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Dorsal hair morphology of some species of bats of southwestern Amazonia (Mammalia: Chiroptera): new descriptions of the cuticular pattern of guard hairs

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ABSTRACT
The present study aims to provide new information on the morphology of bat guard hairs from southwestern Amazonia and to update a preexisting dichotomous identification key based on trichological patterns. Hair samples of 21 bat species were studied. Cuticular impressions were classified into six morphological patterns. We updated a dichotomous key (family and subfamily levels) with the hair structure morphology. We also observed a cuticular pattern not yet described in the literature for *Saccopteryx canescens*.

Keywords: identification hairs key, *Saccopteryx canescens,* trichology

RESUMO - Morfologia dos pelos dorsais de algumas espécies de morcegos do sudoeste da Amazônia (Mammalia: Chiroptera): novas descrições do padrão cuticular dos pelos de guarda. O presente estudo tem como objetivo fornecer novas informações sobre a morfologia dos pelos de guarda de morcegos na região sudoeste da Amazônia e atualizar uma chave de identificação dicotômica preexistente baseada em padrões tricológicos. Amostras de pelo de 21 espécies de morcegos do sudoeste da Amazônia foram estudadas. As impressões cuticulares foram classificadas em seis padrões morfológicos. Atualizamos uma chave dicotômica (família e subfamília) com a morfologia da estrutura capilar e observamos um padrão cuticular ainda não descrito na literatura para Saccopteryx canescens.

Palavras chave: Chave de identificação dos pelos, *Saccopteryx canescens,* tricologia

The hair is a structure exclusive to mammals and has thermoregulation, camouflage, and mechanical protection functions (Miranda et al. 2014). Morphological differences in scale patterns and structure of the mammalian hair vary significantly from species

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to species (Hausman 1920; Teerink 1991; Quadros & Monteiro-Filho 2006a). These characteristics can help identify species for taxonomic, ecological, forensic studies, and archaeological applications (Tobin 2005; Sessions et al. 2009; Silveira et al. 2013; Miranda et al. 2014; Marchioro & Miranda 2017).

The guard hairs are long and coarse, forming the topcoat of the fur. The cuticular scale structure varies at different positions along the hair shaft (Kumar et al. 2016). The study of the microstructure of hairs was developed by Hausman (1920), who provided diagnostic characters for 166 species belonging to different species of mammals, and formulated a nomenclature system for eight cuticle patterns and eight medulla patterns. Cuticular and medullar patterns, and the shape of the cross-sections of the guard hairs are important characteristics in differentiating families, genera, and, sometimes, species (Teerink 1991).

Regarding bats, Benedict (1957) initially concluded that the structure of their hair, as seen under a light microscope, provided reliable identification only for categories above the species level. Although a range of morphological characteristics and molecular techniques are used for taxonomy and species recognition of bats, the ultrastructural hair morphology can be used as an additional taxonomic tool to differentiate families and even genera of the order Chiroptera.

In Brazil, studies of trichology for taxonomic purposes are still scarce (Ingberman & Monteiro-Filho 2006; Quadros & Monteiro-Filho 2006a; Martin et al. 2009; Silveira et al. 2013; Miranda et al. 2014; Marchioro & Miranda 2017; Tremori et al. 2018). More recently, the studies of Marchioro & Miranda (2017) and Meurer & Pereira (2020) specifically deal with trichological analyses of bats. Marchioro & Miranda (2017) analyzed 67 species of chiropterans and provided an identification key based on the cuticular patterns of the studied hairs.

The aim of this study was to describe the cuticular characteristics of guard hairs of 21 species of bats (see Table 1) of southwestern Amazonia, to provide new information, and update the previous identification key proposed by Marchioro & Miranda (2017) based on hair characteristics for some species of bats.

The method proposed by Quadros & Monteiro-Filho (2006a) was used to collect the guard hairs. First, we removed the hair from one specimen per species in the bat collection of the Mammal Ecology Laboratory (LABEM) of the Federal University of Acre, Brazil. Bats were collected in the southwestern region of the Amazon (see Marciente & Calouro 2009; Calouro et al. 2010; Santos et al. 2018; Verde et al. 2018; Silva et al. 2020). The taxonomic arrangement and number of taxa followed Garbino et al. (2020). We collected guard hair tufts from the interscapular region to analyze the cuticle pattern according to the characters described by Quadros & Monteiro-Filho (2006b). We only studied the cuticular pattern because the medulla is usually absent in the hair of bat species.

