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

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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...
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 larval-stage nematodes. 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) Uropygial gland area increased in size. Arrows show roundworms present in the tissues extracted from the affected locations.
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 spirurid nematode species were recovered from around the leg and tail joints of the necropsied bird.

Identification to subfamily level (Diplogasteridae, Dicellonematinae 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 epaulettes-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 oesophagus; 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. 2. 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 support the caudal alae; unpaired papilla sessile on posterior cloacal 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 atrrophied. 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 brasiliiana* by Stossich in 1897, with specimens from Brazil and Paraguay (Caballero, 1937). *Hamatospiculum flagellispiculosum* (Schuurmans Stekoven, 1952) was described for the first time from *Asio clamator* (Strigidae, cited as *Rhinopteryx clamator maculatus*) and *Myiodynastes solitarius* (Tyrannidae), in Tucuman, northern Argentina. The two congeners 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 epaulette-like formation), and most of the morphometric characteristics, were
samples. Uropygial gland cells showed necrosis and lysis, leaving empty fuse mononuclear in placing fi

Fig. 4 A). Under microscope examination, eggs and larvae clustered occasionally observed contiguous to parasitised tissue (upper portion in with normal tissue architecture, with no parasitic content, were occa-

sions of the left tibiotarsus (Fig. 1A), the uropygial gland area (Fig. 1 –

C). Muscular degeneration and necrosis, re-

3.3. Histopathological analyses

Histopathological analyses were carried out on tissues from the three affected locations: the mass extracted from the distocranial extre-

mity 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, re-

placing fibrous tissue, and chronic inflammation with widespread dif-

fuse 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 occasion-

ally observed contiguous to parasitised tissue (upper portion in Fig. 4 A). Under microscope examination, eggs and larvae clustered

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 (Schaumans 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 insigniae* (Schneider, 1866) Skrjabin, 1916, the type of the genus, has been reported in subepithelial connective tissue of the neck of *Spoeotyto cucullata* hypogaea (Strigi-

forms) from Morelos, Mexico (Caballero, 1937), in muscles of the nape of *Colaptes campestrpes* (Piciformes) from Misiones, Argentina; in *Pi-

culus* sp. (Piciformes) from Brazil (Vicente et al., 1995) and in *Picus* sp. (Piciformes) from Brazil and Paraguay (Pinto et al., 1996). *Hamatospi-

culum pauloi* has been reported in general cavity in *Tyto alba tudara* (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 (Oncho-

cercidae), as for example *Pelecitus* spp. (Greve et al., 1982; Bartlett and Greener, 1986; Maiels 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 Magellanian Woodpecker was found moribund, with se-

vere locomotory dysfunction, anorexia and dehydration. In the nec-

cropsed bird, numerous adults, eggs with undeveloped larva in uterus, and well developed L1, were housed in muscles surrounding articula-

tions 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-in-

duced host harm) is proportional to the levels of parasite growth and reproduction (Atkinson et al., 2008; Viney and Cable, 2011). The de-

rived locomotory dysfunction impeded vital activities like foraging, so this multifocal macroparasite infection can be considered lethal. Con-

comitant and predisposing pathologies probably lead to host immu-

nosuppression, favouring parasite growth and increased reproduc-

tion (Viney and Cable, 2011).

The immune response here observed is noteworthy. On the one hand, eosinophils, which normally fight helminth colonization, were absent (Maiels 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 im-

munomodulation by the parasite, as observed for filarial parasites (Maiels 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 like-
lihood that a specific (anti-worm) response may kill them (Viney and
Cable, 2011). This way, life-histories of macroparasites respond dyna-
mically 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
flagellispicosum* (1952) is complemented with SEM images, and a new
location of the parasite (skeletal muscle around joints), and a new lo-
cality (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 wood-
pecker 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 loca-
tions 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 de-
manding, but doing so might change the way in which researchers think
about bird ecology. Wildlife health and disease ecology should be ad-
dressed as part of life history studies, especially because ecosystems are
changing, which affects the physiology and behaviour of wild animals
(Viney and Cable, 2011).

Acknowledgements

We thank Lic. S. Seijas and J. Nielsen from Nahuel Huapi National
Park Conservation Department. We appreciate the assistance of Fabián
Tricarico from the Museo Argentino de Ciencias Naturales “Bernardino
Rivadavia” (MACN, Buenos Aires) at operating the SEM. Bird primary
care and necropsy were conducted by MWG under competent author-
ization (APN). The animal remains are kept under Disposición 003/2017
of the SAyDS, Río Negro Province, granted to VO. This research
did not receive any specific grant from funding agencies in the public,
commercial, or not-for-profit sectors.

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