International Journal for Parasitology 43 (2013) 1119–1132

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Molecular systematics of pinniped hookworms (Nematoda: *Uncinaria*): species delimitation, host associations and host-induced morphometric variation

Steven A. Nadler[a,*], Eugene T. Lyons[b], Christopher Pagan[a], Derek Hyman[a], Edwin E. Lewis[a], Kimberlee Beckmen[c], Cameron M. Bell[d], Aurelie Castinel[e], Robert L. DeLong[f], Padraig J. Duignan[g], Cher Farinpour[a], Kathy Burek Huntington[h], Thijs Kuiken[i], Diana Morgades[j], Soraya Naem[k], Richard Norman[l,1], Corwin Parker[a], Paul Ramos[m,l], Terry R. Spraker[n], Bárbara Berón-Vera[o]

[a] *Department of Entomology and Nematology, University of California, One Shields Avenue, Davis, CA 95616, USA*
[b] *Department of Veterinary Science, University of Kentucky, Gluck Equine Research Center, Lexington, KY 40546, USA*
[c] *Alaska Department of Fish and Game, Division of Wildlife Conservation, 1300 College Road, Fairbanks, AK 99701, USA*
[d] *Department of Environment and Primary Industries, PO Box 2500, Bendigo DC, VIC 3554, Australia*
[e] *Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand*
[f] *National Marine Mammal Laboratory, AFSC, NNMS, 7600 Sand Point Way NE, Seattle, WA 98115, USA*
[g] *UCVM, University of Calgary, 3280 Hospital Dr. NW, Calgary, Alberta T2N 9B5, Canada*
[h] *23834 The Clearing Dr., Eagle River, AK 99577, USA*
[i] *Viroscience Lab, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands*
[j] *Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Science, University of the Republic, Alberto Lasplaces 1550, Montevideo, Uruguay*
[k] *Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, PO Box 1177, Urmia, Iran*
[l] *School of Veterinary Science, University of Melbourne, 250 Princes Highway, Werribee, VIC 3030, Australia*
[m] *Zoos Victoria, PO Box 74, Parkville, VIC 3052, Australia*
[n] *Colorado State University Diagnostic Laboratory, College of Veterinary Medicine, Colorado State University, 300 West Drake Road, Fort Collins, CO 80526, USA*
[o] *Laboratorio de Mamíferos Marinos, Centro Nacional Patagónico, CONICET, Blvd. Brown 3600, Puerto Madryn, 9120 Chubut, Argentina*

ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 28 August 2013
Accepted 31 August 2013
Available online 23 October 2013

Keywords:
Hookworm
Pinniped
Phylogenetics
Molecular systematics
Cophylogeny
Species
Morphometrics

ABSTRACT

Hookworms of the genus *Uncinaria* have been widely reported from juvenile pinnipeds, however investigations of their systematics has been limited, with only two species described, *Uncinaria lucasi* from northern fur seals (*Callorhinus ursinus*) and *Uncinaria hamiltoni* from South American sea lions (*Otaria flavescens*). Hookworms were sampled from these hosts and seven additional species including Steller sea lions (*Eumetopias jubatus*), California sea lions (*Zalophus californianus*), South American fur seals (*Arctocephalus australis*), Australian fur seals (*Arctocephalus pusillus*), New Zealand sea lions (*Phocarctos hookeri*), southern elephant seals (*Mirounga leonina*), and the Mediterranean monk seal (*Monachus monachus*). One hundred and thirteen individual hookworms, including an outgroup species, were sequenced for four genes representing two loci (nuclear ribosomal DNA and mitochondrial DNA). Phylogenetic analyses of these sequences recovered seven independent evolutionary lineages or species, including the described species and five undescribed species. The molecular evidence shows that *U. lucasi* parasitises both *C. ursinus* and *E. jubatus*, whereas *U. hamiltoni* parasitises *O. flavescens* and *A. australis*. The five undescribed hookworm species were each associated with single host species (*Z. californianus*, *A. pusillus*, *P. hookeri*, *M. leonina* and *M. monachus*). For parasites of otarids, patterns of *Uncinaria* host-sharing and phylogenetic relationships had a strong biogeographic component with separate clades of parasites from northern versus southern hemisphere hosts. Comparison of phylogenies for these hookworms and their hosts suggests that the association of *U. lucasi* with northern fur seals results from a host-switch from Steller sea lions. Morphometric data for *U. lucasi* shows marked host-associated size differences for both sexes, with *U. lucasi* individuals from *E. jubatus* significantly larger. This result suggests that adult growth of *U. lucasi* is reduced within the host species representing the more recent host–parasite association. Intraspecific host-induced size differences are inconsistent with the exclusive use of morphometrics to delimit and diagnose species of *Uncinaria* from pinnipeds.

