

Scientific Note

Genetic variation is predominantly structured by geography rather than host in feather mites (Acariformes: Sarcoptiformes) associated with tanagers (Aves: Thraupidae) in Brazil

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Abstract. Feather mites are the most common ectosymbionts on birds. These obligatory symbionts are mainly transmitted during their host's parental care, which creates high host specificity. Due to this intimate relationship, it is thought that their geographic distribution is restricted by their host distribution, or that a host species harbors the same mite composition across its whole range. However, our knowledge regarding the geographic distribution of feather mites remains scarce, with only a few studies indicating disconnections between mite and host distributions, especially in widespread hosts. Here, we investigate the feather mites distribution on four tanager species, three widespread – *Thraupis sayaca* (L.), *T. palmarum* (Wied), and *Stilpnia cayana* (L.) from Northern and Southern Brazil; and the Amazonian *T. episcopus* (L.). Feather mites were identified using the molecular barcode marker COX-1 using K2P genetic distances. We found a strong genetic structure between Northern and Southern populations of tanagers of more than 10%, even among conspecific hosts. Therefore, the mite distribution on Brazilian tanagers is predominantly shaped by geography rather than by host species. These features in turn reflect historical horizontal transmissions among the hosts, suggesting a high potential for frequent host switches in these symbionts.

Keywords: cryptic species, biogeography, symbionts, coevolution, Psoroptidia.

Analoidean feather mites (Sarcoptiformes: Analoidea) are the most abundant ectosymbionts associated with passerine birds (Aves: Passeriformes) (Gaud & Atyeo 1996). These mites have high levels of host specificity and most mite species are found on a single or closely related host species. In addition, different mites species can co-exist in different microhabitats of the same host individual (Dabert & Mironov 1999). Despite being common and abundant avian ectosymbionts, many aspects of the feather mite ecology remain underexplored. One such aspect is their geographic distribution with respect to their host ranges. Does a feather mite distribution mirror that of its host? Does a host species have the same set of feather mites across its whole distribution? Only a few studies addressed those questions; in general they indicate that the feather mite assemblage on a bird species may be affected by climatic variables, such as humidity and temperature (Grossi & Proctor 2021; Meléndez et al. 2014); or reflect vicariance due to historical isolation of host populations (Dabert & Mironov 1999).

In ubiquitous hosts spread by humans, such as rock pigeons, *Columba livia* Gmelin, 1789 and domestic chickens, *Gallus gallus domesticus* (L., 1758), mite assemblages apparently change in new localities, indicating that some mite species have been acquired in recent events of interaction with native and phylogenetically similar host species (Gaud 1992). As for feather mites on naturally widespread host species, *i.e.* generalist hosts with high adaptive plasticity, a molecular identification of mites is often required to investigate their patterns of distribution, either for a proper identification of different mite haplotypes across different localities, as for the identification of potential cryptic species (Doña et al. 2015). Notwithstanding, assessing the geographic distribution of symbionts is a background needed to measure their dispersal potential and their specificity level. Yet,

almost no effort has been made to properly understand the patterns of distribution of these symbionts, especially in the Neotropics, where many feather mite species are yet to be named (Valim et al. 2011; Pedroso & Hernandez 2016). Our knowledge is especially scarce for Brazil, a country with various landscapes and one of the richest bird faunas in the World (Pacheco et al. 2021). Therefore, here we investigate the preliminary distribution of feather mites on tanagers (Thraupidae Cabanis, 1847), comparing samples from Brazilian Northern and Southern territories.

Feather mites were collected from four common and widespread tanager species: *Thraupis sayaca* (L., 1766), *T. palmarum* (Wied, 1821), *Stilpnia cayana* (L., 1766) from both Northern and Southern territories of Brazil, and the Amazonian *T. episcopus* (L., 1766). Birds were captured using mist nets in: Amazon Forest (AMF), Northeast Atlantic Forest (NAF), and Southeast Atlantic Forest (SAF) (Tab. 1). Feather mites were collected by plucking infested feathers (permit MMA/SISBIO 57944), then their DNA was extracted and a barcode gene, the mitochondrial cytochrome oxidase subunit-1 (COX-1) was sequenced following the protocols, primers, and parameters described in Klimov & OConnor (2008). A Maximum Likelihood phylogenetic tree was inferred using RaxML, also using sequences from previous studies, including samples of mites on tanagers from Mexico (Klimov et al. 2017). Mite's exoskeletons (vouchers) were slide-mounted for morphological identification using Hoyer's Medium (Gaud & Atyeo 1996). All specimens were deposited at the Department of Ecology and Zoology of the Universidade Federal de Santa Catarina (ECZ-UFSC).