Tufts of hair were removed with the thumbs, preserving from the bulb to the apex. First, we washed the hairs in a petri dish with 70% alcohol. We then placed the hairs under a stereomicroscope where a single hair was sorted for slide preparation. We washed the selected hair with 70% alcohol and dried it with a hair drier and toilet paper. We applied colorless enamel to a microscope slide in a single direction and let it dry for 20 to 25 minutes at room temperature. The hair was placed over the enamel and pressed with a vise (Lathe 90MM IMP FIX90) and two wooden plates with E. V. A. (Ethyl Vinyl Acetate) coating. The slide remained in the press for 2 to 5 minutes for the hair to become adhered, then removed and exposed to a temperature of 17 °C to 20 °C so that the enamel layer would not damage the hair impression. After 48 hours, the sample was removed gently so the impression did not get damaged. The impressions of the cuticular patterns were observed through an OLYMPUS BX41 optical microscope (model BX41TF) at a 40-fold magnification. Then, they were photographed with an OLYMPUS SC30 camera attached to the microscope, with a 50 µm reference scale.

We classified the guard hairs according to the cuticle patterns (with a description of a new pattern) for 21 bat species of the following families: Phyllostomidae (18 spp.), Emballunoridae (2 spp.), and Molossidae (1 sp.) (see Table 1). We found three different cuticle patterns: a leaf-shaped pattern subdivided into three other types, which included narrow leaf-shaped, intermediate, and wide; a narrow imbricated leaf-shaped pattern; and an imbricated conoidal cuticle pattern. The last two are the most common in phyllostomids (found in 55.5% of the 18 species of this family). The genera *Micronycteris* and *Artibeus* have a conoidal pattern with bracts (Fig. 2a, b and i, j, respectively). However, within Phyllostomidae, there are also species with hairs with an ornamented shape so that they can be described as triangular with alternating layers (Fig. 2). The hairs of *Vampyrum spectrum* and *Phyllostomus elongatus* have a wavey squamous shape (Fig. 2r and k, respectively). *Vampyressa thyone* and *Choeroniscus minor* do not show the same cuticular pattern as the other phylostomids (i.e., presence of multiple bracts), since they have more open scales, an alternate and elongated ornamented conoid shape with straight layers at the end, and lack bracts (Fig. 2e and p, respectively). We observed a new cuticular pattern for the Emballunoridae family, characterized by having hair with obliquely oriented cones. We defined this new pattern as oblique conoidal cuticular. This new form is present in the guard hairs of *Saccopteryx bilineata* and *Saccopteryx canescens* (Fig. 1b and c). In the Molossidae, represented by the species *Promops nasutus*, we observed a shape of ornamented conoidal (Fig. 1a).

Dichotomous key for identifying families and subfamilies of bats from southwestern Amazonia using the cuticular pattern of guard hairs.

1. Edges of squamous scales of wavy shape and oblique orientation........Phyllostomidae: Desmodontinae (see Machioro & Miranda 2017)

1’. Edges of imbricated scales leaf-shaped or conoidal shape..2

2. Leaf-shaped and narrow dimension.......Vespertilionidae (see Machioro & Miranda 2017)

2’. Conoidal shape..3

3. Scales with straight edges...4
3'. Ornamented scale edges..5
4. Scales of equal length and width (intermediate)..............Thyropteridae (see Machioro & Miranda 2017)
4'. Scales with the length larger than the width (narrow).....................Mormoopidae (see Machioro & Miranda 2017)
5. Scales with the length shorter than the width (wide), and in oblique layers ....Emballonuridae (Fig. 1b and c)
5'. Scales of similar length (intermediate) or larger (narrow) than their width............6
6. Scales with the length larger than the width (narrow), with discontinuous edges.... ....Noctilionidae (see Machioro & Miranda 2017)
6'. Scales of different lengths and widths 7
7. Scales of ornamented conoidal and/or cogged edges....................Molossidae (Fig. 1a)
7'. Scales with smooth edges and a bract...8
8. Leaf-shaped pattern........Phyllostomidae (Subfamilies: Stenodermatinae, Carolliinae, Glossophaginae)
8'. Conoidal pattern with scale length shorter than the width (wide), usually long and open....................Glyphonycterinae and Stenodermatinae (*Artibeus*, *Vampyriscus*, and *Vampyressa* (Fig. 2a, b, and p).