---

[*] Corresponding author. Tel.: +1 530 752 2121; fax: +1 530 752 5674.
E-mail address: sanadler@ucdavis.edu (S.A. Nadler).

[1] Present address: Ministry for Primary Industries, Investigation and Diagnostic Centres and Response, PO Box 2526, Wellington 6140, New Zealand.

0020-7519/$36.00 © 2013 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijpara.2013.08.006

1. Introduction

Hookworm disease can have a significant deleterious impact on juvenile pinnipeds and their populations, and although estimates of mortality in pups due to *Uncinaria* hookworm infections vary substantially, one recent study reported that 13% of deaths in New Zealand sea lion pups (*Phocarctos hookeri*) were attributable to hookworms (Castinel et al., 2006). The relationship between hookworm infection intensity and clinical hookworm disease in pinnipeds is not entirely clear, and host body condition is inversely correlated with the number of hookworms present (Sepúlveda, 1998; Lyons et al., 2001), perhaps due to the linkage between nursing and transmammary transmission of these hookworms. Irrespective of questions regarding the exact relationship between the intensity of hookworm infection and pathology, it is clear that *Uncinaria* spp. can cause serious health problems in pinniped pups (Spraker et al., 2004). Recently, hookworms have been reported to interact synergistically with bacterial pathogens, causing enteritis and bacteremia; this emerging disease complex was responsible for 72% of deaths in rookeries of California sea lion pups (*Zalophus californianus*) in one investigation (Spraker et al., 2007). Unexpectedly, California sea lion hosts with enteritis have been found to have hookworm adults deep within muscle layers of the intestine and numerous nematodes free in the peritoneal cavity (Spraker et al., 2004, 2007; Lyons et al., 2005). Hookworm disease in pinnipeds appears to be influenced by host genetics, with homozygosity at a single locus predisposing California sea lion hosts to hookworm anaemia (Acevedo-Whitehouse et al., 2006, 2009). In contrast, genetic studies of different pinniped species offer conflicting results concerning whether increased average homozygosity of hosts is correlated with increased hookworm disease (Acevedo-Whitehouse et al., 2006, 2009). Differences in host responses to hookworms owing to variations in host genetics may help explain the poor predictive value of hookworm infection intensity for pathogenicity and pup condition in certain studies (Lyons et al., 1997, 2001, 2005).

The first species of pinniped hookworm to be formally described was *Uncinaria lucasi* (Stiles, 1901) from the northern fur seal (*Callorhinus ursinus*). This original description was later considered unsatisfactory and because the type specimens were damaged, Baylis (1947) redescribed *U. lucasi* using new specimens obtained from *C. ursinus* collected from the topotype locality (Pribilof Islands, USA). *Uncinaria lucasi* is the only pinniped hookworm for which the life cycle has been experimentally completed (Olsen and Lyons, 1965), and many aspects of its biology have been revealed through subsequent investigations (Olsen, 1958; Lyons and Keyes, 1978, 1984; Lyons and Biggs, 1983; Lyons et al., 1997). Unlike hookworms from many terrestrial mammalian hosts, adult *U. lucasi* establish in hosts only from transmammary transmission of parasitic L_3s acquired by nursing pups from their mother's milk. In northern fur seals, adult *U. lucasi* are eliminated spontaneously from juvenile hosts a maximum of 3 months p.i. (Olsen and Lyons, 1965), and adult seals are not parasitised by adult hookworms. Hookworm eggs in rookery soil hatch as free-living L_3s, and these larvae can penetrate the skin of seals, or enter orally, and persist in tissues as parasitic L_3s. The life cycle is completed when parasitic L_3s are reactivated within lactating fur seals and migrate to the mammary glands (pre-parturition) before transmission to pups through nursing.