Two species delimitation criteria based on the COX-1 were also performed – (i) a distance based delimitation using the Kimura two-parameter (K2P) distance calculated in the R package 'ape' 5.3 (Paradis

Table 1. Detailed information on each feather mite species, host species, collection codes, sampling sites, and GenBank accession numbers used in the present study.

| Mite Haplotype | Host Species | Mite Code | Host Code | Location | Coordinates | GenBank Codes |
|--|---------------------------|-----------|-----------|----------------|---------------------------|---------------|
| <i>Trouessartia</i> sp. | <i>Thraupis sayaca</i> | LGAP77 | SP53 | Rio Claro, SP | 22°23'46.4"S 47°32'50.1"W | MW814607 |
| <i>Trouessartia</i> sp. | <i>Thraupis sayaca</i> | LGAP78 | PE07 | Recife, PE | 8°03'09.7"S 34°57'07.1"W | MW814608 |
| <i>Trouessartia</i> sp. | <i>Thraupis palmarum</i> | LGAP79 | AC08 | Rio Branco, AC | 9°37'23.2"S 67°35'33.1"W | MW814609 |
| <i>Trouessartia</i> sp. | <i>Thraupis palmarum</i> | LGAP80 | RN17 | Natal, RN | 5°50'33.5"S 35°12'05.6"W | MW814610 |
| <i>Trouessartia</i> sp. | <i>Thraupis episcopus</i> | LGAP81 | PA06 | Belem, PA | 1°27'03.8"S 48°26'40.1"W | MW814611 |
| <i>Proctophyllodes</i> sp. | <i>Stilpnia cayana</i> | LGAP101 | SP10 | Rio Claro, SP | 22°23'46.4"S 47°32'50.1"W | MW814629 |
| <i>Proctophyllodes</i> sp. | <i>Stilpnia cayana</i> | LGAP102 | RN22 | Natal, RN | 5°50'33.5"S 35°12'05.6"W | MW814630 |
| <i>Proctophyllodes</i> sp. | <i>Stilpnia cayana</i> | LGAP103 | PE03 | Recife, PE | 8°03'09.7"S 34°57'07.1"W | MW814631 |
| <i>Proctophyllodes</i> cf. <i>thraupis</i> | <i>Thraupis sayaca</i> | LGAP104 | SP22 | Rio Claro, SP | 22°23'46.4"S 47°32'50.1"W | MW814632 |
| <i>Proctophyllodes</i> cf. <i>thraupis</i> | <i>Thraupis sayaca</i> | LGAP105 | RN20 | Natal, RN | 5°50'33.5"S 35°12'05.6"W | MW814633 |
| <i>Proctophyllodes</i> cf. <i>thraupis</i> | <i>Thraupis palmarum</i> | LGAP107 | AC07 | Rio Branco, AC | 9°57'00.0"S 67°44'00.0"W | MW814636 |
| <i>Proctophyllodes</i> cf. <i>thraupis</i> | <i>Thraupis palmarum</i> | LGAP108 | RN17 | Natal, RN | 5°50'33.5"S 35°12'05.6"W | MW814637 |

