

Original Article



Contribution to the knowledge of *Neotrichodectes (Nasuicola) pallidus* (Piaget, 1880) (Phthiraptera: Trichodectidae)

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ABSTRACT

The species in the genus *Neotrichodectes* (Phthiraptera: Ischnocera) infest carnivores. *Neotrichodectes (Nasuicola) pallidus* (Piaget, 1880), which has been primarily found parasitizing Procyonidae mammals, has been recorded in ring-tailed coatis (*Nasua nasua*) in the Brazilian states of Minas Gerais, Pernambuco, Santa Catarina, Rio Grande do Sul and Pernambuco. We report a new record of *N. pallidus* in coatis in the state of Mato Grosso do Sul, central-western Brazil, using morphological (Light and Scanning Electronic Microscopy) and molecular approaches (PCR, sequencing and phylogenetic analysis). Coatis were sampled in two peri-urban areas of Campo Grande city, Mato Grosso do Sul state, Brazil, between March 2018 and March 2019, as well as in November 2021. Lice were collected and examined under light and Scanning Electron Microscopy. DNA was also extracted from nymphs and adults and submitted to PCR assays based on the 18S rRNA and *cox-1* genes for molecular characterization. One hundred and one coatis were sampled from 2018 to 2019 and 20 coatis in 2021 [when the intensity of infestation (II) was not accessed]. Twenty-six coatis (26/101–25.7%) were infested with at least one louse, with a total of 59 lice collected in 2018–2019. The II ranged from one to seven lice (mean $2.2 \pm SD 1.7$). The louse species was confirmed based on the following morphological characteristics: female gonapophyses rounded with the setae along anterior region but not in the medial margin; the male genitalia with a parameral arch not extending beyond the endometrial plate. The same ornamentation was observed on the abdomen of the females, males, and nymphs. The nymphs and the eggs were described in detail for the first time. The obtained 18S rRNA and *cox1* sequences from *N. pallidus* clustered in a clade with other sequences of Ischnocera species. In the present study, a new record of the louse *N. pallidus* in central-western Brazil was provided, along with new insights into the morphological features of this species, with the first morphology contribution of nymphal and eggs stages.

1. Introduction

The Parvorder Phthiraptera (Order Psocodea) comprises approximately 5000 species that parasitize birds and mammals, which consists of four suborders, namely Ischnocera, Amblycera, Anoplura and Rhynphthirina (Johnson et al., 2003; Galloway, 2018; Durden, 2019). The

louse species belonging to the suborders Ischnocera and Amblycera, occur in the Neotropical region and are popularly known as chewing lice, since their diet is mainly composed by necrotic epidermal fragments, hair, feathers and sebaceous secretions from their hosts and blood in some species (Urquhart et al., 1996; Boyd and Reed, 2012; Galloway, 2018; Durden, 2019). Although an infestation by chewing lice

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is not usually considered harmful to the host, lice can cause lesions on the skin and, consequently, open entryways for many pathogens (Marcondes and Linardi, 2017; Benelli et al., 2018). Indeed, some species can cause stress and weight loss to the host (e.g. *Menacanthus stramineus* (Nitzsch, 1818) infesting chicken) (Paliy et al., 2018; Kouam et al., 2022).

Within the Ischnocera, the family Trichodectidae Kellogg, 1896 comprises species associated with domestic and wild animals, such as *Felicola subrostratus* (Burmeister, 1838) and *Trichodectes canis* (De Geer, 1818) that parasitize domestic cats and dogs, respectively. *Felicola subrostratus* and *T. canis* are intermediate hosts for the zoonotic tapeworm *Dipylidium caninum* (Rousseau et al., 2022). Particularly, *Neotrichodectes*, has been associated with species of the order Carnivora. *Neotrichodectes (Nasuicola) pallidus* (Piaget, 1880) is known to infest only species of the *Nasua* (Price et al., 2003). In South America, this species infests ring-tailed coatis, *Nasua nasua* (L., 1766) in the Brazilian states of Minas Gerais (Rodrigues et al., 2006; Estevam et al., 2020), Paraná (Magalhães-Matos et al., 2022), Pernambuco (Valle, 2020), Rio Grande do Sul, and Santa Catarina (Piccoli, 2010), and in Venezuela (Emerson and Price, 1975; Cañizales and Guerrero, 2017), Bolivia, Colombia, and Paraguay (Werneck, 1948). Additionally, this louse species has been recorded in Latin America, including Mexico (Werneck, 1948; Sánchez-Montes et al., 2018; Guzmán-Cornejo et al., 2020), Panama, (Werneck, 1948), and Nicaragua (Emerson, 1971) where it infests white-nosed coati, *Nasua narica* (L., 1766) (Carnivora: Procyonidae).

