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The raptor Chimango Caracara (*Milvago chimango*) (Aves: Falconiformes) - A new host for *Trichomonas gallinae* (protozoa: Trichomonadidae)

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ABSTRACT

This work describes a specimen of Chimango Caracara (*Milvago chimango* - Vieillot, 1816), from southern Brazil, as a new natural host for *Trichomonas gallinae* (Rivolta, 1878). Caseous oral lesions were observed in a young bird, and the parasite was isolated in modified Diamond's media. Morphology of the parasite was evaluated through microscopy and subsequently, sequencing of the internal transcribed spacer 1 (ITS1) of ribosomal DNA was performed to confirm *T. gallinae* identification. As far as authors are concerned, this is the first report of *Milvago chimango* as a natural host for *T. gallinae*.

1. Introduction

Trichomonas gallinae (Rivolta, 1878) is a flagellated protozoan with worldwide distribution that infects several order of birds such as columbiformes, strigiformes, psittaciformes, and passeriformes (Forrester and Foster, 2008). The members of the order Columbiformes are the natural hosts of *Trichomonas gallinae* (Amin et al., 2014) and the transmission can occur from adults to young birds during feeding and between adults during courtship behaviors (Stabler, 1941). Avian trichomonosis is a major cause of death in young pigeons, however the disease also occurs in raptors, as for instance hawks and falcons, which become infected after feeding on infected birds. Additionally, indirect transmission is also possible and happens through contaminated fomites and water (Amin et al., 2014).

The infection usually affects the upper digestive and respiratory tracts and is characterized by lesions that progress to form caseous masses/abscesses. Birds affected by trichomonosis usually display apathy, wheezing, difficulty in closing the beak and, consequently, impaired feeding and drinking, in addition to excessive salivation. Lesions and clinical signs vary largely in severity and might be completely absent in some cases, as for instance in asymptomatic carriers such as pigeons, or lead to death in others (Neimanis et al., 2010; Forrester and Foster, 2008).

Oral trichomonosis is the most frequently diagnosed infectious diseases of raptors and it consequently leads to a decrease in their population (Forrester and Foster, 2008), especially for birds inhabiting urban and peri-urban areas. One example of it was previously observed for urban Cooper's hawks, which as a consequence of the disease presented nesting failures and higher nesting mortality when compared to falcons that exclusively inhabit wild areas (Boal and Mannan, 1999). This dynamic of transmission happens because *Columba livia*, which is the main source of trichomonosis for raptors due to their feeding habits, has a large population in urban areas facilitating and preserving the transmission cycle.

Multiple species of raptors have been documented as hosts for *T. gallinae* in Europe, Africa, the USA, and Mexico (Forrester and Foster,

