or X of article 3 of RDC Anvisa nº 623/2022[14] (Figure 5).

The alveolar pattern of the medulla of *Mus musculus* guard hairs can also be observed in small wild rodents, such as species of Sigmodontinae[32,33]. However, these wild rodents do not have synanthropy as a habit. Also in this sense, although the reticulated pattern observed in rats (*Rattus* spp.) has also been reported for other rodents such as *Nectomys squamipes* and *Holochilus brasiliensis*; and for the marsupial *Chironectes minimus*[33], they are all wild and have a semi-aquatic habit, which reduces the chances of these species contaminating food.

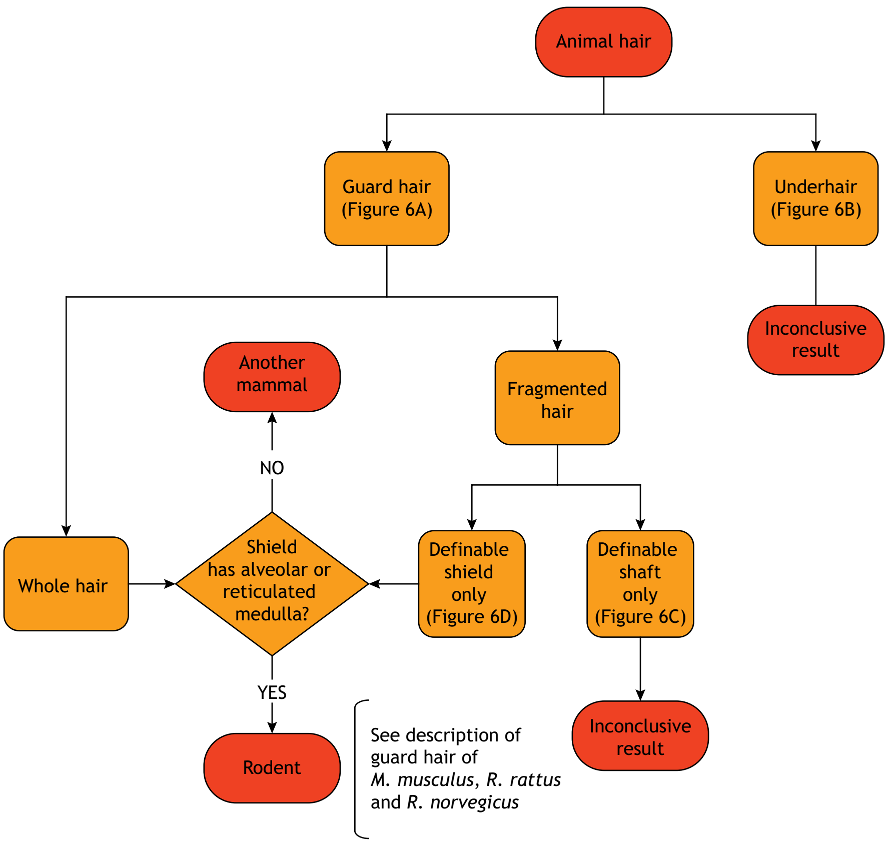
In addition, the medullar patterns observed (alveolar and reticulated) are different from those observed in human hair or those of other mammalian groups to which RDC Anvisa nº 623/2022[14] refers. According to Silveira et al.[23], in humans the medulla is absent or uniseriate; domestic dogs and cats have matrix-like and trabecular medulla, respectively; in bats, the medulla is absent; and in opossums it is of the riddled type[28]. Felix et al.[34], working with Brazilian breeds of cattle used in food production, observed trabecular medulla. De Marinis and Asprea[35], studying the hair morphology of domestic ungulates (cows, sheep, goats, horses and donkeys), reported the presence of uniseriate or multiseriate medulla, with or without the formation of vacuoles, which can be discontinuous and narrow. All these characteristics differ markedly from those observed in rodents.