In the present study, we report a new Brazilian locality for *N. pallidus* associated with the ring-tailed coati. Additionally, we present the first molecular data for this species of louse and the first detailed morphological images using scanning electron microscopy.

2. Material and methods

2.1. Sampling of lice

All captures were performed in two Conservation areas, “Parque Estadual do Prosa” (PEP) (−20.44987, −54.56529) and “Vila da Base Aérea” (VBA) (−20.47163, −54.65405), both located in Campo Grande city, Mato Grosso do Sul state, in midwestern Brazil. PEP is a state National Conservation Park 134 ha in size, representing one of the last remnants of the Cerrado biome within the urban perimeter. The area has become a tourist spot for visitors from cities in the state of Mato Grosso do Sul as well as from different regions of Brazil, so there is a high daily circulation of people. VBA is a residential area, surrounded by three Cerrado forest fragments and one area used for military training, with approximately 484 ha. At least 730 people and their domestic animals inhabited the residential complex.

Ring-tailed coatis were captured every three weeks for 10 consecutive days between March of 2018 and April of 2019. Animals were captured using metal traps (1 m × 0.40 m × 0.50 m) placed arbitrarily according to the possibility of human access and availability of shadow, covering most of the PEP and VBA areas. Animals were anesthetized with an association of Tiletamine hydrochloride and Zolazepam hydrochloride (Telazol, Zoetis® (Parsippany-Troy Hills, NJ, USA) (5–7 mg/kg, intramuscularly). After chemical restraint, animals were marked with numbered colored earrings and had a microchip implanted in the subcutaneous tissue between the shoulder blades in order to identify them for future recaptures. Animals were measured and the age was estimated according to Olifiers et al. (2010). The animals' entire body was inspected for the presence of louse for three minutes. Louse were removed with the aid of a forceps and stored in 100% alcohol (Merck Ensure®, Darmstadt, Germany)- containing RNase/DNase free microtubes. Taxonomic identification of ectoparasites was performed with a stereoscopic microscope (Olympus SZX7, Tokyo, Japan). Overall fur/skin condition and weight of the animals were assessed. The infestation intensity corresponded to the number of lice collected from each animal. In November 2021, a new sampling procedure was performed, but the

infestation intensity was not assessed. Such samplings have already resulted in several other publications about tick-borne agents in coatis and ecological features of the species in urban areas of Central-Western Brazil (Barreto et al., 2021; Perles et al., 2022a, 2022b, 2022c). However, the assessment of infestation by lice on these animals has yet to be addressed.

2.2. Morphological analysis

Louse specimens were studied using three methods: (1) slide-mounting for identification by light microscopy, (2) preparation and observation using scanning electron microscopy (SEM), and (3) molecular characterization. Lice were identified using previously described taxonomic keys (Werneck, 1936; Price et al., 2003) and all the original descriptions for the species into this genus. All these preparations were made based on Barros-Battesti et al. (2021). The material was deposited at the Entomological Collection of the Instituto Butantan, São Paulo, Brazil (IBSP - Ent).

The images were obtained using Leica DFC 500 digital camera coupled to a Leica DM4000B light microscope. Extended focal range images were composed using Leica Application Suite version 2.5.0. The SEM images were obtained with a Digital Scanning Microscope FEI, Quanta 250, located at the Laboratório de Biologia Celular, Instituto Butantan, São Paulo, SP, Brazil (IBSP).