The predominance of the conoidal pattern in the hair cuticle with the presence of a bract coincides with the results of Marchioro & Miranda (2017), described in the hair scales of Phyllostomidae. However, the findings of cuticular pattern variations or the presence of different ornaments as the cases of *Vampyressa thyone*, *Choeroniscus minor*, and *Promops nasutus* is different from the patterns described by Marchioro & Miranda (2017) for Phyllostomidae and Molossidae families. Also, descriptions of the hair of the two species of *Saccopteryx* (Emballonuridae) indicate that the hair shaft is narrower, and its layers are more marked, especially in *S. canescens* where layers are more rounded and have an enlarged conoidal that intersects from the right and left. Our results show a variation in the cuticular morphology pattern of emballonurid bats compared to the pattern described by Marchioro & Miranda (2017) for *Rhynchonycteris naso*. Thus, these differences indicate a diversity of cuticular patterns in the studied families and show that species hairs might differ in shape although belonging to the same family. In *Promops nasutus* we observed an ornamented conoidal pattern, different from the ornamented conoid with cogged edges pattern found in other species of Molossidae by Marchioro & Miranda (2017) and Ibarra et al. (2004).

The variations described within families indicate the need for descriptions of cuticular patterns at a species level, recording patterns in subfamilies or genera/species that are exceptions. The hair morphology of bats in the scale cuticle differs among families and even between genera and possibly species.

Here we described the microstructure of the hair for 21 bat species and defined a new cuticular pattern for one species, contributing to the knowledge of hair structure in bats. Our results show that hair structures can be used to identify Neotropical bats.

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Table 1. List of the 21 bats species sampled, their families/subfamilies, the cuticular shapes of the guard hair observed and vouchers examined.

Family/Subfamily/Species	Cuticular shape	Nº voucher (LABEM)
Emballunoridae		
Saccopteryx bilineata (Temminck, 1838)	Conoidal oblique	447
Saccopteryx canescens Thomas, 1901	Conoidal oblique	410
Phyllostomidae		
Carolliinae		
Carollia benkeithi Solari and Baker, 2006	Narrow imbricated leaf-shaped	205
Glossophaginae		
Choeroniscus minor (Peters, 1868)	Ornamented conoidal alternating	275
Lichonycteris degener Miller, 1831	Imbricated conoidal	270
Phyllostominae		
Phyllostomus elongatus (É. Geoffroy, 1810)	Wavey squamous shape	521
Tonatia maresi Williams, Willig e Reid, 1995	Imbricated conoidal	190
Vampyrum spectrum (Linnaeus, 1758)	Wavey squamous shape	191
Glyphonycterinae		
Glyphonycteris sylvestris Thomas, 1896	Imbricated conoidal	592
Lampronycteris brachyotis (Dobson, 1878)	Imbricated conoidal	595
Micronycteris hirsuta (Peters, 1869)	Imbricated conoidal	772
Micronycteris minuta (Gervais, 1856)	Imbricated conoidal	922
Stenodermatinae		
Chiroderma trinitatum Peters, 1860	Narrow imbricated leaf-shaped	413
Artibeus anderseni (Osgood, 1916)	Imbricated conoidal	1030
Artibeus glaucus (Thomas, 1893)	Narrow imbricated leaf-shaped	1034
Uroderma magnirostrum Davis, 1968	Narrow imbricated leaf-shaped	513
Platyrrhinus infuscus (Peters, 1880)	Narrow imbricated leaf-shaped	1054
Sphaeronycteris toxophyllum Peters, 1882	Imbricated conoidal	1095
Vampyressa thyone Thomas, 1909	Ornamented conoidal	908
Vampyriscus bidens Dobson, 1878	Ornamented conoidal	987
Molossidae		
Promops nasutus (Spix, 1823)	Ornamented conoidal	796