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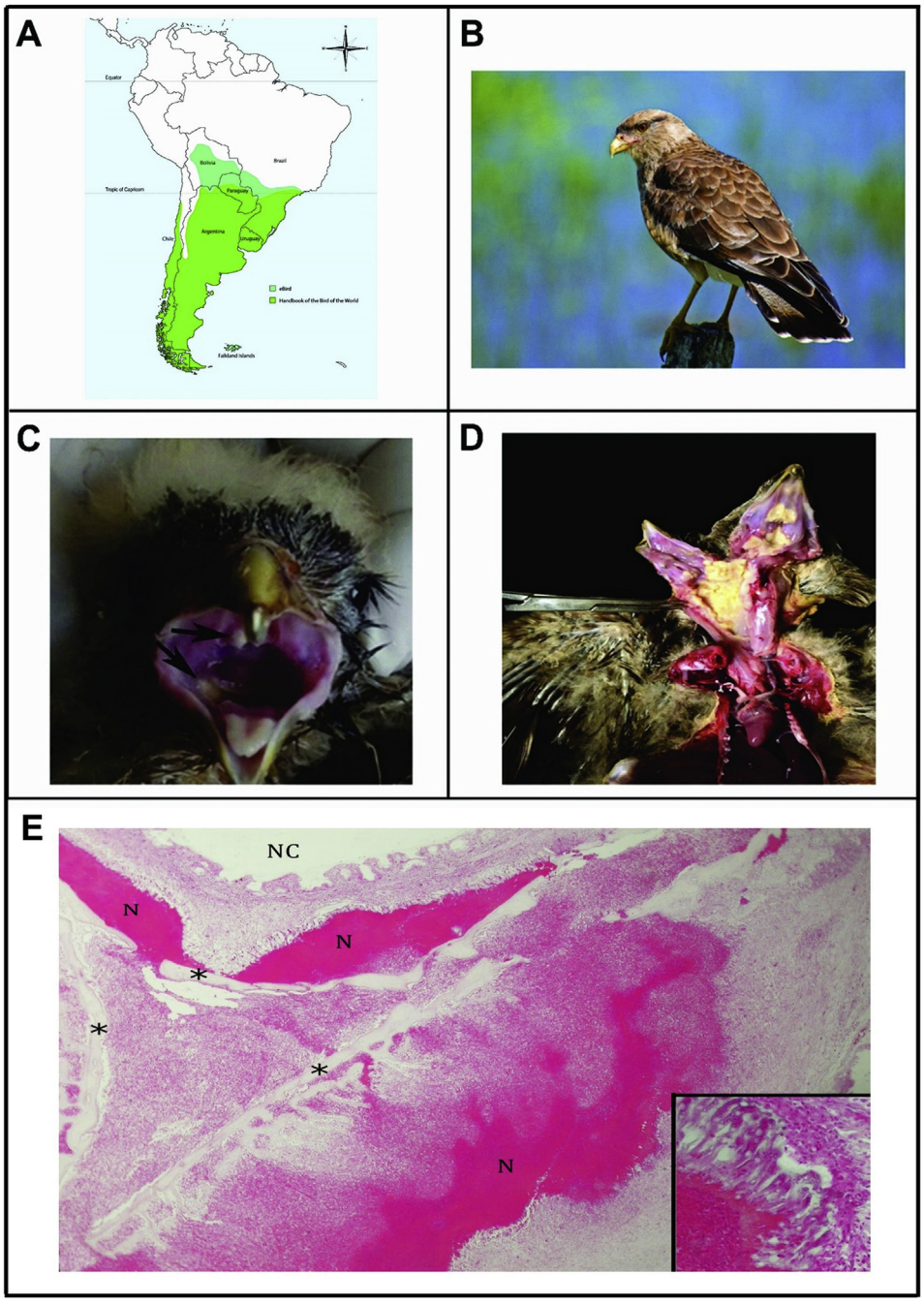

Fig. 1. A) Geographical distribution of *Milvago chimango*, a characteristic raptor from South America; B) Adult specimen of *Milvago chimango* (Timm and Timm, 2016); C) Oral trichomonosis in nestling *Mivalgo chimango*. Oral cavity. The nestling present dirty feathers, sialorrhea and within the oral cavity multifocal, well delimitate pale yellow caseous nodules in the mucosa.; D) Oral trichomonosis in nestling *Mivalgo chimango*. Oral cavity, oropharynx, esophagus and crop. Multifocal to coalescent yellow caseous nodular plaques extending to caudal hard and soft palate obliterating the oropharynx compromising also two thirds of esophagus and crop; E) Oral trichomonosis in nestling *Mivalgo chimango*. Oral cavity, palatal bone and nasal cavity. Focally extensive areas of necrosis (N) surrounded by giant cell areas and severe fibrinoheterophilic inflammatory infiltrate. Bone (*). Nasal cavity (NC). HE 100X. Inset: Caseous necrosis. Hipereosinophilic center surrounded by giant cells. HE 200X. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2008). In South America, Brazil is the only country with reported cases of the disease in falconiforms and several species were described as infected, as for instance the Southern Caracara (*Caracara plancus*) (Joppert, 2007; Andery et al., 2013), the Aplomado Falcon (*Falco femoralis*) (Andery et al., 2013), the American Kestrel (*Falco sparverius*) (Joppert, 2007; Andery et al., 2013), the Yellow-headed Caracara (*Milvago chimachima*) (Andery et al., 2013) and the Roadside Hawk (*Rupornis magnirostris*) (Andery et al., 2013). Cases of the disease have also been reported in strigiforms, such as the Striped Owl (*Asio clamator*) (Joppert, 2007; Ecco et al., 2012; Andery et al., 2013), the Burrowing Owl (*Athene cunicullaria*) (Andery et al., 2013), the Ferruginous Pygmy-Owl (*Glaucidium brasilianum*) (Andery et al., 2013), and the Barn Owl (*Tyto alba*) (Andery et al., 2013).

