

Cyclocoelid (*Morishitium* sp.) Trematodes from an Air Sac of a Purple Sandpiper, *Calidris maritima* (Brünnich)

Dave Shutler, Mark L. Mallory, Mark Elderkin*, and J. Daniel McLaughlin†

Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada B4P 2R6. Correspondence should be sent to e-mail: dave.shutler@acadiau.ca

ABSTRACT: Like many shorebirds, purple sandpipers (*Calidris maritima* [Brünnich] Charadriiformes: Scolopacidae) have experienced population declines in recent years, but causes of these declines have not been established. As part of a larger study to identify causes of these declines, we collected and examined 25 purple sandpipers in coastal Nova Scotia, Canada during March 2013. In the course of dissections to collect tissue samples, we detected a cluster of trematodes in the air sac of 1 bird that were subsequently identified as cyclocoelids belonging to the genus *Morishitium* Witenberg, 1928, which we believe is the first report of this genus of parasites in this host. Cyclocoelids have been reported from other scolopacids and other shorebird families, but we are unaware of reports of serious pathology arising from these trematodes. Given this and the low prevalence (4%) in our sample, our data cannot ascribe declines in purple sandpiper populations to these trematodes, but our sample is limited both spatially and temporally.

Several species of North American shorebirds that underwent significant population reductions in the last half of the 20th Century (e.g., Morrison et al., 2012) have recovered in recent years; nonetheless, most have not reached conservation recovery targets (Andres et al., 2012). Explanations for the plight of shorebirds include changes in food availability caused by anthropogenic changes in habitat (e.g., development of waterfronts that were formerly foraging and nesting areas), climate change, increased predation (e.g., by feral cats; Blancher, 2013; Loss et al., 2013), and natural population cycles (Andres et al., 2012). Relatively less work has been conducted on the possibility of diseases and parasites influencing shorebird populations (Newton, 1998), although High Arctic species (Bennett et al., 1992) and species reliant on marine habitats tend to have lower prevalence of parasitism than those relying on more southerly or freshwater habitats (Mendes et al., 2005). The purple sandpiper *Calidris maritima* (Brünnich) is a circumpolar breeder that has experienced population declines in North America, the causes of which may include any of the aforementioned factors (Gratto-Trevor et al., 2011). Purple sandpipers are the northernmost wintering shorebird species in North America. During winter, these sandpipers inhabit rocky coastlines, often foraging on shoals that are exposed at low tide, and in areas of rockweed (*Ascophyllum nodosum*) where they forage for small molluscs and crustaceans. As a first step in identifying causes of declines, we initiated studies to test whether breeding provenance (to be assessed with stable isotopes; general approaches are described in Rubenstein and Hobson [2004]) was associated with variation in population health. Incidental to this initiative, we discovered trematodes in a purple sandpiper we collected.

Little is known about helminths of purple sandpipers in North America. The first report of parasites from North America was by Arnsdorf (1908), who described *Cyclocoelum* (= *Monostomum*) *vicarium* (Monostomatidae) trematodes but did not provide other details on the infection. The most up-to-date account of purple sandpiper biology

(Payne and Pierce, 2002) cites only a single published report from North America (Hantzsch, 1928) wherein numerous specimens of *C. vicarium* were recovered from 1 of 12 adults shot in northeastern Newfoundland, Canada. Finally, for North America an examination of 8, winter-sampled sandpipers from the Gulf of Maine led to the discovery of 4 species of trematodes, 2 species of cestodes, and 1 larval nematode (A. S. Didyk, pers. comm.), but no cyclocoelids. More information is available on the helminth fauna of purple sandpipers in northern Europe and the former USSR (see Gibson et al., 2005 for a partial list that includes 9 trematode, 11 cestode, 5 nematode, and 2 acanthocephalan species), but very little is known about the parasite fauna of purple sandpipers in general and from North America in particular.

