

## ***Mesocestoides* sp. in Wild Northern Bobwhite (*Colinus virginianus*) and Scaled Quail (*Callipepla squamata*)**

**Bradley W. Kubečka,<sup>1,2,4</sup> Nicole J. Traub,<sup>1</sup> Vasyly V. Tkach,<sup>3</sup> Taylor R. Shirley,<sup>1</sup> Dale Rollins,<sup>2</sup> and Alan Fedynich<sup>1</sup>**  
<sup>1</sup>Caesar Kleberg Wildlife Research Institute, Texas A&M University–Kingsville, 700 University Boulevard, MSC 218, Kingsville, Texas 78363, USA; <sup>2</sup>Rolling Plains Quail Research Foundation, PO Box 220, Roby, Texas 79543, USA; <sup>3</sup>Department of Biology, University of North Dakota Grand Forks, PO Box 9019, Grand Forks, North Dakota 58202, USA; <sup>4</sup>Corresponding author (email: bkubecka@talltimbers.org)

**ABSTRACT:** *Mesocestoides* spp. have a cosmopolitan distribution with zoonotic potential. *Mesocestoides* tetrathyridia were found under the pericardial sac, on the surface of the crop, and in the peritoneal cavity of a hunter-harvested Northern Bobwhite (*Colinus virginianus*) and a Scaled Quail (*Callipepla squamata*) collected during the 2016–17 quail hunting season in northwest and southern Texas, US respectively. Molecular analysis indicated that the tetrathyridia from the birds likely belonged to an undescribed species and are identical to pretetrathyridium stages recently found in *Scincella lateralis* skinks in Oklahoma. This is the first report of *Mesocestoides* from North American birds.

**Key words:** *Callipepla squamata*, *Colinus virginianus*, *Mesocestoides*, molecular analysis, Northern Bobwhite, Scaled Quail, tetrathyridia, Texas.

Tetrathyridia are the second larval stage (or metacystode) of cestodes belonging to the genus *Mesocestoides* (Mesocestoididae), which are characterized by a three-host life cycle (Khalil et al. 1994; Skirnisson et al. 2016). The first intermediate host is unknown but presumed to be a coprophagous arthropod (Foronda et al. 2007). Tetrathyridia have been found in >200 vertebrate hosts, including birds, mammals, reptiles, and amphibians on every continent except Australia (Chertkova and Kosupko 1978; Padgett et al. 2013). Of the seven avian orders known to host *Mesocestoides*, Galliformes and Passeriformes are the most frequently documented (Chertkova and Kosupko 1978; Literák et al. 2004). Known definitive hosts include, but are not limited to, arctic fox (*Vulpes lagopus*), domestic dogs (*Canis familiaris*), and domestic cats (*Felis silvestris catus*; Skirnisson et al. 1993, 2016).

The Northern Bobwhite (*Colinus virginianus*) is a popular game bird of the family

Odontophoridae known for its precipitous population decline over the past century. Their geographic range extends from northern Mexico to Wisconsin, and Colorado to the Carolinas (Guthery 2000). Scaled Quail (*Callipepla squamata*) occur sympatrically with bobwhites in Texas, and are the most closely related taxon to the genus *Colinus* (Hosner et al. 2015).

A Bobwhite and Scaled Quail harvested during the 2016–17 hunting season in northwestern Texas (32°15'35"N, 100°43'23"W) and southern Texas (26°52'53"N, 99°04'20"W), respectively, were infected with tetrathyridia of the genus *Mesocestoides*. The condition of the quails was within normal limits on gross examination (i.e., no lesions or morphological aberrations). Tetrathyridia were present on the surface of the crop, under the pericardial sac (Fig. 1), and attached to the serous membrane of (and free-floating throughout) the coelomic cavity. Identification to genus was performed by J. M. Kinsella (HelmWest Laboratory, Missoula, Montana, USA) using morphological characteristics. Metacystodes had all morphological characteristics typical of *Mesocestoides* tetrathyridia with apical end and suckers invaginated (Fig. 2).