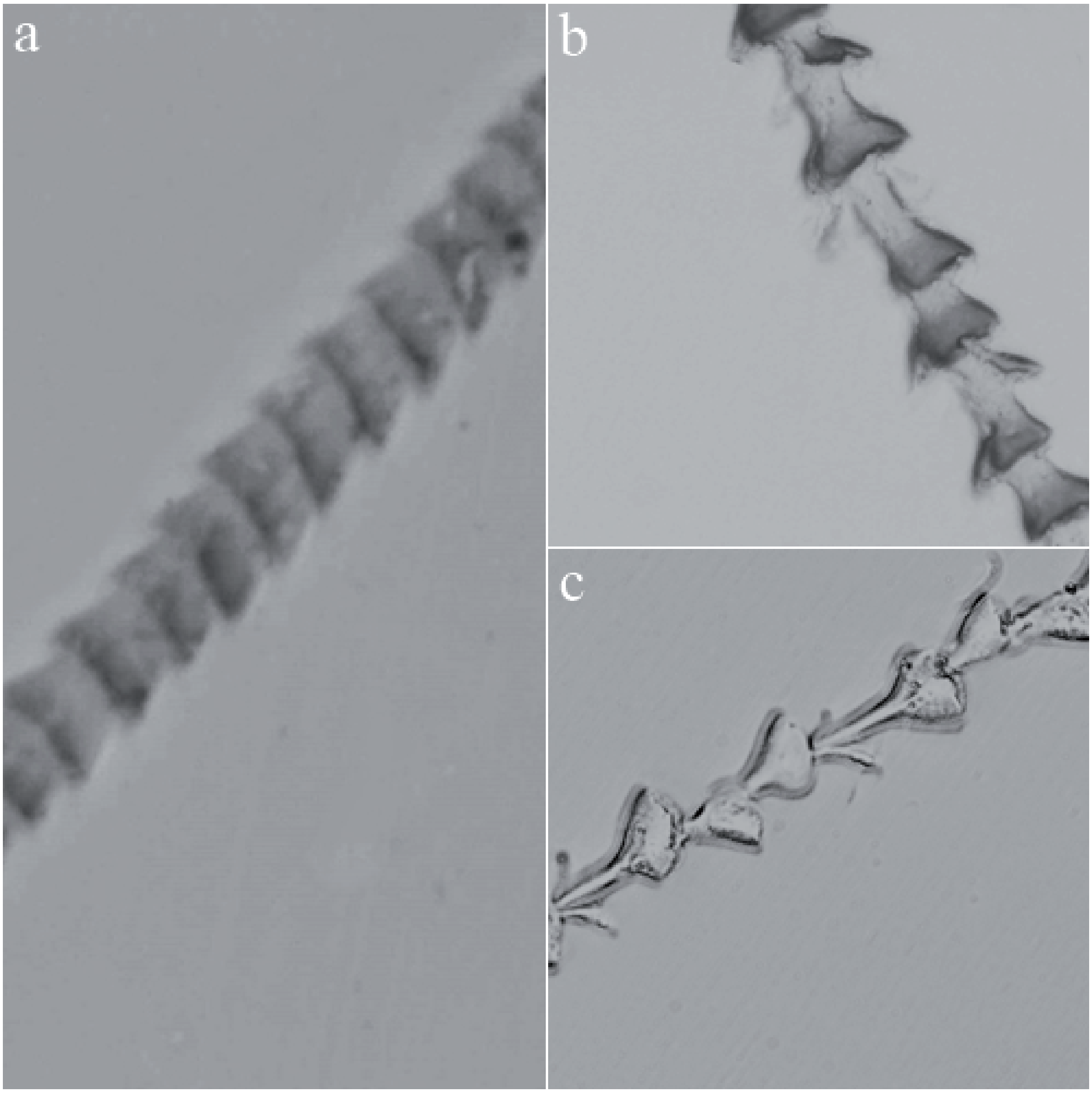

Figure 1. a) Ornamented conoidal shape (*Promops nasutus*); b, c) conoidal oblique shape alternated with layers separated and inserted in the broad part of the hair, thin and elongated bracts after the layers (*Saccopteryx bilineata* and *Saccopteryx canescens* respectively).