As part of our larger study, we collected 25 purple sandpipers in March 2013 in coastal Nova Scotia. Carcasses were frozen at -40°C until processed. At time of processing, birds were weighed to the nearest 0.01 g. We examined carcasses for gross parasites in the body cavity during dissection and discovered a cluster of trematodes in an air sac of 1 bird. Ten intact flukes were recovered from this air sac, 5 of which were badly distorted and 3 of which were fragmented. An additional intact fluke and fragments of another 3 were recovered from the preserving fluid, presumably from a different air sac damaged during necropsy. These were preserved in 30% ethanol for later examination. Specimens were stained in acetic acid alum carmine for 24 hr, dehydrated in a graded series of ethanol, cleared in xylene, and mounted in Canada balsam for microscopic study. Attempts to obtain DNA data from these specimens were unsuccessful, possibly due to the initial treatment of the material. Voucher specimens have been deposited in the New Brunswick Museum, Saint John, New Brunswick, Canada: NBM 010310.1–010310.18.

Examination of intact specimens revealed that they were a species of *Morishitium* Witenberg, 1927. These specimens lacked a discernible oral sucker and an acetabulum, which is consistent with the “rauschi” body type as defined by Dronen and Tkach (2014). Only 2 species of *Morishitium*, *M. rauschi* Gupta and Gupta, 1979 and *Morishitium texanense* Dronen, 2007, display the rauschi body type (Dronen and Tkach, 2014), but only *M. texanense* is known from North America. Detailed study of the specimens was hampered by their general condition and by the extensive uterus that obscured the anterior testis and the ovary in many specimens. Our observations suggest that these flukes are not conspecific with either species currently included in the rauschi group. However, specimens on hand were frozen and were not, in our opinion, suitable for a proper description of a new species. Hence, our objectives here are limited to distinguishing these specimens from *M. texanense* and providing sufficient data on them for a detailed comparison with more suitable specimens, should they be encountered by other workers in the future.

General description: Body elongate, oral sucker and acetabulum not observed. Pharynx large, esophagus short and bifurcates at or near the posterior margin of the pharynx. Intestinal caeca have inwardly directed swellings along their length that are obscured posteriorly by uterine coils. Cirrus sac present; genital pore located at the posterior margin of the pharynx. Anterior testis variable in shape, longer than wide, and lies to 1 side of the midline and is partially obscured by the uterine coils in many specimens. Posterior testis varies from oval to spherical in most specimens and is situated on the midlines posterior to the uterine coils. In several specimens, one of the testes is shrunken or otherwise distorted. Ovary oval to spherical, lies anterior and oblique to the posterior testis, and is often

* Nova Scotia Department of Natural Resources, 136 Exhibition Street, Kentville, Nova Scotia, Canada B4N 4E5.

† Department of Biology, Concordia University, 7141 Sherbrooke St. W., Montreal, Quebec, Canada H4B 1R6.

