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Hamatospiculum flagellispiculosum (Nematoda: Diplotriaenidae) causing severe disease in a new host from Argentine Patagonia: *Campephilus magellanicus* (Aves: Picidae)

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A B S T R A C T

We describe pathological aspects of an infection caused by parasitic nematodes in skeletal muscles of a Magellanic woodpecker (*Campephilus magellanicus*), providing the first description of any disease findings in this species. A weakened female with locomotory dysfunction was rescued near Bariloche city (Argentine Patagonia), which soon died. At the necropsy, unexpected masses of tissue were located at three joints (legs and tail). A dissection of these masses exposed numerous nematodes in the musculature surrounding the joints that were identified as *Hamatospiculum flagellispiculosum* (Nematoda: Diplotriaenidae), a species that was not previously found in Piciformes (woodpeckers, toucans, and allies) of the Neotropical Region. In this report, we complement the original parasite description from 1952 with SEM images, and extend the species range about 2000 km southwards. Histopathological analysis (tissues sectioned 4–6 microns, stained with hematoxylin and eosin) of the affected tissues revealed parasitic myositis with muscle fibrosis. Severe muscle degeneration and necrosis, fibrous tissue replacing muscle tissue, chronic inflammation with widespread diffuse mononuclear infiltration, and parasitic content (adult roundworms, eggs, and eggs with first-stage larvae) were present in all samples. The multifocal nature of these lesions was consistent with the locomotory dysfunction exhibited by the bird. Both the immune response (mononuclear infiltration without eosinophils, which normally fight helminth colonization) and the clinical severity of this case (a lethal, multifocal macroparasite infection) are noteworthy. The expected immune response may have been suppressed through immunomodulation by the parasite, as observed for filarial parasites. Based on their demography and life history traits (i.e., long-lived picids that produce a single nestling every 1–2 years, and live in sparse populations), Magellanic Woodpeckers do not seem to be obvious hosts of an obligately killing parasite, and other (more regular) hosts should be expected to occur in the same region.

1. Introduction

Parasites are detrimental to host fitness, affecting morphology, behaviour, and other life history traits, even when sub-lethal (Jovani, 2003). Although birds are commonly parasitised by several taxa, data on parasite distribution, prevalence, and life cycles lack for most free-ranging wild birds (Friend and Franson, 1999; Atkinson et al., 2008; Miller and Fowler, 2014). In particular, parasitic diseases of woodpeckers (Picidae) and other forest-specialists remain little studied (Foster et al., 2002). In South America, where woodpecker diversity is highest, only a few endoparasites have been described for these birds, mostly in Brazil (Vicente et al., 1995; Pinto et al., 1996; Pinto and Noronha, 2003).

For a long time, parasitic nematodes have been associated with pathology and mortality in wild birds (mostly, waterfowl and grouse), and in poultry (Friend and Franson, 1999). For example, some helminths cause disease by physical disruption of the tissues as they migrate, provoking an intense, often eosinophilic, inflammatory response

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(Friend and Franson, 1999; Maizels and Yazdanbakhsh, 2003; Atkinson et al., 2008). In woodpeckers, nematodes have been found mostly in the body cavities, air sacs, digestive tube, and main organs, but rarely other body parts are examined for parasites (Pinto et al., 1996; Foster et al., 2002; Atkinson et al., 2008; Siegel et al., 2012; Miller and Fowler, 2014). Hence, nematodes that might be hosted in sites such as muscles, tendons, and joints of the leg and feet (or foot pant), as for example the filarioid (Onchocercidae) *Pelecitus* sp., have not been routinely reported (Pinto and Noronha, 2003).

In the present article we describe an infection caused by parasitic nematodes (*Hamatospiculum flagellispiculosum*) in skeletal muscles around the joints of the legs and tail of a free-ranging female Magellanic woodpecker (*Campephilus magellanicus*) (Aves, Picidae) from Argentine Patagonia. We also contribute novel elements (SEM images) that complement the original description of *H. flagellispiculosum*, published in 1952.