2.3. DNA extraction and molecular characterization

Five representatives of each stage (female, male and nymph) obtained during the sampling performed between 2018 and 2019 were selected randomly and submitted individually to DNA extraction using the DNeasy Blood and Tissue Kit (Qiagen®), according to manufacturers' instructions. Vouchers were preserved to confirm the identification and corroborate the morphological results. Conventional PCR assays targeting fragments of the 18S rRNA and *cox1* genes were performed following previously described protocols (Folmer et al., 1994; Otto and Wilson, 2001). Amplicons were purified using Exosap (Life Technologies®, Carlsbad, CA, USA) and subjected to sequencing by the Sanger method using ABI PRISM 3730 DNA Analyzer (Applied Biosystems) at the Human Genome and Stem Cell Research Center, Instituto de Biociências, University of São Paulo (USP), São Paulo, SP, Brazil. Due to economic limitations, only one sequence from each life stage (male, female and nymph) was sequenced for *cox1* gene. Using the BLASTn tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), we could determine the Query-coverage %, E-value, and % identity of the obtained sequences. The phylogenetic analyses were based on Bayesian inference (BI) and performed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) via the CIPRES Science Gateway. The phylogenetic tree edition and rooting (outgroup) were performed using TreeGraph 2.0 beta software (Stover and Muller, 2010).

3. Results

In total, 101 coatis were sampled [51 in PEP (30 females and 21 males) and 60 in VBA (34 females and 26 males)] during 2018–2019. Twenty-six coatis [26/101–25.7% (13 females and 13 males)] were infested with at least one louse, with a total of 59 lice obtained (27 nymphs, 15 females and 17 males; see Table 1). The intensity of infestation ranged from 1 to 7 lice (mean $2.2 \pm SD 1.7$). From PEP, 10 (19.6%) animals were infested [three females (IR: one - two lice) and seven males (IR: one - four)]. From VBA, 16 (26.6%) animals were infested [10 females (IR: one - five) and six males (IR: one - seven)].

Regarding weight, all parasitized animals presented good body condition, and weights were within the normal range for the species. Regarding overall skin/full conditions, 10 animals showed skin lesions (4 from PEP and 6 from VBA) consisting of scars in different body regions (nose, legs, etc.), suggestive of aggressive interactions but not related

Table 1

Identification of South American coatis (*Nasua nasua*) infested with *Neotrichodectes (Nasuicola) pallidus* sampled in Campo Grande city, Mato Grosso do Sul state, Brazil, during 2018 and 2019. Information is shown about sample localities, date of collection, sex of the hosts, intensity of infestation and life stages of lice collected.

Identification	Localization	Sampling data	Sex	Infestation rate	Stages
ID04	PEP ¹	14/03/2018	M ³	4	1F, 3N ⁵
ID08	PEP	15/03/2018	F ⁴	1	1 N
ID09	PEP	16/03/2018	F	1	1 M
ID10	PEP	16/03/2018	M	2	1F, 1 M
ID11	PEP	16/03/2018	F	2	1 N, 1F
ID13	PEP	20/03/2018	M	1	1 M
ID88	PEP	07/08/2018	M	2	2F
ID93	PEP	09/08/2018	M	1	1 M
ID121	PEP	02/10/2018	M	1	1 N
ID123	PEP	02/10/2018	M	1	1 N
ID48	VBA ²	18/06/2018	F	2	1 N, 1 M
ID155	VBA	23/01/2019	F	2	2 N
ID165	VBA	20/03/2019	F	1	1 N
ID98	VBA	21/08/2018	M	3	1 N, 2F
ID61	VBA	25/06/2018	M	1	1 N
ID104	VBA	27/08/2018	F	4	2F, 2 M
ID105	VBA	27/08/2018	F	1	1 N
ID107	VBA	28/08/2018	F	5	1 N, 2F, 2 M
ID109	VBA	28/08/2018	M	1	1 N
ID110	VBA	28/08/2018	M	1	1 N
ID112	VBA	29/08/2018	F	4	1F, 3 M
ID153	VBA	23/01/2019	F	1	1F
ID154	VBA	23/01/2019	F	1	1F
ID161	VBA	29/01/2019	M	3	2 N, 1 M
ID167	VBA	21/03/2019	F	6	3 N, 3 M
ID169	VBA	21/03/2019	M	7	5 N, 1F, 1 M
			Total	59	27 N, 15F, 17 M

¹ Parque Estadual do Prosa.

² Vila da Base Aérea.

³ Male.

⁴ Female.

⁵ Nymph.

to the presence of lice.

In November 2021, twenty animals were sampled (the infestation intensity was not accessed). All collected specimens (nymphs and adults) we identified to species level. At this moment, louse eggs were also sampled.