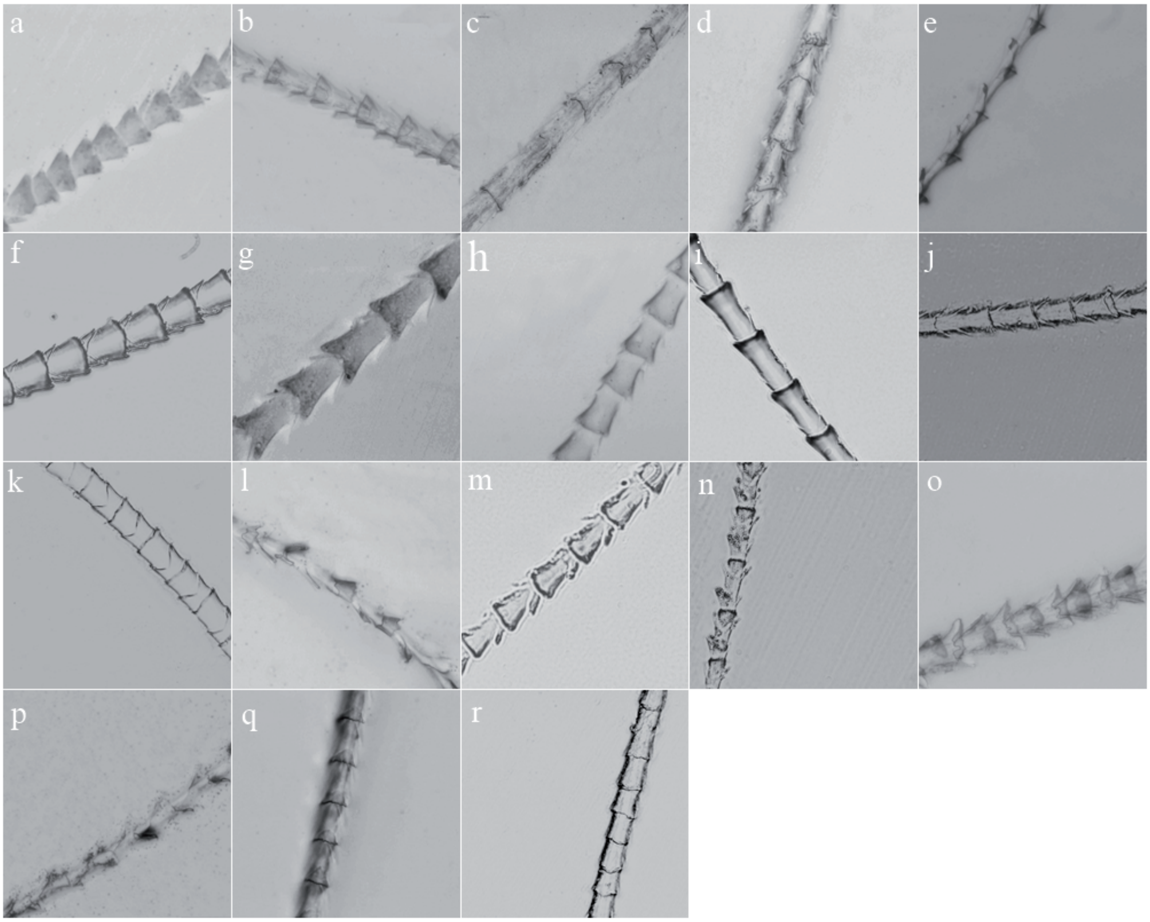

Figure 2. a) Imbricated conoidal shape with shorter and wider layers and thin bracts away from the layers, arising from the previous layers (*Artibeus anderseni*); b) narrow imbricated leaf-shaped with overlapping layers and at the end of the layers the tone darkens in the form of waves (*Artibeus glaucus*); c) narrow imbricated leaf-shaped with overlapping layers with an overlapping layer with not accentuated curvature and straighter divisions (*Carollia benkeithi)*; d) narrow imbricated leaf-shaped with light layers overlapping over shorter layers and others more elongated (*Chiroderma trinitatum*); e) ornamented conoidal shape alternating and elongated with straight layers at the end (*Choeroniscus minor*); f, g) imbricated conoidal shape with broader and shorter bracts, united to the layers and arising from the layers (*Glyphonycteris sylvestris* and *Lampronycteris brachyotis* respectively); h) imbricated conoidal shape with thin lateral bracts (*Lichonycteris degener*); i) imbricated conoidal shape with short and thin bracts, slightly away from the bracts (*Micronycteris hirsuta*); j) imbricated conoidal shape, with medium bracts and adhered to layers (*Micronycteris minuta*); k) wavey squamous shape with alternating direction, where one is straight, and the other is inclined in a bell shape (*Phyllostomus elongatus*); l) narrow imbricated leaf-shaped with light layers overlapping over shorter layers and others more elongated (*Platyrrhinus infuscus*); m) imbricated conoidal shape, with medium bracts and adhered to layers (*Sphaeronycteris toxophyllum*); n) imbricated conoidal shape with bracts in the middle portion of the layers; o) narrow imbricated leaf-shaped with overlapping layers and at the end of the layers the tone darkens in the form of waves (*Uroderma magnirostrum*); p) ornamented conoidal shape alternating and elongated with straight layers at the end (*Vampyressa thyone*); q) elongated conoidal imbricated shape with small bracts and tightly joined to the layers (*Vampyriscus bidens*); r) wavey squamous shape, with gentle slopes in each layer (*Vampyrum spectrum*).

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