The Magellanic woodpecker is the largest South American picid (*ca.* 300 g). These birds are endemic to the temperate forests that extend along the Patagonian Andes shared between Chile and Argentina, where they are a conservation target. They live in small family groups with extended parental care (2–3 years), occupying stable territories year-round (Ojeda and Chazarreta, 2014). These woodpeckers are specialist predators of large, wood-boring insect larvae that constitute their primary food throughout their range. But they are also opportunistic foragers that will eventually consume vegetable matter, and vertebrates (e.g., lizards, bats, and avian eggs and nestlings) (Ojeda and Chazarreta, 2006; Chazarreta et al., 2012).

Species of *Hamatospiculum* Skrjabin, 1916 (Nematoda: Diplotriaenidae) were found throughout the world, parasitizing six orders of birds: Strigiformes, Falconiformes, Accipitriformes, Passeriformes, Coracliformes and Piciformes, mainly in subcutaneous connective tissues (Chabaud et al., 1964; Yorke and Maplestone, 1926). In the Neotropical Region, this genus was found in different Strigidae, Tyrannidae, Tytonidae and Picidae from Brazil, Paraguay and Argentina (Caballero, 1937, 1948; Chabaud et al., 1964; Rodrigues and Franco, 1964; Vicente et al., 1995), with three known species: *H. insigne* (Schneider 1866) Skrjabin, 1916 (*H. brasilianum*) (=*Filaria brasiliana* Stossich 1897), in Brazil, Paraguay and México (Morelos); *H. flagellispiculosum* Schuurmans Stekhoven, 1952, in Argentina; and *H. pauloi* (Rodrigues and Franco, 1964) (=*Tytofilaria pauloi* Rodrigues and Franco, 1964), in Brazil (Caballero, 1937, 1948; Schuurmans Stekhoven, 1952; Rodrigues and Franco, 1964; Vicente et al., 1995; Pinto et al., 1996).

2. Materials and methods

In February 2015, a weakened Magellanic woodpecker adult female was found in a natural forest at the outskirts of Bariloche city (41.179S, 71.415°W). The woodpecker was on the ground, unable to fly or climb; a few other woodpeckers were nearby (presumably, its family clan). A circumstantial witness rescued the bird and called wildlife authorities, who transferred it for qualified attention. Despite routine primary care (rehydrating with glucose solution, etc.), it died within a few hours.

A forensic necropsy performed by a specialised wildlife veterinarian detected parasitic development at specific host locations (joints of the legs and tail, see section 3), which were studied by means of both parasitological and histopathological analyses. In addition, avian parasitologists carried out a parasite-screening of the gastrointestinal tract, and the respiratory system of the necropsied bird.

2.1. Taxonomic identification

Twelve nematodes that were obtained around the joints of the legs and tail during the necropsy were fixed in 70% alcohol. Some specimens were fixed in 5% formalin and cleared in Aman's lactophenol for light microscope investigation. The remaining specimens were dehydrated through graded ethanol, dried in a critical point drier, coated with gold, and examined with a Philips XL 30 scanning electron microscope (Philips, Amsterdam, Netherlands) available at the Museo Argentino de Ciencias Naturales (MACN, Buenos Aires). The morphometric data were based on mature males and on females with larvigerous eggs. All measurements are expressed in micrometers (μm) unless otherwise stated, with the mean followed, in parentheses, by the range.

2.2. Histological processing

Tissues were dissected from the affected locations and fixed in 10% neutral buffered formalin. Tissues were then embedded in paraffin, sectioned (4–6 microns), and stained with hematoxylin and eosin for routine histologic examination. An Olympus BX51 equipment was used for microscopic examination (40–400 x).