After slide-mounting the chewing louse specimens sampled, the

species was identified as *N. pallidus* based on the following set of morphological characteristics: female gonapophyses rounded with the setae along the anterior region but not in the medial margin (Fig. 1); male genitalia with a parameral arch not extending beyond the endometrial plate (Fig. 2). Furthermore, for the entire genus, the spiracles are absent in the abdomen; the endometrial plate and parameral arch have an apical pointed process in the male genitalia. For the first time, the nymphs and the eggs of this chewing louse species (Fig. 3A-C and 3D, respectively) were photographed and their details recorded. The elliptical eggs have a protective cover in the anterior region, with subtle small balls adorning the entire surface (Fig. 3D). This ornamentation resembles a scale-like ornamentation that the females (Fig. 1A), males (2A), and nymphs (Fig. 3A) have.

Only one 18S rRNA sequence from one male collected from a coati from PEP was obtained. BLAST results showed 100% query-coverage, 98% identity and 0.0 E-value with *Bovicola bovis* (Linnaeus, 1758) (AY077769), and 90% identity from *F. subrostratus* (AY077770). BI phylogenetic analyses based on the 18S rRNA gene clustered the obtained sequence in a large clade, composed of *Neotrichodectes arizonae* Werneck, 1948, *F. subrostratus*, and *Bovicola ovis* (Schrank, 1781) (Fig. 4A). Three sequences were obtained from the *cox1* gene from one female, one male and one nymph, collected from animals from PEP. Sequences were 100% identical and presented 99% of query-coverage, 76% identity and 0.0 E-value with *Geomydoecus* sp. (JF342603, JF342605, JF342569). BI phylogenetic analyses based on the *cox-1* gene clustered the sequences of *N. pallidus* in a large clade composed of sequences of different *Geomydoecus* sp., *Thomomydoecus* sp. and *Bovicola caprae* (Gurlt, 1843) (Fig. 4B). All obtained sequences were deposited at GenBank under accession numbers OP831279 (18SrRNA) and OP852364-OP852366 (*cox1* gene).

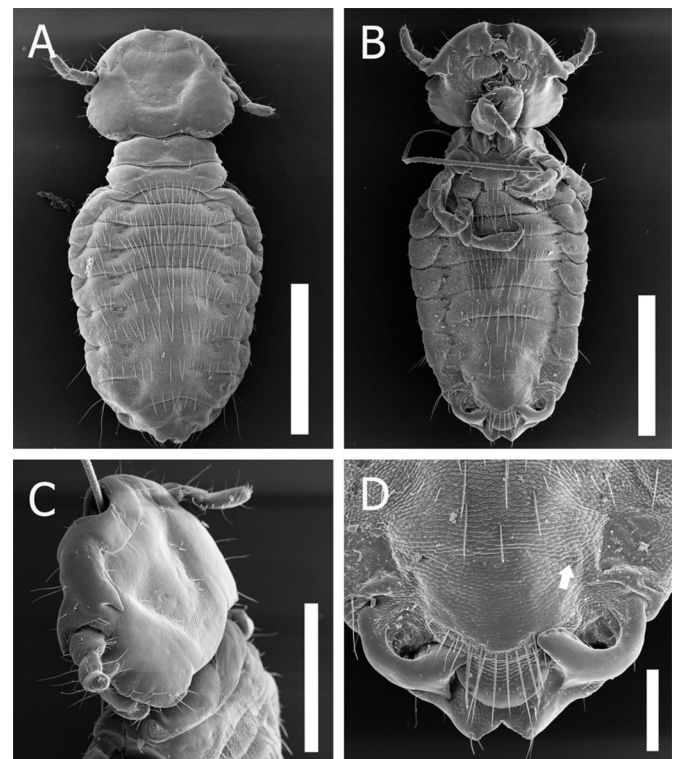


Fig. 1. SEM images of the *Neotrichodectes (Nasuicola) pallidus* (Piaget, 1880) female. A – dorsal; B – ventral; C – head; D – distal part of the ventral region. White arrow highlights the scale-like ornamentation. Scale bars: A and B 500 μm, C 300 μm and D 100 μm.