DOI: 10.1645/15-808

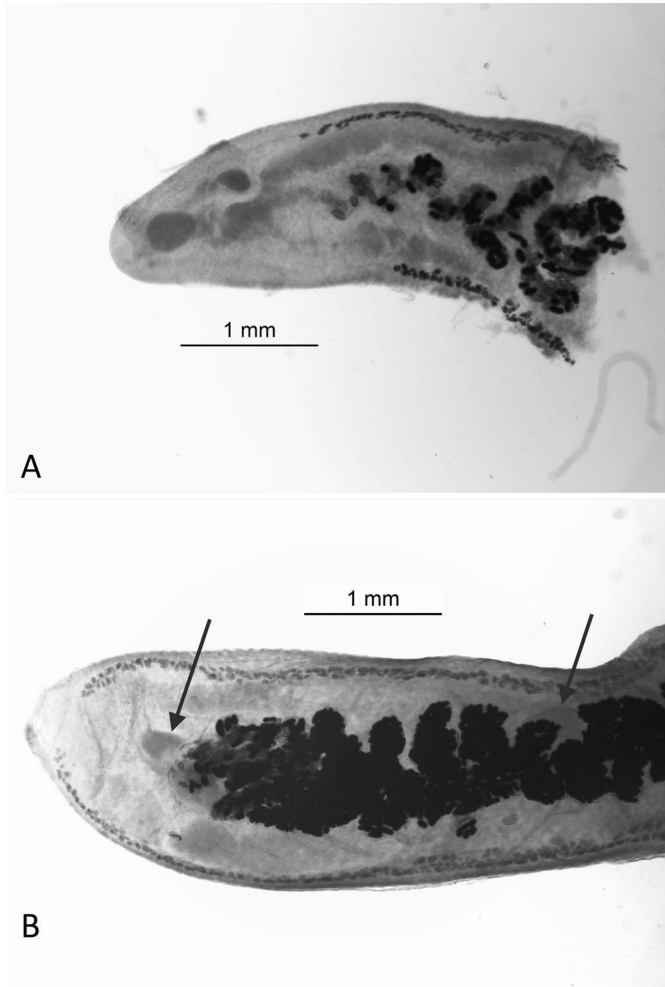


FIGURE 1. *Morishitium* sp. from purple sandpipers (*Calidris maritima*) from Nova Scotia, Canada. (A) Anterior end of specimen NBM-010310.6 showing the extent of, and the asymmetry between, vitelline bands. (B) Posterior half of specimen NBM-010310.3 showing the typical positions of the anterior and posterior testis (arrows). The ovary and Mehlis gland lie anterior and to the right of the posterior testis in this specimen and are partly obscured by the uterus.

obscured by uterine coils. Vitelline follicles occur in thin lateral bands that extend from a point posterior to the pharynx and esophageal bifurcation in these specimens. The anterior termination points are generally asymmetrical (Fig. 1A). Except for 1 specimen, the vitellaria do not become confluent at the posterior end of the worm (Fig. 1B). The uterus occupies most of the body length and overlaps the caeca, the anterior testis, and the ovary (Fig 1B). Eggs in the anterior uterus each contain a fully developed miracidium.

The morphometric data from the Nova Scotia specimens and from *M. texanense* are presented in Table I. Average lengths of the body, the inter- and post-testicular spaces, the cirrus sac, and the pharynx are larger in specimens from Nova Scotia than in *M. texanense* and, except for the cirrus sac, there is little overlap of measurements between them. The inter-testicular distance of the Nova Scotian specimens is greater owing to the distinctly more anterior position of anterior testis (Fig. 1B). In contrast, average size of the testes and dimensions of the eggs are greater in *M. texanense*. Overlap in testis size of flukes from both sources is extensive while virtually none exists among the eggs.

Several factors make species-level differentiation of adult digeneans difficult. These include, but are not limited to, their small size, general lack

of useful taxonomic characters, and uncertainty over the reliability of such characters for species-level differentiation (Nolan and Cribb, 2005). Other factors, including host-induced variation (Blankespoor, 1974; Kinsella, 1974), age or developmentally-related differences, crowding, and artifacts induced during fixation of specimens may affect morphological or morphometric data.

Species-level identification within the Cyclocoelidae presents additional challenges (Dronen and Blend, 2015). Cyclocoelids generally occur in low numbers within an individual host, so few replicates are available for most species. Most species within a genus are similar morphologically, and measurements of specific structures often overlap extensively, limiting their usefulness in species determination (Dronen, 2007). Dronen and Blend (2015) provide a comprehensive assessment of the usefulness of morphological and morphometric data used for species level identification within the family. They stressed the need for proper fixation of material and concluded that some criteria commonly used to distinguish trematode species are too variable on their own to distinguish species whereas others are more useful when corroborated by another variable.

The specimens from our purple sandpiper resemble *M. texanense* in general morphology, and the range in dimensions of most structures overlap to varying degrees. Unfortunately, our specimens had been frozen, potentially compromising organ measurements and distances between them (N. O. Dronen, pers. comm.). Nevertheless, certain features, including size of the pharynx, the intertesticular space, dimensions of eggs, and the anterior extent of vitelline follicles, differ between the two. Unfortunately, none of these by themselves are definitive and, given the condition of our material, it would be inappropriate to assign these specimens to *M. texanense* at this time.

Adult cyclocoelids are common in aquatic birds where they infect air sacs or other sites within the body cavity (Dronen et al., 2008; Ahmed et al., 2011). There is a single gastropod intermediate host in cyclocoelid life cycles that supports the production and accumulation of metacercariae within the snail over time. Thus, ingestion of an infected snail by a final host may result in establishment of several adults (Kanev et al., 2002). Thus, the infected sandpiper we examined may have only needed to ingest a single, heavily infected snail to develop the infection we observed. Alternatively, the bird may have experienced multiple exposures but, because all specimens were ovigerous, there is no way to distinguish separate cohorts.