3. Results and discussion

3.1. Bird examination and necropsy

At clinical examination, the bird was emaciated, showed low body condition, depressed sensorium and dehydration. No external injuries were visible, plumage was complete, and a subluxation of the proximal joint of the right humerus was noted at touch.

At the necropsy, pectoral muscles were decreased, with no subcutaneous fat. Left thoracic limb (radius and ulna) presented hematoma and edema. Left pelvic limb contained a *ca.* 3 × 1.5 cm mass of firm, orange tissue with fibrous appearance, under the peroneus longus fascia, near the tibiotarsus distal articulation. When dissected, the mass showed a central cavity with white coloured roundworms (Fig. 1 A). After these findings, all muscles and articulations were carefully examined. The uropygial gland area was increased in size; when this tissue was incised, a serous fluid flowed, and white roundworms

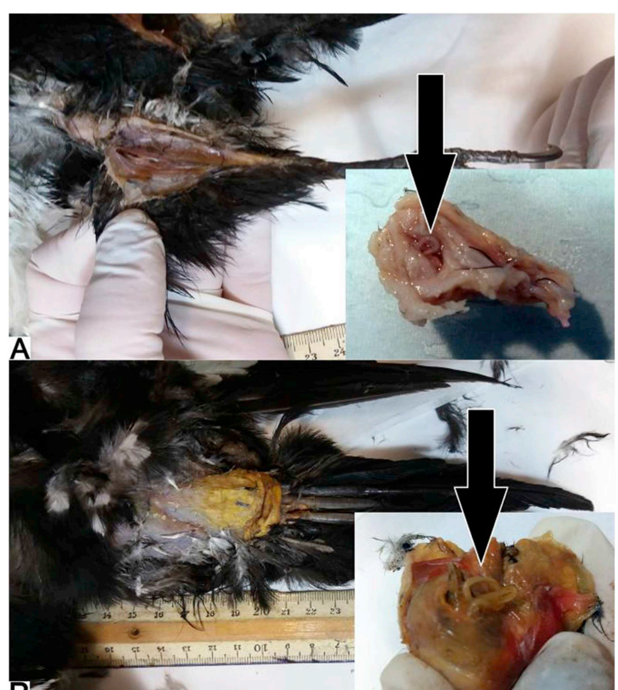

Fig. 1. Parasitic infections at joints of a necropsied Magellanic woodpecker (*Campephilus magellanicus*) adult female from Argentine Patagonia: (A) Dissected distocranial extremity of the left tibiotarsus. (B) Urogygial gland area increased in size. Arrows show roundworms present in the tissues extracted from the affected locations.