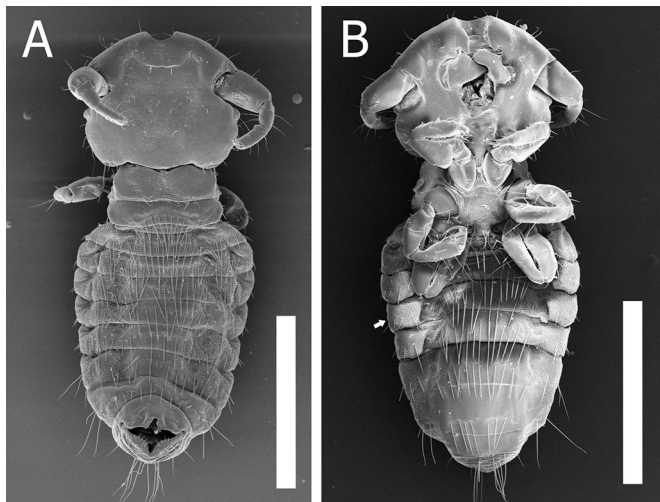


Fig. 2. SEM images of the *Neotrichodectes (Nasuicola) pallidus* (Piaget, 1880) male. **A** – dorsal; **B** – ventral. White arrow highlights the absence of spiracles. Scale bars: **A** and **B** 500 μ m.

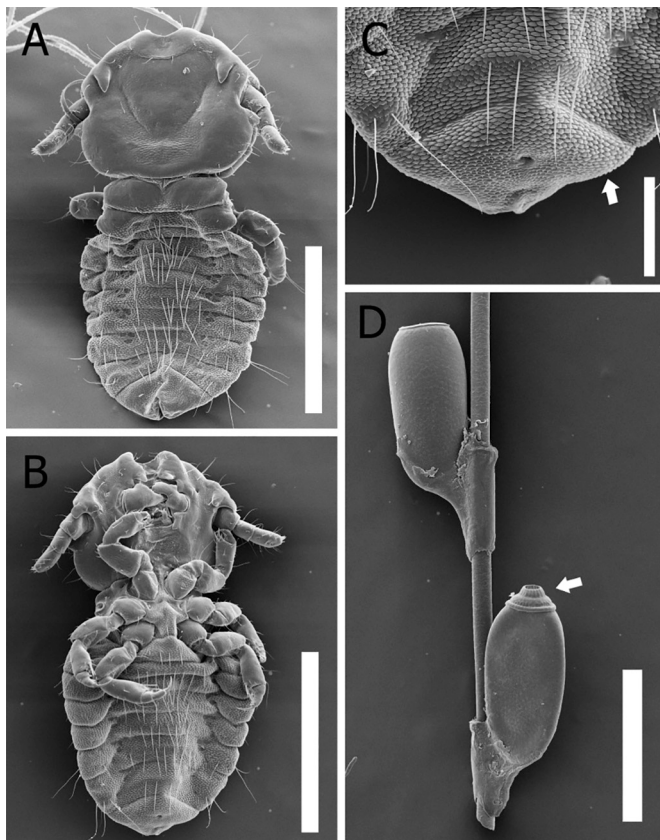


Fig. 3. SEM images of the *Neotrichodectes (Nasuicola) pallidus* (Piaget, 1880) eggs and nymph. **A** – nymph, dorsal; **B** – nymph, ventral; **C** - distal part of the ventral region; **D** - eggs attached to a hair. White arrows highlight the scale-like ornamentation and the protective cover in the anterior region. Scale bars: **A** and **D** 500 μ m, **B** 400 μ m and **C** 100 μ m.

4. Discussion

Herein, we expand the Brazilian geographical distribution of *N. pallidus* for the Midwest region of Brazil. This species has been previously recorded in some Brazilian regions with different prevalence. It infested 52.6% (10/19) ring-tailed coatis sampled in a fragment of

Atlantic Forest, Juiz de Fora city, Minas Gerais state, Brazil ($n = 61$) (Rodrigues et al., 2006). Piccoli (2010) found *N. pallidus* infesting one coati in an area of Atlantic Forest in a border area between the states of Rio Grande do Sul and Santa Catarina, with no mention of the number of specimens. Magalhães-Matos et al. (2022) reported a prevalence of 13% (11/86) in Atlantic Forest (Iguaçu National Park, Foz do Iguaçu city, Paraná state), ($n = 40$; 3 females, 6 males and 31 nymphs). In areas of Atlantic Forest and Cerrado biomes in Mangabeiras Municipal Park, Belo Horizonte city, Minas Gerais state, Estevam et al. (2020) collected 51 specimens (prevalence of infestation was 24.4%). Recently, in *Centro de Endemismo*, an area of Atlantic Forest in the state of Pernambuco, Valle (2020) reported a prevalence of infestation by *N. pallidus* of 54.6% (30/55) among the sampled coatis. These findings suggest that the coatis analyzed in Juiz de Fora (prevalence of 52.6%) and in *Centro de Endemismo* (54.6%) may have a more important epidemiological role in the maintenance of *N. pallidus* when compared to coatis sampled in other studied regions (prevalence varying from 13 to 25.75%), including those sampled in present work. Even within the same biome, each location sampled has its own characteristics of climate, phytophysiology, faunistic composition and anthropic influence that might influence the differences in the occurrence of parasites.

