Morphologic Observations and Novel 18S rRNA Sequences of Abbreviata hastaspicula and Abbreviata antarctica from Varanus spp. Lizards in Australia

Authors: King, Chloe, Tay, Chin Y., and Jones, Hugh I.

Source: Journal of Wildlife Diseases, 55(1) : 149-152

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/2017-11-272

DOI: 10.7589/2017-11-272

Journal of Wildlife Diseases, 55(1), 2019, pp. 149–152

Morphologic Observations and Novel 18S rRNA Sequences of *Abbreviata hastaspicula* and *Abbreviata antarctica* from *Varanus* spp. Lizards in Australia

Chloe King,[1,2] Chin Y. Tay,[1] and Hugh I. Jones[1] [1]The Marshall Centre for Infectious Diseases Research and Training, The University of Western Australia, M504, 35 Stirling Hwy., Crawley, Western Australia 6009, Australia; [2]Corresponding author (email: chloe.king@research.uwa.edu.au)

ABSTRACT: The nematodes *Abbreviata antarctica* von Linstow, 1899, and *Abbreviata hastaspicula* Jones, 1979, are predominant spirurid nematodes in species of *Varanus* lizards in Australia. However, genetic knowledge of these two species of nematode is lacking. In this study, nematodes removed from *Varanus gouldii* were examined using integrated morphologic and molecular methods. We extracted DNA from *A. hastaspicula* and *A. antarctica* for PCR and sequencing. Specific 18S small subunit ribosomal RNA (rRNA) primers were designed on the basis of existing 18S rRNA sequences of Physalopterinae strains. Species of *Abbreviata*, which are closely similar morphologically, may be misidentified, especially the larvae of different species of *Abbreviata* that cannot be differentiated. The findings of this study will improve the accuracy in identification of *A. hastaspicula* and *A. antarctica*, both morphologically and molecularly.

Key words: *Abbreviata* spp., morphology, Physalopterinae, Spirurina nematodes, SSU rRNA, small subunit, 18S rRNA.

Abbreviata antarctica and *Abbreviata hastaspicula* (Spirurida: Physalopteridae) are predominant gastrointestinal nematodes parasitizing *Varanus* lizards of Australia (Jones 2014). They are spirurid nematodes and exhibit a heteroxenous life cycle (Anderson 2006). Their life cycles can only be completed by the final hosts feeding on arthropod intermediate hosts containing infective third stage larvae (King et al. 2013; King and Jones 2016). Physalopterine nematodes infecting humans had been previously reported in different countries (Ortlepp 1926; Morgan 1945; Singh and Rao 1954; Vandepitte et al. 1964). In Australia, an 11-mo-old Caucasian male baby was reported suffering from gangrene of the distal portion of the small bowel as a result of ingesting larval *Physaloptera* sp. (Nicolaides et al. 1977). Unfortunately, because of the lack of diagnostic tools, the species of nematode involved could not be identified.

Viable nematodes of *A. antarctica* and *A. hastaspicula* were collected from six freshly killed and dissected *Varanus gouldii* captured (Western Australian Department of Environment and Conservation License SF009524) in the wild in an arid area near Sandstone, Western Australia (–27°S, 119°E). We euthanatized the *V. gouldii* by the injection of sodium pentobarbital (≥100 mg/kg) before they were dissected (University of Western Australia Animal Ethics Reference RA/3/100/1248).

The nematodes were firmly attached to the stomach wall of the *V. gouldii*. Live nematodes for photographic imaging were removed from the stomach by forceps, cleared in chlorolactophenol, placed on a clean microscope slide, and examined under a compound microscope at 100×, 200×, and 400× magnifications. Specimens for further molecular analysis were cleared in glycerol instead of chlorolactophenol to avoid damage to DNA. The taxonomic criteria distinguishing the nematodes *A. hastaspicula* and *A. antarctica* are the male copulatory spicules, egg shell thickness, form of the vulva in females, and variations in cephalic morphology.

After the nematodes were morphologically identified, single adult nematodes were cleaned with distilled water and preserved in 70% ethanol for DNA extraction. Within 48 h after collection, each nematode was first submerged in 300 µL of distilled water, in a 2-mL centrifuge tube containing a 0.5-cm stainless steel ball, and homogenized with a TissueLyser II (Cat#85300, Qiagen, Doncaster, Victoria, Australia). Homogenized sam-

ples were then processed according to the manufacturer's protocol (Qiagen DNeasy® Animal Tissues Mouse Tail, Spin-Column Protocol) for DNA extraction.