However, the fact that only a single bird out of 25 was infected suggests that multiple exposures are unlikely. In winter, purple sandpipers feed mainly on gastropods whereas summer diets consist mostly of insects (Payne and Pierce, 2002). Five potential gastropod intermediate hosts were identified in gut contents within our sample of purple sandpipers: *Littorina littorea*, *Littorina saxatilis*, *Littorina obtusata*, *Margarites helicinus*, and *Lacuna vineta* (M. L. Mallory, unpubl. data). However, these are all marine species whereas freshwater and terrestrial snails are the only known intermediate hosts for cyclocoelids (Kanev et al., 2002).

There are numerous records of cyclocoelid infections of shorebirds in the suborder Charadrii (e.g., Hannum and Wilson, 1934; Zelif, 1946; Dronen and Tkach, 2013), and their geographic distribution appears to include most of the globe. However, none of the published reports, at least for shorebirds, cite evidence of pathology associated with cyclocoelid infections. The infected purple sandpiper we observed had a mass of 69.4 g whereas the uninfected birds had a mean mass of 71.6 ± 7.4 g SD. Despite the large number of flukes (14) present in this individual, no gross lesions were observed and its body mass was close to the sample mean, so there was no evidence of serious pathology. A prevalence of 4% in our sample also suggests that declines of purple sandpiper populations are unlikely to be attributable to this parasite, although we note that our sample represents only a single spatial and temporal snapshot. Further information is required to properly assess the potential effect of this parasite on populations of this host.

Research was conducted under permits issued from Environment Canada. Pamela Mills helped collect birds and Nathalie Leblanc, Pamela Mills, José Lefebvre, and Nora Spencer helped with dissections. Andrée

TABLE I. Comparison of the mean measurements and their respective ranges of *Morishitium texanense* from Dronen (2007) and those from specimens collected from a purple sandpiper, *Calidris maritima*, in Nova Scotia (Fig. 1). All measurements are in micrometers (μm) unless stated otherwise. Data on body length, body width, and position of the testes in the body in the Nova Scotian material are based on 4 intact specimens. The remaining measurements are pooled from the complete sample. Sample sizes for measurements from the Nova Scotian samples are given following the range in values. The post-uterine space for *M. texanense* was calculated from figure 7 in Dronen (2007). L denotes length and W denotes width.

Metric	Mean sizes		Ranges		n
	<i>M. texanense</i>	Nova Scotia	<i>M. texanense</i>	Nova Scotia	
Length (mm)	10.6	13.2	9.3–11.0	11.7–14.8	7
Width (mm)	2.0	2.3	1.9–2.3	2.0–2.6	7
Prepharynx	175	128	150–220	97–170	5
Pharynx L	278*	370	250–270	330–385	13
Pharynx W	220	290	210–230	260–310	13
Cirrus sac	510	652	400–750	525–855	6
Anterior testis L	528	435	460–630	355–500	8
Anterior testis W	452	255	370–550	190–285	8
Posterior testis L	578	355	410–690	260–525	13
Posterior testis W	454	329	350–550	261–390	13
Intertesticular space	1,090	2,225	650–1,370	1,910–2,490	6
Ovary L	248	280	210–300	215–428	6
Ovary W	248	334	210–300	233–431	5
Post-uterine space	1,122	1,210	1,122†	955–1,660	13
Post-testicular space	550	825	450–670	665–995	13
Egg L	140	125	130–155	119–130	55
Egg W	72	58	60–80	50–66	55

* sic; Dronen (2007) transposed the upper length and mean length values for pharynx length; in any event, the 2 measurements are so close as to not matter.

† Estimated from drawing in Dronen (2007).

Gendron, Environment Canada, provided photographic assistance and Dr. Paul Albert, Concordia University, helped arrange the plate. We thank Dr. Norman Dronen, Department Wildlife and Fisheries Sciences, Texas A&M University, for his critical contributions and an anonymous reviewer for additional contributions. Dr. Andrew Didyk, University of New Brunswick (Moncton), kindly provided unpublished information on purple sandpiper parasites and access to an unpublished manuscript. Funding was provided by Environment Canada (STAGE), the Natural Sciences and Engineering Research Council (NSERC), and the Nova Scotia Department of Natural Resources. Research in parasitology at Concordia University is funded by NSERC Discovery Grant A6979 to J. D. McLaughlin.

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