Morphological features of tetrathyridia do not allow for their reliable identification; therefore, molecular techniques are recommended to be utilized for identification (Padgett et al. 2005). Moreover, this is also true for adult stages and there is disagreement on the number of valid species in this genus (Webster 1949; Zalesný and Hildebrand 2012). Therefore, we obtained partial sequences of mitochondrial *12S*, *cox1*, and *nad1* genes from the tetrathyridia obtained from the bobwhite following the procedures

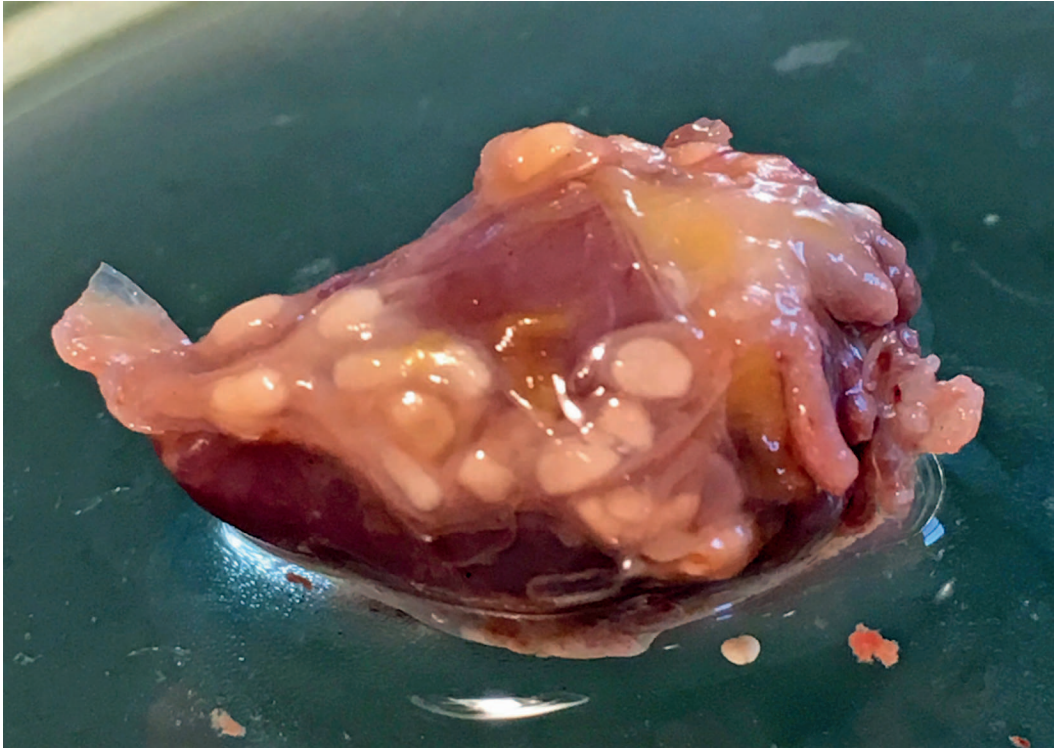


FIGURE 1. Northern Bobwhite (*Colinus virginianus*) heart with tetrathyridia of *Mesocestoides* sp. under the pericardial sac collected during February 2017 in Mitchell County, Texas, USA.