In this sense, the presence of the alveolar and reticulated patterns on the shield of whole guard hairs leads to the diagnosis of rodent (Figure 5) and makes it possible to classify them under item IX of article 3 of RDC Anvisa nº 623/2022[14]. In addition, another pattern that leads to the diagnosis of rodent because it has been observed exclusively in this group of mammals is the striped pattern. Although this has not been described for the three species of synanthropic rodents studied here, it has been described for other species of small wild rodents (Sigmodontinae)[32,33].

CONCLUSIONS

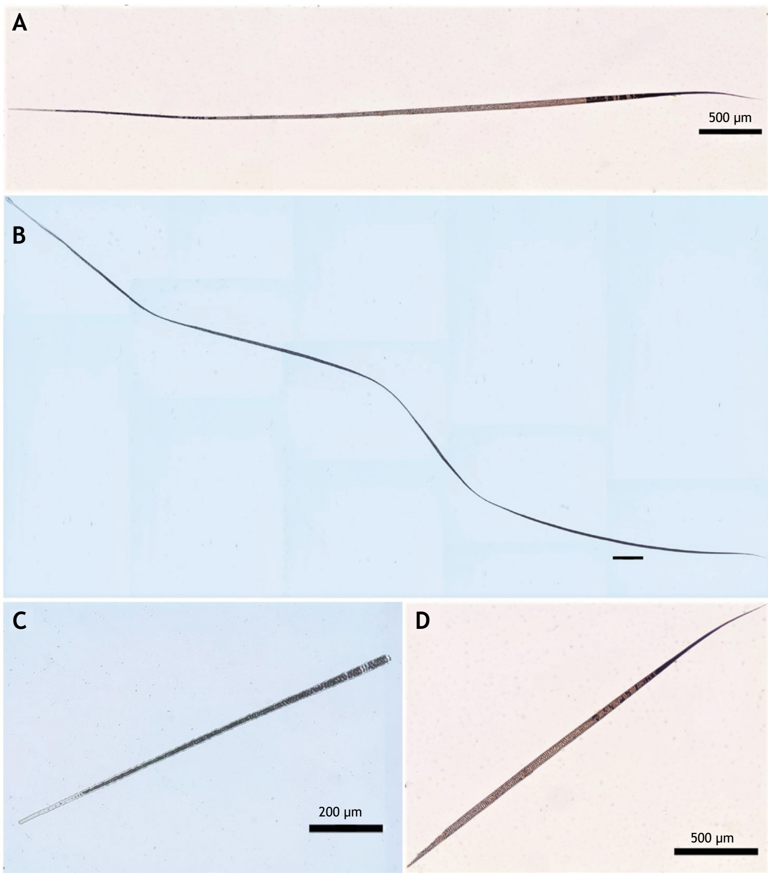
Trichological analysis of the rodent hair studied showed that these synanthropic species can be differentiated from other mammal species of health interest by the presence of alveolar and reticulated medullar patterns in the guard hair shield. The cuticular patterns on the shaft, on the other hand, show interspecific overlaps, making them useless for this diagnosis. Therefore, the analyst is more specifically interested in mastering the recognition of guard hairs and the identification of medullar patterns on the shield portion of these hairs.

The trichological analysis protocol, containing the flowchart shown, highlights those cases in which the result is



Source: Prepared by the authors, 2021.

Figure 5. Flowchart applied to the identification of rodent hair contaminating food.



Source: Prepared by the authors, 2021.

Figure 6. Photomicrographs of the hair types and their fragments: (A) guard hair, (B) underhair, (C) guard hair fragment with definable shaft only and (D) guard hair fragment with definable shield only (scale bar A, D = 500 µm; B, C = 200 µm).

inconclusive (underhair and shaft fragments) and, in particular, makes it possible to classify, solely and exclusively, whole guard hairs or shield fragments as foreign matter indicative of a health risk (rodent hair) or as indicative of good practice failures (human and other mammal hair) in accordance with RDC Anvisa No. 623/2022.

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Authors' Contribution

Aquino CI - Conception, planning (study design) and writing of the work. Quadros J - Conception, planning (study design), analysis, data interpretation, and writing of the work. All the authors have approved the final version of the work.

Conflict of Interest

The authors inform that there is no potential conflict of interest with peers and institutions, political or financial, in this study.