Chimango Caracara (*Milvago chimango*) (Aves: Falconiformes) is a neotropical bird originated in South America that belongs to the Falconidae family and it is found in Brazil, Argentina, Uruguay, Chile, Bolivia, and Peru (Fig. 1A) (Bierregaard et al., 2019; eBird, 2018). In Brazil, the largest populations of *M. chimango* are found in the Southeast and Central-west regions of the country (Táxeus, 2018) and regarding its conservation status, it is a species that has less concern because it is adapting to urban environments where it finds places to nest even with environmental degradation and consequently, its traditional prey is replaced by urban pigeons (Amin et al., 2014; Anderson et al., 2009). The Chimango Caracara is closely related to the Yellow-headed Caracara (*M. chimachima*), however it can be distinguished from it through its brownish coloration, which is lighter in the ventral region, and through a clear white colored region, which is more evident during the flight. This bird is a small raptor that weighs between 250 and 300g (Fig. 1B), has diurnal habits and opportunistic feeding habits, which consist of dead animals, turtle eggs, fish, insects, rodents, nesting and adult birds, and bird eggs (Timm and Timm, 2016). Although *M. chimango* preferentially feeds scavenges on carrion, it can also attack

injured and diseased animals, such as sheep and horses (Sick, 1993). Here, we report a specimen of *Milvago chimango* infected with *T. gallinae*, adding this bird of prey to the list of hosts for this protozoan species.

2. Case history

In the south region of Brazil, a young bird from the species *Milvago chimango* was rescued at the Oceanographic Museum Prof. Eliézer de Carvalho Rios, Federal University of Rio Grande, Rio Grande do Sul, Brazil in March 2016 and sent to the Wildlife Rehabilitation Center and Wild Animals Triage Center (Wildlife Rehabilitation Center and Wild Animals Triage Center - NURFS/CETAS) at the Federal University of Pelotas (UFPel), state of Rio Grande do Sul. The bird was in a cachectic state, presented sialorrhea and had caseous lesions on the palate, altogether these clinical signs constitute a clinical profile suggestive of oral trichomonosis (Fig. 1C).

The full diagnosis was performed on the Parasitology Laboratory of the Biology Institute at UFPel. Samples of the lesions were collected with sterile swabs and a wet mount was carried out, however the technique did not allow visualization of the parasite. Another swab containing the sample was used for *in vitro* culture and isolation of the parasite (Diamond, 1957). After a 24-h incubation at 37 °C, culture tubes were centrifuged at 1500 rpm for 10 min and then, an aliquot of the pellet was analyzed under optical microscopy. During analysis, trophozoites demonstrating characteristic rotating movements were visualized. Besides that, the visualization of morphological characteristics, such as piriform to oval shape with a size of about 7–11μm, clear undulating membrane, which did not extend itself to the posterior extremity, and four unequal anterior flagella reinforced the hypothesis of the parasite being *Trichomonas gallinae*.

Even after receiving specific treatment with metronidazole, the *M. chimango* specimen died after nine days of treatment and was referred to the Pathology Department of the Regional Diagnostic Laboratory of the Veterinary School at UFPel, where it was necropsied. Nodules and caseous abscesses were detected on the soft palate, glottis, esophagus, and initial portion of the crop. Fragments of the lesions and all organs were collected and fixed for 24 hours in buffered 10% formalin solution. Bone fragments were decalcified in 8% nitric acid. All fragments were routinely processed for histology, cut in 5μm and stained with hematoxylin and eosin. Tissue sections were stained with Giemsa for protozoal visualization and all tissue sections were analyzed under a light microscope (Figure 1E). All lesions found in the specimen reinforced that the death of the animal was caused by trichomonosis. Grossly, the bird was in poor body condition and have multifocal caseous nodules within the oral cavity that extent to the oropharynx, esophagus and crop. The esophagus and crop were obliterates whit yellow caseous material. Histologically, the areas of caseous necrosis were characterized by a hypereosinophilic center containing cellular debris, surrounded by giant cells and a discrete proliferation of fibroblasts. Adjacent to those areas a severe focally extensive inflammatory infiltrate of lymphocytes, plasmocytes and macrophages was observed. Additionally, the palatine bone with massive presented osteolysis, necrosis and loss of it architecture. Despite the expressive inflammatory response, the parasite was not present within the lesions.