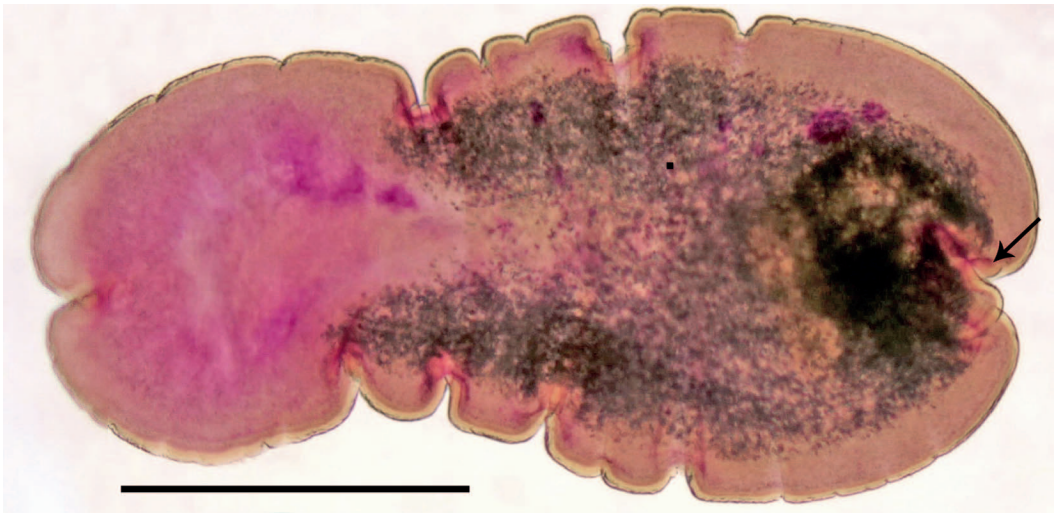


FIGURE 2. Total mount of tetrathyridium of *Mesocestoides* sp. from a Northern Bobwhite (*Colinus virginianus*) collected during February 2017 in Mitchell County, Texas, USA. Note the invaginated anterior (indicated by arrow) and posterior end, and numerous calcareous corpuscles in parenchyma of the metacystode that appear as dark inclusions in the stained specimen. Bar=1 mm.