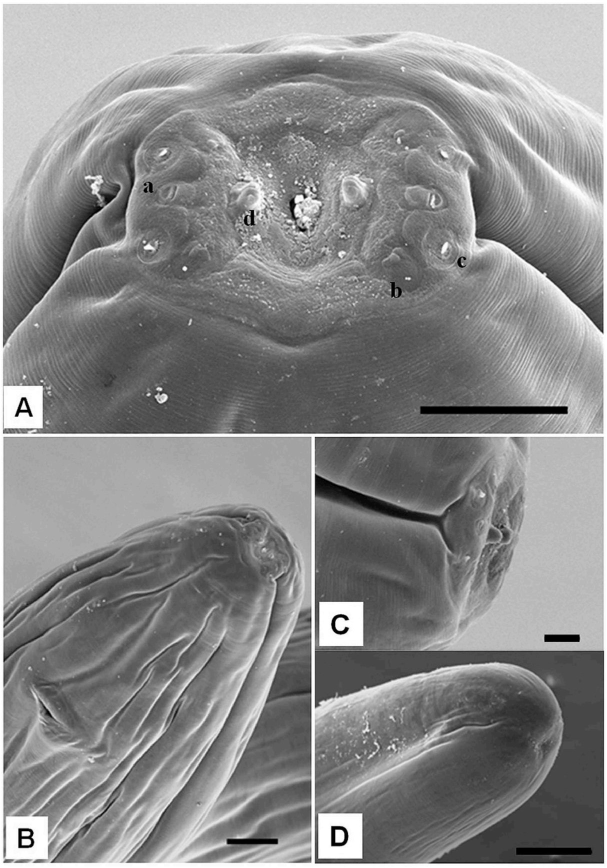

Fig. 2. Scanning electron micrograph (SEM) of female *Hamatospiculum flagellispiculosum*: (A) Detail of epaulette in anterior end (frontal view): a: amphid, b: cephalic papilla in inner circle, c: cephalic papilla in outer circle, d: tooth. (B) Anterior end with vulva (ventral view), Bar = 100 µm. (C) Anterior end (lateral view), Bar = 20 µm. (D) Detail of anal region (caudal view) with atrophied anus, Bar = 200 µm.

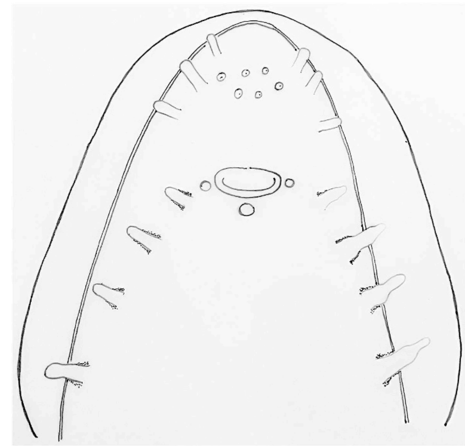

Fig. 3. Schematic outline of the tail of male *Hamatospiculum flagellispiculosum*.

appeared (Fig. 1 B). The tail muscles exhibited an orange colouration and firm consistency. Similar tissues and worms were found in the musculature surrounding the right knee. Digestive tract was empty. The multifocal nature of these lesions was consistent with the locomotory dysfunction exhibited by the bird.

3.2. Parasite determination

Screening of the gastrointestinal tract, and the respiratory system, detected no nematodes. Six males (3 complete, 3 posterior) and six females (2 complete, 3 broken, 1 anterior) of a single spiruroid nematode species were recovered from around the leg and tail joints of the necropsied bird.

Identification to subfamily level (Diplotriaenidae, Dicheilonematinae Wehr, 1935) was made using representative specimens on the basis of the following characters (Anderson et al., 2009, Fig. 2A–D): body cylindrical, large and rounded at both ends of female; cuticle with transverse striation; mouth dorsoventrally elongated; cephalic end armed with two lateral tooth-like elevations beside oral opening; lateral eupaulette-like formation present; cephalic papillae arranged in inner and outer circles of four each; conspicuous amphids, interspersed with the papillae of the outer circle; cervical papillae absent; lateral canals conspicuous; buccal capsule absent; oesophagus clearly divided into short, muscular, anterior portion, and long glandular portion; nerve ring and excretory pore anterior to glandular portion of oesophagus; vulva anterior, but posterior to muscular portion of esophagus; caudal alae of male present with elongated papillae; spicules markedly unequal and dissimilar, left spicule long, and right spicule short; gubernaculum absent; oviparous and didelphic.

Identification to genus level (*Hamatospiculum*) was made on the basis of the following characters of the diagnosis: mouth with 2 lips, cuticle slightly striated, divided esophagus, cephalic limb with 4 pairs of submedian papillae and a pair of lateral papillae. Male with caudal end provided with 2 lateral wings. The left spicule is almost 10 times the length of the right spicule. About 5 pairs of preanal papillae and 6 pairs of postanal papillae all pedunculated. Amphidelphic females, vulva in the esophageal region, with rounded posterior extremity and terminal or subterminal anus.