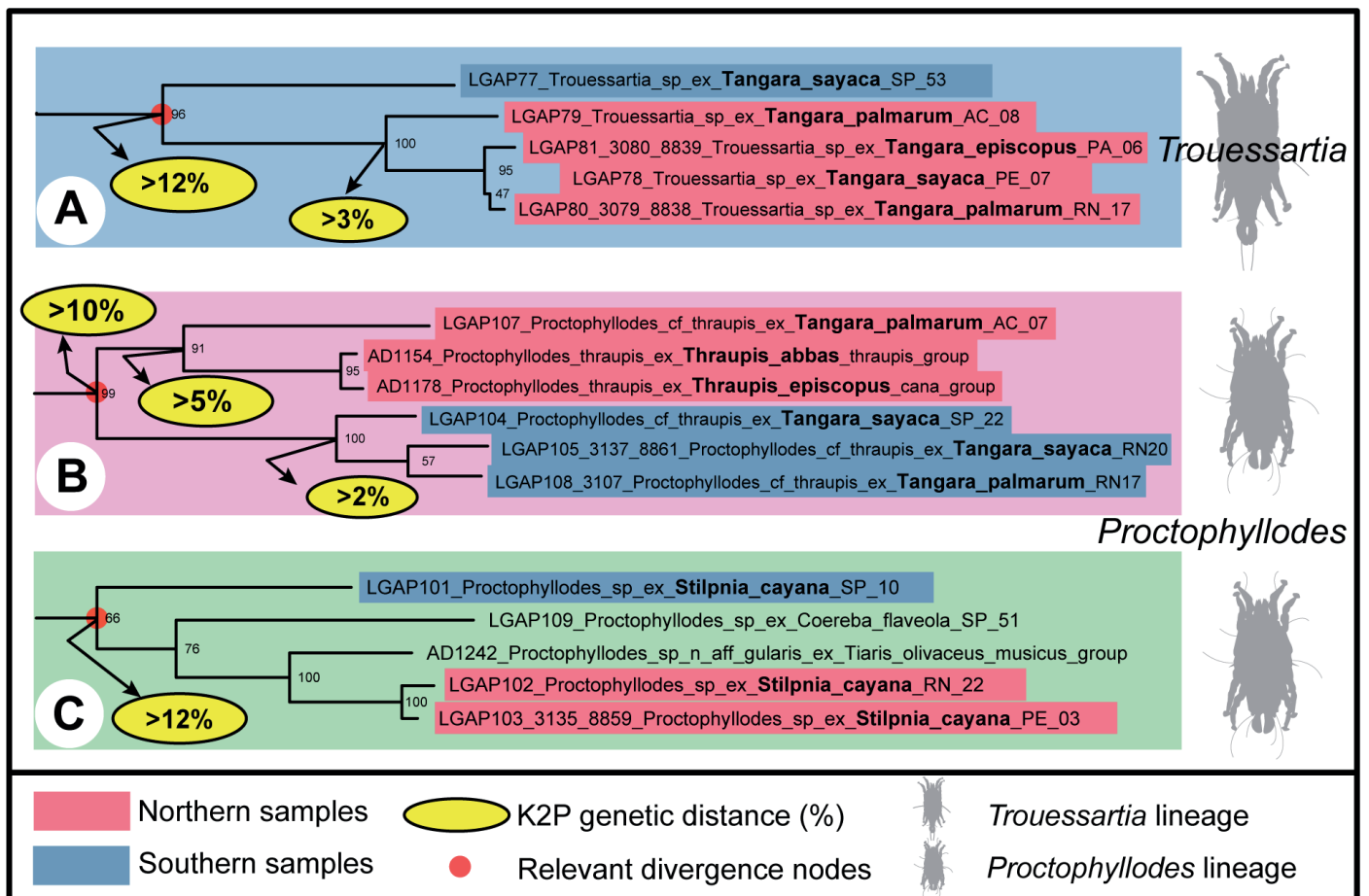
& Schliep 2019), and (ii) a barcoding gap analysis, using the Automatic Barcode Gap Discovery (ABGD), which groups putative species based on the barcoding gap among samples (Puillandre et al. 2012).

We recorded three feather mite lineages showing cases of geographic partitioning with large genetic distances on tanagers in Brazil, one representing the mite genus *Trouessartia* Canestrini, 1899, and two other lineages in the mite genus *Proctophyllodes* Robin, 1868 (Fig. 1A, B, C). The mite lineages presented similar patterns of geographic distribution, defined by the separation of mite populations between birds from Northern and Southern territories in Brazil. *Trouessartia* species yielded a genetic distance higher than 12% between the NAF and AMF vs. SAF host populations (Fig. 1A; 2A). In *Proctophyllodes*, one lineage (*Proctophyllodes thraupis* Atyeo & Braasch, 1966, described from *Thraupis abbas* (Deppe, 1830)), had genetic distance above 10%

between AMF vs. NAF and SAF (Fig. 1B; 2B); while the other lineage, which included solely mites from *Stilpnia cayana*, had genetic distances above 12% between NAF vs. SAF populations (Fig. 1C; 2C).

Both species delimitation methods (ABGD and K2P) inferred to a split in the mite populations on tanagers in Brazil following a geographic pattern. The distribution pattern differed among the mite lineages, as both the *Trouessartia* and *Proctophyllodes* lineages from *S. cayana* presented a clear division between samples from Southern territories (SAF) from samples from Northern territories (NAF and AMF) (Fig. 1A, C; 2A, C). A different pattern of distribution was observed for the *P. thraupis* lineage, where the mite populations in the Atlantic Forest (SAF and NAF) presented connections between them, while the mite population from Amazon composed a separate group (Fig. 1B; 2B).