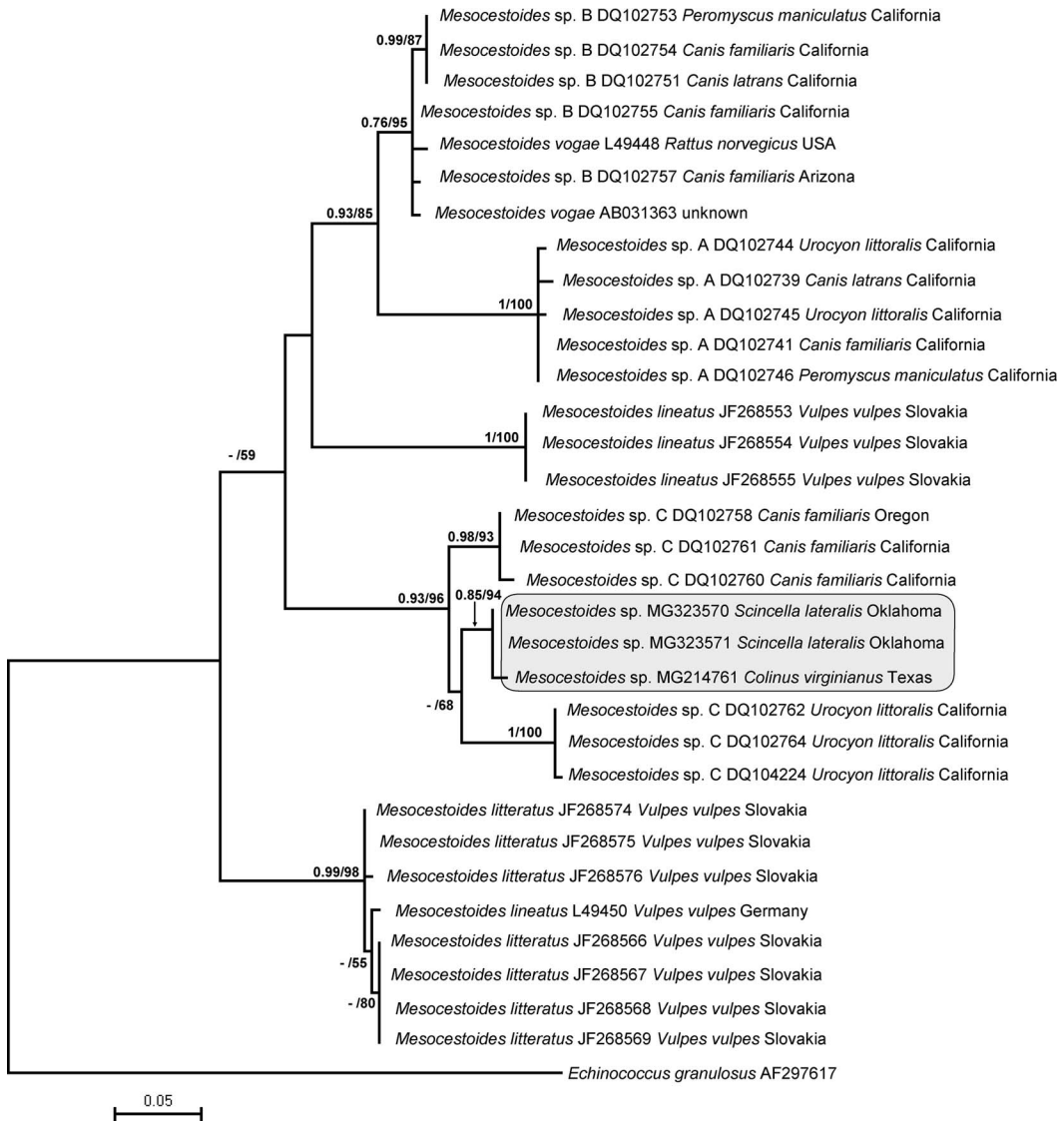


FIGURE 3. Phylogenetic tree resulting from Bayesian analysis and maximum likelihood analysis of 12S alignment of *Mesocestoides* showing the position of the specimens found in Northern Bobwhite (*Colinus virginianus*) collected during February 2017 in Mitchell County, Texas, USA. Branch support values are shown at internodes as Bayesian analysis posterior probabilities/maximum likelihood bootstrap percentages. Only posterior probabilities greater than 0.75 and bootstraps greater than 50% are shown. The scale bar indicates the number of substitutions per site.

described by McAllister et al. (2018). Sequences were submitted to the GenBank under accession numbers MG214761–MG214763. Phylogenetic analysis of 12S data was carried out using Bayesian inference as implemented in the MrBayes program version 3.1 (Ronquist and Huelsenbeck 2003) and maximum likelihood algorithm as implement-

ed in MEGA 7 (Kumar et al. 2016). All analyses were performed as described by McAllister et al. (2018); the only difference was the addition of the new sequence of *Mesocestoides* from the bobwhite.

Pairwise sequence comparisons as well as the results of molecular phylogenetic analysis (Fig. 3) have confirmed identification of our

metacestodes from quail as *Mesocestoides* sp. Interestingly, these larvae were conspecific with the pretetrathyridium stages recently reported from the ground skink *Scincella lateralis* in Oklahoma (McAllister et al. 2018). The two forms were nearly completely identical genetically with only 0.7% (only two nucleotides) divergence in the *12S* gene, 0.8% divergence in *cox1* gene, and 1.6% divergence in the highly variable *nad1* gene. According to McAllister et al. (2018), these metacestodes do not have a match among currently sequenced adult or metacestode stages of *Mesocestoides* and might represent a yet undescribed species that is closely related (Fig. 3) to two lineages previously reported as *Mesocestoides* sp. C by Padgett et al. (2005). The important difference between our specimens and those described by McAllister et al. (2018) is that the specimens from the quail were fully-formed tetrathyridia with invaginated apical ends (Fig. 2), whereas the specimens from the skinks were pretetrathyridia with evaginated scolex and neck region.