Physalopterinae-specific 18S small subunit ribosomal RNA (SSU rRNA) primers were designed on the basis of the alignment of 18S rRNA from six Physalopterinae (GenBank): *Physaloptera alata* (AY702703), *Physaloptera apivori* (EU004817), *Physaloptera turgida* (DQ503459), *Physaloptera* sp. JSL-2010 (HM067978), *Turgida torresi* (EF180069), and *Physaloptera* sp. SAN-2007 (EF180065). The forward 5′-GCGCGCAAATTAACCCA ATCTC-3′ and reverse 5′-CGGGCGTCTCG CTACGG-3′ primers were synthesized commercially (Sigma-Aldrich, St. Louis, Missouri, USA). Each PCR reaction contained 2 µL (30 ng/µL) of DNA template, 5 µL (10 pmol/µL) of each primer, 25 µL of GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA), and nuclease-free water to a total volume of 50 µL. The PCR thermal cycle was as follows: initial denaturation at 94 C for 1 min, 35 cycles of denaturation at 94 C for 18 s, annealing at 45 C for 30 s, extension at 72 C for 1 min, and final extension at 72 C for 10 min. After analyzing on 2% agarose gels, the successfully amplified PCR products were purified using PCR clean up kit (Qiagen). Purified PCR products were then sequenced using Sanger sequencing method by Australian Genomic Research Facility, Perth, Western Australia, Australia. All the molecular procedures were duplicated to confirm the reliability and consistency of the findings.

Abbreviata hastaspicula was the only species of nematode that occurred in five out of the six dissected *V. gouldii*; the sixth *V. gouldii* had concurrent infection of *A. antarctica* and *A. hastaspicula*. Microscopic morphologic examination confirmed that the nematodes removed from the stomach of the varanid lizards were adult stage *A. antarctica* and *A. hastaspicula*. The two species of nematodes can be morphologically distinguished as follows: 1) mouth corner denticles are present in the mouthparts in *A. antarctica* but inconstant in *A. hastaspicula* (Fig. 1A). 2) The spicule of male *A. antarctica* is thicker and shorter than that of *A. hastaspicula*, with no spearhead-like tip at the anterior end of the right speculum (Fig. 1B). 3) For *A. antarctica*, the vulva is situated in a slight depression between one-fifth and one-fourth from the anterior end, behind the esophago-intestinal junction. Coils of uterus extend anteriorly to the vulva (Fig. 1C) in the female uterus under the microscope, and the eggs of *A. antarctica* are darker than those of *A. hastaspicula*. 4) A thin-walled narrow tube of *A. hastaspicula* projects out from the body wall in the vulva of *A. hastaspicula*, pointing posteriorly or at right angles to it, or sometimes slightly anteriorly (Fig. 1D). 5) *Abbreviata antarctica* eggs are oval in shape, 60–65 by 30 µm, with smooth and thick shells. The eggs of *A. hastaspicula* are slightly less elongated, 53–40 by 32 µm, are smaller than those of *A. antarctica*, and have conspicuously thinner shells (King et al. 2017).

Identification of the two species of nematode was confirmed morphologically and provided guidelines for the molecular analyses. Given a lack of existing genomic data on the nematodes *A. antarctica* and *A. hastaspicula*, accurate identification of the species by morphologic features is essential and fundamental for an accurate phylogenetic analysis. Both *A. antarctica* and *A. hastaspicula* conform to the basic pattern of physalopterine nematodes. Cephalic dentition is a valuable character in the identification of these nematodes. They possess a large single external apical tooth and a small bifid internal apical tooth, doubled submedian teeth at the dorsal and ventral lip margin. Mouth corner denticles are present in *A. antarctica* but are inconstant in *A. hastaspicula*. The amphids are situated at the base of the lateral pseudolabia (Anderson 2006; Anderson et al. 2009). In males, the caudal bursa is ornamented, and the caudal alae are on the ventral surface of the body.