Clinical signs associated with chewing louse infestation are well known in domestic animals. In dogs and cats, *T. canis* and *F. subrostratus*, respectively, can cause cutaneous crust and hair loss, especially in breeds with long ears (e.g. Basset, Cocker spaniel) and longhair (e.g. Persian cats) (Benelli et al., 2018). In farm animals, *B. bovis* and *B. ovis* can cause cutaneous irritation, itching, and hair loss in several parts of the body of cattle and sheep, respectively (Benelli et al., 2018). Large chewing louse infestations may cause reduced food consumption, and consequently interfere with weight gain (Kettle and Lukies, 1982; Rony et al., 2010). On the other hand, little is known about the impact of louse infestation on wild animals. In wild birds, previous studies have reported reductions in the potential attractiveness of louse-infested males to females, since the presence of lice can cause feather damage (Clayton, 1991; Borgia et al., 2004; Garamszegi et al., 2005). The presence of lice can also affect the metabolic rates of avian hosts. Free-ranging birds can increase metabolic rates and heavily infested birds need to draw on fat reserves to maintain these energetic costs, leading to a chronic decline in body mass over several months (Booth et al., 1993).

To the author's knowledge, there is no information about the impacts and possible clinical alterations due to the presence of *N. pallidus* in procyonids. In this host group, information available is mainly associated with updates on checklists and molecular detection of pathogens in lice (Ford, 2004; Reeves et al., 2005; Torres-Mejía and De La Fuente, 2006; Ouarti et al., 2021). Estevam et al. (2020) found that 13.2% (20/151) of coatis presented some clinical abnormalities, such as dull and ruffled fur, scars, and other clinical alterations, but with no mention of the presence of lice in those animals. In the present study, although some animals presented skin lesions, they were unlikely to have been associated with louse infestations. The infestation of *N. pallidus* at low levels did not seem to interfere with parasitized coatis' skin/fur condition. All animals examined presented good body condition, with weight within normal expectations for the species (Barreto et al., 2021; Perles et al., 2022b). It is essential to highlight that none of the captured animals presented high infestation rates. Large infestations can cause problems to the host, such as those already demonstrated in domestic animals (Kettle and Lukies, 1982; Rony et al., 2010).

We provide the first sequences of two molecular markers and phylogenetic inferences from specimens of *N. pallidus*. Before the advent of molecular techniques, traditional phylogenetics based on morphology suggested that parasitic lice had a monophyletic radiation, mainly because their permanent parasitic lifestyle. Lice were at one time classified into two orders: Mallophaga (all chewing lice) and Anoplura (all sucking lice) (Kim and Ludwig, 1978, 1982). Lyal (1985) and Smith (2004) challenged the monophyly of the Mallophaga by supporting a topology of two sister clades, one clade containing the Amblycera, and