Adult *Mesocestoides vogae* (reported as *Mesocestoides corti*) and *Mesocestoides lineatus* have been documented in canids, felids, and mustelids from Texas (Custer and Pence 1981; Pence and Windberg 1984; Pence et al. 2003). In South Texas, coyotes (*Canis latrans*) and ocelots (*Leopardus pardalis*) had 48% and 7% prevalence of *M. lineatus*, respectively (Pence and Windberg 1984; Pence et al. 2003), whereas bobcats (*Lynx rufus*) and American badgers had 36% and 23% prevalence of *M. vogae* (reported as *M. corti*) in northern Texas, respectively (Stone and Pence 1978; Pence and Dowler 1979; Etges 1991).

Our study appears to be the first record of tetrathyridia in wild North American birds. During the 2016–17 hunting season we noted two other instances of tetrathyridia in bobwhites from North Texas, but disregarded their occurrence as proglottids of more robust cestodes. Recent helminth surveys of quails from Texas have been extensive, and comprehensive helminth surveys have been conducted across their geographic range (Stoddard 1931; Kellogg and Calpin 1971; Moore et al. 1986; Davidson et al. 1991). However, none of

these studies have documented *Mesocestoides* spp. in quails. It is likely that the occurrence of *Mesocestoides* in quail, or any New World birds, is rare and incidental.

The pathogenicity of *Mesocestoides* is low (Atkinson et al. 2009); however, human infections have been documented to occur from eating raw or undercooked meat containing tetrathyridia (Eom et al. 1992; Fuentes et al. 2003; Centers for Disease Control and Prevention 2016). Hunters should assure their harvest is washed and cooked thoroughly before consumption. Additionally, we suggest that internal organs be disposed in a place not accessible to pets or other wildlife.

This study was funded by the Rolling Plains Quail Research Foundation, Caesar Kleberg Wildlife Research Institute, South Texas and Park Cities Chapters of Quail Coalition, and the Reñe Barrientos Fund for Graduate Student Tuition. We thank Mike Kinsella for morphological identification of specimens and appreciate the studious nature of the hunters who submitted their harvested samples. Necropsies were conducted in accordance to the TAMUK Institutional Biosafety Committee protocol 2015-12-01. This is manuscript 18-111 of the Caesar Kleberg Wildlife Research Institute.

#### LITERATURE CITED

- Atkinson CT, Thomas NJ, Hunter DB. 2009. *Parasitic diseases of wild birds*. John Wiley and Sons, Hoboken, New Jersey, 592 pp.
- Centers for Disease Control and Prevention. 2016. DPDx-Laboratory identification of parasitic diseases of public health concern, *Mesocestoidiasis*. <https://www.cdc.gov/dpdx/mesocestoidiasis/index.html>. Accessed November 2017.
- Chertkova AN, Kosupko GA. 1978. The suborder Mesocestoidata Skryabin, 1940. In: *Principles of cestodology*, Ryzhikov KM, editor. Nauka Publishers, Moscow, Russia, pp. 118–229.
- Custer JW, Pence DB. 1981. Ecological analyses of helminth populations of wild canids from the Gulf coastal prairies of Texas and Louisiana. *J Parasitol* 67: 289–307.
- Davidson WR, Kellogg FE, Doster GL, Moore CT. 1991. Ecology of helminth parasitism in bobwhites from northern Florida. *J Wildl Dis* 27:185–205.
- Eom KS, Kim SH, Rim HJ. 1992. Second case of human infection with *Mesocestoides lineatus* in Korea. *Korean J Parasitol* 30:147–150.