Identification to species level (*H. flagellispiculosum*) was made on the basis of the following characters of the diagnosis:

Male (n = 3). Fig. 3. Body length 31.3 (28–37) mm, maximum body width 564 (530–630). Distance of nerve ring 153 (140–154) and excretory pore 250 (250) from anterior end. Muscular esophagus length 305 (290–320); greatest width 68 (60–77). Glandular esophagus length 9.85 (9.7–10.0) mm; greatest width 365 (340–390) from anterior end. Caudal alae length 230 (170–270); width 40 (20–60) that extend beyond the terminal end of the tail. Tail 90 (90-90). Caudal end with precloacal and postcloacal papillae arranged as follows: 4 pairs of precloacal, larger, and pedunculate papillae supportin the caudal alae; unpaired papilla sessile on posterior clocal lip; 1 pair of paracloacal, sessile papillae; 3 pairs of postcloacal, pedunculate, lateral papillae, and 6 small, post cloacal, sessile papillae at terminal end. Large (left) spicule 2.7 (2.4–2.9) mm long; small (right) spicule 0.32 (0.31–0.32) mm long. Ratio of spicule length (1:8.5).

Female (n = 4). Fig. 2. Body length 79 (76–82) mm, maximum body width 958 (890–990). Distance of nerve ring 160 (144–168) and excretory pore 205 (156–245) from anterior end. Muscular esophagus length 344 (317–350); greatest width 74 (58–96). Glandular esophagus length 14.5 mm; greatest width 480 (471–489) from anterior end. Anus atrophied. Vulva 945 (672–1476) from anterior end with lower margin slightly elevated.

The genus *Hamatospiculum* was created by Skrjabin in 1916, having as a type species *Filaria insignis* (Schneider, 1866), called *Filaria brasiliana* by Stossich in 1897, with specimens from Brazil and Paraguay (Caballero, 1937). *Hamatospiculum flagellispiculosum* (Schuurmans Stekhoven, 1952) was described for the first time from *Asio clamator* (Strigidae, cited as *Rhinoptynx clamator maculatus*) and *Myiodynastes solitarius* (Tyrannidae), in Tucumán, northern Argentina. The two congeneric species (*H. pauloi* and *H. insigne*) differ from *H. flagellispiculosum* in the number of cephalic papillae and caudal papillae, the caudal alae shape, and ratio of spicule length, among other differences.

After comparison of the collected parasites with the original description of *H. flagellispiculosum*, morphology (especially eupaulette-like formation), and most of the morphometric characteristics, were