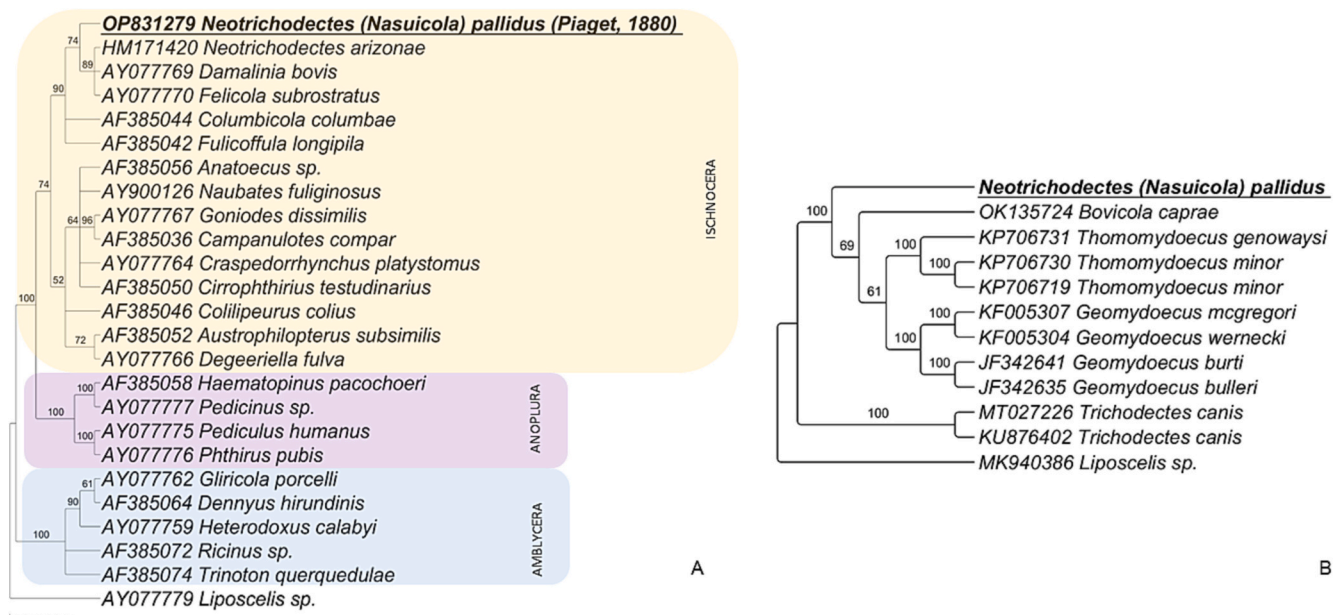


Fig. 4. Bayesian phylogenetic trees, with numbers at nodes that corresponding to the bootstrap. Accession numbers are indicated in the sequences. Sequence of *Neotrichodectes (Nasuicola) pallidus* detected in the present study is highlighted in bold/underlined. *Liposcelis* sp. was used as outgroup. **A)** Alignment of 677 bp fragment 18S rRNA sequences, TN + G4 evolutionary model and gamma shape = 0.22; **B)** Alignment of 495 bp fragment 18S rRNA sequences, TN + G4 evolutionary model and gamma shape = 0.22. *Liposcelis* sp. was used as outgroup.

the other containing the Ischnocera, Rhynchophthirina, and Anoplura. With the advance of sequencing technologies, the evolutionary relationships of lice and their taxonomy have changed considerably over multiple revisions (Boyd and Reed, 2012). Johnson and Whiting (2002) and Barker et al. (2003) were the first to examine Phthiraptera phylogeny by using molecular data from nuclear and mitochondrial markers and supported the polyphyletic origin. Despite studies regarding phylogenetic analyses of lice parasitizing domestic animals, there is a paucity of information on molecular data from lice from wild animals, especially from Brazil. Sequences from only one louse species *Neotrichodectes arizonae* Werneck, 1948 (18S rRNA, *cox1* and EF1-alpha genes), a parasite of the American hog-nosed skunk (*Conepatus leucurus* (Lichtenstein, 1832)), are available in the GenBank database. All previous studies on lice from coatis in Brazil reported only prevalence and life stages, with no attempts to perform molecular inferences of the louse species. Future molecular studies, aiming at achieving sequences of both nuclear and mitochondrial genes from lice found parasitizing wild animals should be performed, to achieve an increased database to allow new comparative and evolutionary relationships within Phthiraptera.

5. Conclusions

The present work extends the Brazilian geographic range of distribution for *N. pallidus*, and includes the first molecular contribution with sequencing and phylogenetic inference based on two molecular markers (18S rRNA and *cox1*). Also, for the first time, SEM images from all life stages (eggs, nymphs and adults from both sexes), with the first morphological description of eggs. The infestation by *N. pallidus* with low parasite burden did not seem to produce any alterations on body condition and overall skin/fur condition of the sampled coatis in Mid-western Brazil.

Ethics statement

All methods were carried out in accordance with relevant guidelines and regulations and were approved by the "Instituto Chico Mendes de Biodiversidade" (ICMBio, protocol number 49662-8) and by the Ethics

Committee on Animal Use of the School of Agricultural and Veterinary Sciences, UNESP (CEUA FCAV/UNESP, protocol number 06731/19), Ethics Committee on Animal Use of the Universidade Católica Dom Bosco (CEUA UCDB, protocol number 001/2018) and Air force cooperation agreement (N°01/GAP-CG/2018).

Declaration of Competing Interest

None.

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