- Etges FJ. 1991. The proliferative tetrathyridium of *Mesocestoides vogae* sp. n. (Cestoda). *J Helminthol Soc Wash* 58:181–185.
- Foronda P, Rivero AP, Santana Morales MA, Kabdur A, González AC, Quispe Ricalde MA, Feliu C, Valladares B. 2007. First larval record of *Mesocestoides* in Carnivora of Tenerife (Canary Islands). *J Parasitol* 93:138–142.
- Fuentes MV, Galan-Puchades MT, Malone JB. 2003. A new case report of human *Mesocestoides* infection in the United States. *Am J Trop Med Hyg* 68:566–567.
- Guthery FS. 2000. *On bobwhites*. Texas A&M University Press, College Station, Texas, 213 pp.
- Hosner PA, Braun EL, Kimball RT. 2015. Land connectivity changes and global cooling shaped the colonization history and diversification of New World quail (Aves: Galliformes: Odontophoridae). *J Biogeogr* 42:1883–1895.
- Kellogg FE, Calpin JP. 1971. A checklist of parasites and diseases reported from the bobwhite quail. *Avian Dis* 15:704–715.
- Khalil LF, Jones A, Bray RA. 1994. *Keys to the cestode parasites of vertebrates*. CAB International, Wallingford, UK, 751 pp.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874.
- Literák I, Olson PD, Georgiev BB, Spakulova M. 2004. First record of metacestodes of *Mesocestoides* sp. in the common starling (*Sturnus vulgaris*) in Europe, with an 18S rDNA characterization of the isolate. *Folia Parasitol* 51:45–49.
- McAllister CT, Tkach VV, Conn DB. 2018. Morphological and molecular characterization of post-larval pre-tetrathyridia of *Mesocestoides* sp. (Cestoidea: Cyclophyllidea) from ground skink, *Scincella lateralis* (Sauria: Scincidae), from southeastern Oklahoma, USA. *J Parasitol* doi: 10.1645/17-178.
- Moore J, Freehling M, Simberloff D. 1986. Gastrointestinal helminths of the northern bobwhite in Florida: 1968 and 1983. *J Wildl Dis* 22:497–501.
- Padgett KA, Crosbie PR, Boyce WM. 2013. *Mesocestoides*. In: *Molecular detection of human pathogens*, Liu D, editor. CRC Press, Taylor and Francis Group, Boca Raton, Florida, pp. 277–285.
- Padgett KA, Nadler SA, Munson L, Sacks B, Boyce WM. 2005. Systematics of *Mesocestoides* (Cestoda: Mesocestoididae): Evaluation of molecular and morphological variation among isolates. *J Parasitol* 91:1435–1443.
- Pence DB, Dowler RC. 1979. Helminth parasitism in the badger, *Taxidea taxus* (Schreber, 1778), from the western Great Plains. *J Helminthol Soc Wash* 46:245–253.
- Pence DB, Tewes ME, Laack LL. 2003. Helminths of the ocelots from southern Texas. *J Wildl Dis* 39:683–689.
- Pence DB, Windberg LA. 1984. Population dynamics across selected habitat variables of the helminth community in coyotes, *Canis latrans*, from south Texas. *J Parasitol* 70:735–746.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- Skirnisson K, Eydal M, Gunnarsson E, Hersteinsson P. 1993. Parasites of the arctic fox *Alopex lagopus* in Iceland. *J Wildl Dis* 29:440–446.
- Skirnisson K, Sigurðardóttir ÓG, Nielsen ÓK. 2016. Morphological characteristics of *Mesocestoides canislagopidis* (Krabbe 1865) tetrathyridia found in rock ptarmigan (*Lagopus muta*) in Iceland. *Parasitol Res* 115:3099–3106.
- Stoddard HL. 1931. *The bobwhite quail: Its habits, preservation, and increase*. Charles Scribner's Sons, New York, New York, 559 pp.
- Stone JE, Pence DB. 1978. Ecology of helminth parasitism in the bobcat from west Texas. *J Parasitol* 64:295–302.
- Webster D. 1949. Fragmentary studies on the life history of the cestode *Mesocestoides latus*. *J Parasitol* 35:83–90.
- Zalesny G, Hildebrand J. 2012. Molecular identification of *Mesocestoides* spp. from intermediate hosts (rodents) in central Europe (Poland). *Parasitol Res* 110:1055–1061.

Submitted for publication 15 November 2017.

Accepted 1 February 2018.