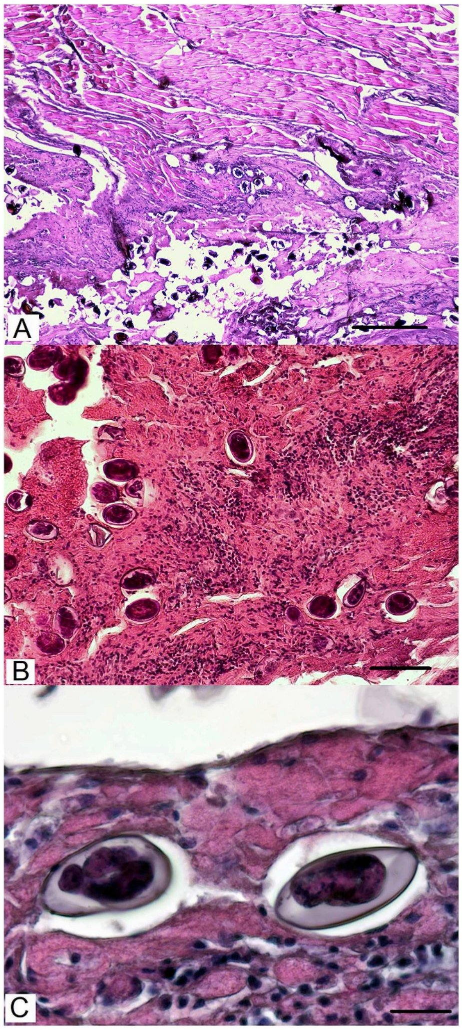

Fig. 4. Optical microscope micrograph of histopathological assessment of muscular tissues dissected from articulations affected by a parasitic infection in *Campephilus magellanicus*: (A) Sample from the right knee exhibiting loss of the skeletal muscle architecture and nematode eggs (dark dots at lower half), contiguous to muscle fibers with normal tissue architecture (upper half), Bar = 200 μm. (B) Sample from the left tibiotarsus mass showing myofibers of variable shape and size, diffuse mononuclear infiltration, and several eggs, Bar = 100 μm. (C) Two thin-shelled eggs with fully differentiated L1 at the centre of the image, surrounded by mononuclear cells, Bar = 20 μm.

coincident with that taxon. Never the less, we observed differences in ratio of spicule length (1:8.5 vs 1:7) and presence of sessile papillae, not observed in the original description.

3.3. Histopathological analyses

Histopathological analyses were carried out on tissues from the three affected locations: the mass extracted from the distocranial extremity of the left tibiotarsus (Fig. 1 A), the uropygial gland area (Fig. 1 B), and muscles around the right knee. Microscope examination of all these tissues denoted loss of the skeletal muscle architecture, along with parasitic content (Fig. 4A–C). Muscular degeneration and necrosis, replacing fibrous tissue, and chronic inflammation with widespread diffuse mononuclear infiltration (without eosinophils), were evident in all samples. Uropygial gland cells showed necrosis and lysis, leaving empty spaces in the glandular acini (i.e., reduced cellularity). Muscle fibers with normal tissue architecture, with no parasitic content, were occasionally observed contiguous to parasitised tissue (upper portion in Fig. 4 A). Under microscope examination, eggs and larvae clustered

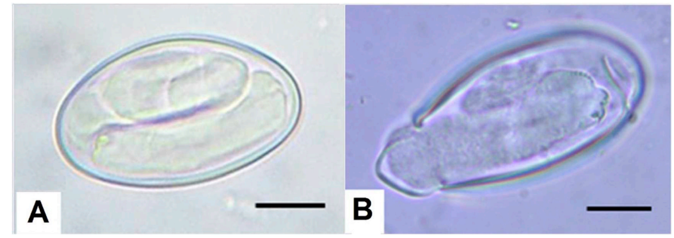

Fig. 5. *Hamatospiculum flagellispiculosum*, optical microscope micrograph of eggs: (A) Egg with first-stage larvae (L1), Bar = 15 μm. (B) Larvae hatching, Bar = 15 μm.

surrounded by damaged skeletal muscle, or inside parasitic tissue (presumably, remains of the parasite uterus). Eggs with first-stage larvae (L1) were ellipsoidal and thin-shelled, 56 (55–57) long and 31 (30–32) wide, and were two- or three-fold creased (Figs. 4C and 5). Larvae were 203 (170–215) long and 13.7 (12–14.4) wide (Fig. 5).

The target body organs of *H. flagellispiculosum* are barely known, as only the original description is available (Schuurmans Stekhoven, 1952), which describes the organs as "neck" (*Asio flammeus*) and "anus" (*Myiodynastes solitarius*), with no information on the affected tissues. Other members of the same genus recovered from birds were hosted in subcutaneous connective tissues, and secondarily, in skeletal muscles and general body cavity. *Hamatospiculum insigne* (Schneider, 1866) Skrjabin, 1916, the type of the genus, has been reported in subepithelial connective tissue of the neck of *Speotyto cunicularia hypogaea* (Strigiformes) from Morelos, México (Caballero, 1937), in muscles of the nape of *Colaptes campestroides* (Piciformes) from Misiones, Argentina; in *Piculus* sp. (Piciformes) from Brazil (Vicente et al., 1995) and in *Picus* sp. (Piciformes) from Brazil and Paraguay (Pinto et al., 1996). *Hamatospiculum pauloi* has been reported in general cavity in *Tyto alba tuidara* (Strigiformes) from Brazil (Rodrigues and Franco, 1964, based only on two males). Most frequently, adult parasitic nematodes found in birds' skeletal muscle and tendons of the legs and feet are filarioid (Onchocercidae), as for example *Pelecitus* spp. (Greve et al., 1982; Bartlett and Greener, 1986; Maizels and Yazdanbakhsh, 2003; Maxie, 2007; Atkinson et al., 2008), which have been found in several American woodpeckers (Vicente et al., 1995; Pinto et al., 1996; Pinto and Noronha, 2003; Siegel et al., 2012).