Different host species may contribute to the different genetic composition of the nematodes: *A. antarctica* and *A. hastaspicula* are nematodes of reptiles (King et al 2013; King and Jones 2016), *P. alata* and *P. apivori* are found in birds (Ali 1961; Anderson 2006), and

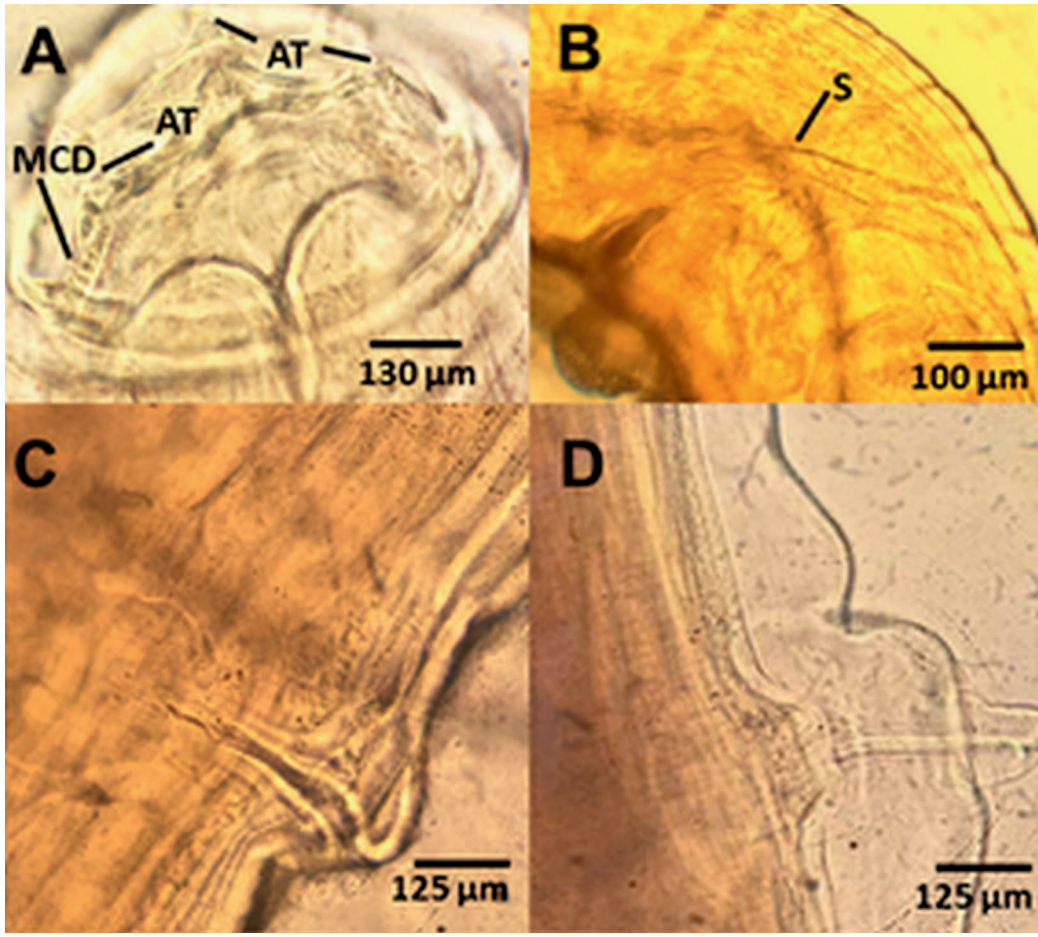

FIGURE 1. The distinguishing morphologic features between *Abbreviata antarctica* and *Abbreviata hastaspicula* are as follows: (A) Anterior end from a lateral view of cephalic features of *A. antarctica*; the mouthparts, mouth corner denticles are present. AT=apical tooth; MCT=mouth-corner denticles. (B) The spicule of male *A. antarctica* is thicker and shorter, with no spearhead-like tip at the anterior end of the right spicule (=S). (C) Lateral view of female *A. antarctica*, vulva; it is behind the esophago-intestinal junction. Coils of uterus extend anterior to the vulva. (D) Lateral view of female *A. hastaspicula*, vulva; a thin-walled narrow tube is projecting out anteriorly from the body wall.

P. turgida and *T. torresi* are nematodes of marsupials and rodents, respectively (Smythe et al. 2006; Nadler et al. 2007). *Physaloptera turgida* Travassos, 1920, was considered synonymous with *T. torresi* Schulz, 1927 (Morgan 1945); yet, molecular studies have shown that they are different species (Smythe et al. 2006; Nadler et al. 2007). *Physaloptera thalacomys* is hosted by rabbit-eared bandicoots (Beveridge and Spratt 1996) and *Physaloptera* sp. SAN-2007 is hosted by striped skunks (Nadler et al. 2007). The resulting sequences were submitted to GenBank under accession number KX255660 for *A. antarctica* and KX255661 for *A. hastaspicula*.