This pattern of distribution where the Northern Atlantic Forest is


Figure 1. Genetic distance of three feather mite lineages – one *Trouessartia* (A) and two *Proctophyllodes* (B and C) on tanagers in Brazil. The relevant diverging nodes have their genetic distances in percentage highlighted. The relevant taxa were highlighted according to their sampling location: Northern and Southern territories.

more related to the Amazon Forest than with the Southern Atlantic Forest has been recorded in other taxa (Santos et al. 2007; Sobral-Souza et al. 2015; Thomé et al. 2016; Ledo & Colli 2017) including birds (Batalha-Filho et al. 2013). Many studies suggest that the “dry diagonal” formed by the biomes Chaco, Cerrado, and Caatinga, is an important barrier preventing biotic exchange between the rain forests (Amazon and Atlantic Forest) (Werneck et al. 2011). Our data corroborate these biogeographic hypotheses and give valuable evidences for obligate symbionts in this regard. A similar allopatric pattern of feather mites were recorded in Europe, where different species of the mite genus *Scutulanysus* Mironov, 1985 (Analgoidea: Pteronyssidae) were identified occurring on populations of the House Martin, *Delichon urbicum* (L., 1758), separated by the Ural Mountains (Dabert & Mironov 1999; Dabert 2004). In that study, however, the morphological differentiation of the mite species was evident, while in our study this pattern could be detected by using molecular data only.

The smallest genetic distance between samples from different territories was found in the *P. thraupis* clade, where mites from AMF differed in at least 10% with Atlantic Forest mites (SAF and NAF). This COX-1 genetic variation was expressively above the threshold of 3.4%

found for species delimitation of feather mites in European passerines (Doña et al. 2015). Yet, in these European passerines, no genetic structure among mites collected from different geographic territories and different host species was found, and instead, putative cryptic species for feather mites on close related hosts were recognized (Doña et al. 2015). In North America, a minimum COX-1 distance of 6.6% among proctophylloid species was identified on parulid warblers, with mites phylogenetically related according to their host’s nesting ecology (Matthews et al. 2018). In our study, the distribution of feather mites among tanagers were geographically shaped instead of following a host-specific pattern of distribution, i.e. close related mites were recorded on different host species instead among conspecific hosts, reflecting the relevance of horizontal pathways for their dispersion. Quantitative and thorough morphological analyses are needed to see if there are any morphological discontinuities in the cryptic species detected by molecular methods. Further studies, increasing the sample size and geographic coverage, are necessary to properly understand the pattern of genetic distribution of feather mites observed on Brazilian tanagers.

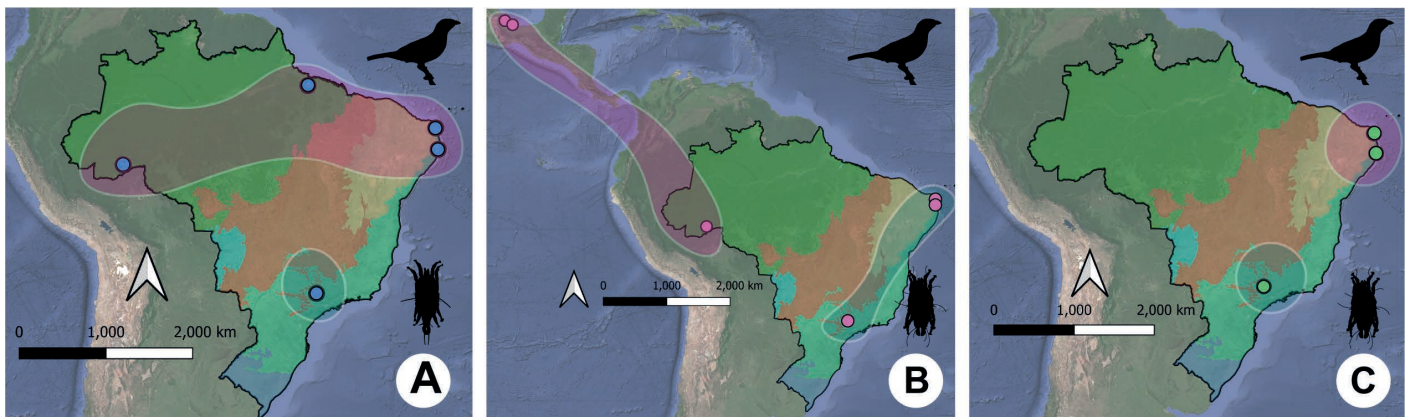


Figure 2. Schematic representation of geographic partition observed in different lineages of feather mites on tanagers in Brazil. A – *Trouessartia* on *Thraupis*, B – *Proctophyllodes* on *Thraupis*, and C – *Proctophyllodes* on *Stilpinia*. Phylogeographic clusters are represented.

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Authors’ Contributions

LGAP collected the mite samples and wrote the first draft of the manuscript. Molecular work and analysis was done by L.G.A.P. and P.B.K. at the University of Michigan Museum of Zoology. PBK and FAH critically reviewed the final version of the manuscript.

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