Trichomonas gallinae identification was confirmed by molecular analysis, more specifically total genomic DNA from *T. gallinae* sample was extracted using a commercial DNA extraction kit – GenElute Mammalian Genomic DNA Miniprep (Sigma-Aldrich, St. Louis, MO, USA) according to manufacturer's instructions. To confirm the identity of the suspected *T. gallinae* DNA sample, ITS rDNA sequences were amplified by conventional PCR using primers ITS1F and ITS1R that were modified by Cepicka et al. (2005). The PCR reaction was carried out using Promega PCR Master Mix (Promega Corporation, WI, USA) in a final volume of 25 μL, containing 20 pM of each primer and 100–150 ng of DNA template. DNA amplification was constituted of a denaturation step performed at 95 °C for 5 min, followed by 40 cycles at 95 °C for 60 s, 56 °C for 30 s, and 72 °C for 30 s and by a final extension at 72 °C for 10 min. Positive and negative controls were included in the amplification reaction. Amplicons were analyzed by agarose (2%) gel electrophoresis with DNA ladder 100pb and visualized by staining with DNA Blue Green loading dye 1X (LGC Biotecnologia, São Paulo, Brazil) to confirm the size and integrity of the DNA samples. Subsequently, amplicons were purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) and the DNA strands were directly sequenced. After sequencing, the resulting sequences were compared with the NCBI/Genbank database using the Basic Local Alignment Search Tool - BLASTn. The identity of *T. gallinae* was confirmed by similarity with Genbank isolates. The isolated sample showed 99% identity with *T. gallinae* strains *Acridotheres tristis* (common mynah), in Iran, *Melopsittacus undulatus* (budgerigar) in Iran, *Columba livia, in* China (Genbank: KT869157.1, KT869155.1, MH733817.1, respectively) and isolates *Streptopelia decaocto*, in Malta, *Columba oenas* in Germany, *Columba oenas*, in Germany, *Columba oenas*, in Germany, Columba oenas, in Germany, *Bubo bubo*, in Spain, *Serinus canaria f. domestica*, in Slovenia , and *Aquila pennata*, in Spain (Genbank: KX844991.1, KX459445.1, KX459444.1, KX459443.1, KX459440.1, KX514378.1, KX584000.1, KP900042.1, respectively).

3. Discussion

The vast biodiversity of Brazil is being threatened by the intensification of human activities and by the expansion of the areas destined to agriculture, both of which cause environmental changes, alter wild species' biomes, and consequently lead to the reduction or even loss of these species' natural habitat (Soares et al., 2008). As a result, many species of raptors have approached urban areas in search of food and have also broaden their feeding habits, as for instance by widening their group of preferential preys, due to the reduction of natural populations of prey (Amin et al., 2014). Many cities and suburban areas have reported an increase in populations of Rock Pigeons (*Columba livia*), which, as other Columbiformes, are important reservoirs of the protozoan *Trichomonas gallinae* (Chi et al., 2013). By feeding on nesting birds or adult pigeons, raptors are highly susceptible to the protozoan and tend to get infected, which in most cases can be considered as the cause of death (Forrester and Foster, 2008; Chi et al., 2013).

In Brazil, so far, *T. gallinae* infection has been diagnosed in predatory birds in the states of São Paulo (Joppert, 2007) and Minas Gerais (Ecco et al., 2012; Andery et al., 2013), including the following species: *Asio clamator*, *Athene cunilaia*, *Caracara plancus, Falco femoralis, Falco sparverius*, *Glaucidium brasilianum*, *Milvago chimachima, Rupornis magnirostris* e *Tyto alba*. As far to the knowledge of the authors, this work is the first to describe the falconiform *Milvago chimango* as a host for *Trichomonas gallinae*. Therefore, the present study concludes that *M. chimango* should be added to list of raptors reported as *T. gallinae* hosts.

We declare that this work was conducted with the formal approval of the Animal Experimentation Ethics Committee from the Federal University of Pelotas (CEEA/UFPel nº 5206-2017).

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Declaration of competing interest

No conflicts of interest declared by any author.

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