We provided a useful piece of information for the identification and understanding of physalopterans in Australia. The 18S SSU rRNA was chosen for the molecular analysis in this study because it is the only gene available in the database, and the gene is highly conserved and a suitable choice for differentiating species that are not closely related. Although infection with Physalopterinae is considered rare in humans, who are usually reported as accidental hosts to physalopterid

nematodes, they may be underdiagnosed and underreported (particularly if they are immature larvae stage) because of the poor understanding of physalopterans and the lack of identification tools. The combined morphologic-molecular detection tools developed in this study can certainly provide a sound basis for further investigation of other *Abbreviata* spp. in Australian reptiles and aid in diagnosing infections in humans.

We are very grateful to the School of Animal Biology, University of Western Australia, for providing housing for the lizards; Rick Roberts and Husnan Ziadi for catching the lizards in the wild; the Marshall Centre for Infectious Diseases Research and Training for providing facility and materials for the molecular experiments; and Mao Zhu for assisting in the collection of nematodes from the lizards. The Vice-Chancellor of the University of Western Australia partially funded this project.

LITERATURE CITED

Ali SM. 1961. On some new species of *Physaloptera* from Indian birds. *J Helminthol* 35:49–60.

Anderson R, Chabaud A, Willmott S, editors. 2009. *Keys to the nematode parasites of vertebrates: Archival volume*. CABI Publishing, Wallingford, UK, 463 pp.

Anderson RC. 2006 *Nematodes parasites of vertebrates. Their development and transmission*. 2nd Ed. CABI Publishing, Wallingford, UK, 650 pp.

Beveridge I, Spratt DM. 1996. The helminth fauna of Australasian marsupials: Origin and evolutionary biology. *Adv Parasitol* 37:162–163.

Jones HI. 1979. Gastrointestinal nematodes, inclcuding three new species, from Australian and Papua New Guinean pythons. *Proc Helminthol Soc Wash* 46:1–14.

Jones HI. 2014. Physalopterine nematodes in Australian lizards: Interactions and patterns of infection. *Aust J Zool* 62:180–194.

King C, Jones HI. 2016. The life cycle of the reptile-inhabiting nematode *Abbreviata hastaspicula* (Spirurida: Physalopteridae: Physalopterinae) in Australia. *Int J Parasitol Parasites Wildl* 5:258–262.

King C, Jones HI, Tay CY. 2013. Arthropod intermediate hosts of *Abbreviata antarctica* (Nematoda: Physalopteridae) in Australia. *J Parasitol* 99:708–711.

King C, Jones HI, Tay CY. 2017. If termite *Coptotermes acinociformis* is not an intermediate host, could it be a safe food source for humans? *J Insects Food Feed* 3: 139–143.

Morgan BB. 1945. The nematode genus *Abbreviata* (Travassos, 1920) Schulz, 1927. *Am Midl Nat* 34: 485–490.

Nadler SA, Carreno RA, Mejía-Madrid H, Ullberg J, Pagan C, Houston R, Hugot JP. 2007. Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. *Parasitology* 134:1421–1442.

Nicolaides NJ, Musgrave J, McGuckin D, Moorhouse DE. 1977. Nematode larvae (Spirurida: Physalopteridae) causing infarction of the bowel in an infant. *Pathology* 9:129–135.

Ortlepp RJ. 1926. On the identity of *Physaloptera caucasica* v. Linstow, 1902, and *Physaloptera mordens* Leiper, 1908. *J Helminthol* 4:199–202.

Singh S, Rao V. 1954. On a species of *Physaloptera* causing a subcutaneous abscess in the neck of an Indian. *J Helminthol* 28:155–158.

Smythe AB, Sanderson MJ, Nadler SA. 2006. Nematode small subunit phylogeny correlates with alignment parameters. *Syst Biol* 55:972–992.

Vandepitte J, Michaux J, Fain A, Gatti F. 1964. Premières observations Congolaises de physaloptérose humaine. *Ann Soc Belges Med Trop Parasitol Mycol* 44:1067–1076. French.

Submitted for publication 5 November 2017.
Accepted 6 April 2018.