The only other species of hookworm from pinnipeds that has been formally described is *Uncinaria hamiltoni*, obtained from the South American sea lion, *Otaria flavescens* (syn *Otaria byronia*) in the Falkland Islands (Baylis, 1947). Baylis (1933) originally suggested that specimens of *U. hamiltoni* from *O. flavescens* were conspecific with hookworms recovered from a California sea lion, *Z. californianus* (see Nadler et al., 2000), and this has led to representation of hookworms from *Z. californianus* as *U. hamiltoni*. However, other researchers (Dailey and Hill, 1970) reported that specimens of *Uncinaria* from *Z. californianus* had morphometric characteristics intermediate between *U. lucasi* and *U. hamiltoni*, thereby questioning the conspecificity of hookworms from South American and California sea lions.

Morphological, mainly morphometric, differences have been reported between *Uncinaria* individuals from different pinniped species, but it is unclear whether these are species-level differences, reflect intraspecific variation or are host-induced morphological differences (George-Nascimento et al., 1992; Nadler et al., 2000; Castinel et al., 2006; Ramos et al., 2013). Based on their formal descriptions, morphological differences between the two described species, *U. lucasi* and *U. hamiltoni*, are minor (Baylis, 1933, 1947; Nadler et al., 2000). Nadler et al. (2000) reported statistically significant differences in some morphometric features (e.g., total body length, spicule length) of male *U. lucasi* from northern fur seal pups obtained from two different geographic regions. This observation is consistent with previous suggestions that differences in nematode body size and certain characteristics of infection may reflect host-induced variation. For example, Olsen (1952) noted the larger body size of hookworms from Steller sea lions (*Eumetopias jubatus*) versus hookworms from northern fur seals, although he believed both host species were infected with *U. lucasi*. Similarly, George-Nascimento et al. (1992) reported that differences in nematode body size, prevalence of host skin lesions and infection intensity for adult hookworms parasitising South American sea lions (*O. byronia*) and South American fur seals (*Arctocephalus australis*) represented host-induced variation within one hookworm species.

The species-level systematics of some pinniped *Uncinaria* has recently been investigated using a molecular systematic approach (Nadler et al., 2000; Nadler, 2002; Ramos et al., 2013), yielding evidence independent of morphology for delimiting species and providing a phylogenetic framework for understanding intraspecific and interspecific morphological variation. This approach has been used to evaluate the specific status of *Uncinaria* parasitising California sea lions and northern fur seals (Nadler et al., 2000), with lineage exclusivity and species status determined by molecular phylogenetic analysis. These studies revealed that northern fur seals and California sea lions, species that share the same rookery space in parts of their breeding ranges, host different *Uncinaria* spp. (Nadler et al., 2000; Nadler, 2002). Similarly, molecular characterisation of *Uncinaria* sp. from Australian fur seals, Australian sea lions, and New Zealand fur seals indicates that these three hosts share a distinct species of hookworm that, based on morphology, most closely resembles *U. hamiltoni* (Ramos et al., 2013). However, developing a more complete understanding of the species diversity of *Uncinaria* in pinniped hosts and investigation of their host ranges requires molecular comparisons of hookworms from many additional host species, together with characterisation of hookworms representing both described species.

In the present study, we investigate the specific status of *Uncinaria* parasitising nine pinniped species using evolutionary analysis of nuclear and mitochondrial gene sequences amplified from more than 100 individual hookworms. Phylogenetic trees were reconstructed for the hookworms and compared with published phylogenies for their hosts, yielding new hypotheses for pinniped hookworm evolution and host associations. In addition, an evaluation of the utility of morphometrics is investigated through comparisons of a hookworm species that infects two host species. In addition to providing specific conclusions regarding pinniped hookworms, the approaches used herein are applicable to other investigations of parasites designed to test hypotheses concerning species and to evaluate their relationships.