3.4. Clinical disease

The female Magellanic Woodpecker was found moribund, with severe locomotory dysfunction, anorexia and dehydration. In the necropsied bird, numerous adults, eggs with undeveloped larva in uterus, and well developed L1, were housed in muscles surrounding articulations of the legs and tail that are key for woodpecker locomotion. Such high virulence levels may explain the clinical severity of this case: since worms greatly harm target tissues, virulence (i.e., macroparasite-induced host harm) is proportional to the levels of parasite growth and reproduction (Atkinson et al., 2008; Viney and Cable, 2011). The derived locomotory dysfunction impeded vital activities like foraging, so this multifocal macropasasite infection can be considered lethal. Concomitant and predisposing pathologies probably lead to host immunosuppression, favouring parasite growth and increased reproduction (Viney and Cable, 2011).

The immune response here observed is noteworthy. On the one hand, eosinophils, which normally fight helminth colonization, were absent (Maizels and Yazdanbakhsh, 2003; Maxie, 2007; Atkinson et al., 2008). On the other hand, mononuclear cells led the immune response, which is more typical of viral and bacterial infections. The expected immune response may have been suppressed through immunomodulation by the parasite, as observed for filarial parasites (Maizels and Yazdanbakhsh, 2003). In several macroparasites, host anti-parasite immune response is used as a major cue for sexual reproduction, so maturation time is adjusted depending on the likelihood that a specific (anti-worm) response may kill them (Viney and Cable, 2011). This way, life-histories of macroparasites respond dynamically to conditions within the host, and parasites that normally show low pathogenicity (i.e., at sub lethal, or even subclinical levels) can cause severe clinical disease (Friend and Franson, 1999; Jovani, 2003; Maxie, 2007; Atkinson et al., 2008; Miller and Fowler, 2014).

4. Conclusions

In the present work, the original description of *Hamatospiculum flagellispiculosum* (1952) is complemented with SEM images, and a new location of the parasite (skeletal muscle around joints), and a new locality (in north Patagonia, about 2000 km south of previously known distribution), are provided. This nematode is cited for the first time in Neotropical Piciforms (woodpeckers, toucans and allies), causing parasitic myositis with muscle fibrosis in a female Magellanic woodpecker from north Patagonia.

Health of South American woodpeckers is a highly unexplored field. In fact, this work provides the first description of any disease findings in the Magellanic Woodpecker These are long-lived picids that produce few offspring (a single nestling every 1–2 years), and thus live in sparse populations (Ojeda and Chazarreta, 2014). Their demography and life history traits make them bad candidate hosts for co-evolving with an obligately killing parasite; in view of this, other (more regular) hosts should be expected to be available for this parasite in the same locality or region, as this woodpecker species does not seem to be an obvious host. Alternatively, skeletal muscle may not be the woodpecker tissue where these parasites normally develop, while other tissues and locations that are not so key for vital activities, may be regular targets of this parasite.

The fatality here reported drew our attention towards parasites that would not be otherwise (i.e., at sub lethal levels) detected. Assessing the potential effects of parasites in wild birds will be challenging and demanding, but doing so might change the way in which researchers think about bird ecology. Wildlife health and disease ecology should be addressed as part of life history studies, especially because ecosystems are changing, which affects the physiology and behaviour of wild animals (Viney and Cable, 2011).

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