

## First Report of an Adult Tapeworm (Cestoda: Diphyllbothriidea) in a Southern Sea Otter (*Enhydra lutris nereis*)

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**abstract:** We present a novel case of an intestinal cestode infection in a southern sea otter (*Enhydra lutris nereis*). The cestode species *Diplogonoporus tetrapterus* was confirmed genetically. Stable isotope analysis of whiskers collected from the sea otter did not confirm the consumption of fish as the route of exposure.

An adult female southern sea otter (*Enhydra lutris nereis*; collection SO 7106-14) was found dead in Monterey County (36°35'42", -121°57'43", California) on 2 April 2014 with trauma consistent with a shark bite. The decomposed carcass was frozen before post-mortem examination. During necropsy, a single, ~15-cm segment of the posterior portion of cestode strobila containing gravid proglottids was recovered from the intestine and preserved in pure 70% ethanol for identification.

Selected proglottids were paraffin embedded, and 5- $\mu$ m-thick hematoxylin and eosin-stained serial sections were prepared for microscopic examination. Pieces of strobila were prepared as whole mounts stained with Mayer's hydrochloric carmine solution (Sigma-Aldrich, St. Louis, Missouri, USA) and mounted in Canada balsam. Each proglottid contained double genital organs, with genital and uterine pores located medially on the ventral surface (Fig. 1A, B), indicating a diphyllbothriid tapeworm of the genus *Diplogonoporus*. However, this material was incomplete, partially decomposed, and except for the above-mentioned characteristics and the presence of nonembryonated, thick-walled eggs (64–79 $\times$ 41–49  $\mu$ m;  $n=25$ ; Fig. 1C, D), the sample was not suitable for species confirmation based solely on these characteristics. This specimen was compared with voucher specimens (hol-

ogenophores) deposited at the helminthologic collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS C-682) and museum material loaned from the Smithsonian National Museum of Natural History collected from northern sea otters (*Enhydra lutris kenyoni*) by Rausch (1964) and Margolis et al. (1997; USNM 1356136, 1381986). Museum specimens identified as *Diplogonoporus tetrapterus* had close morphologic similarity with our material, but for more precise identification molecular markers were used (Kuchta et al. 2015).

A segment of the tapeworm was used for DNA extraction, PCR amplification, and sequencing of the nuclear large subunit ribosomal RNA gene (lsrDNA) and mitochondrial cytochrome c oxidase subunit 1 (*cox1*) were conducted following the methodology described in Hernández-Orts et al. (2015). Pairwise sequence comparisons of lsrDNA (1,470 bp; GenBank KX227386) and *cox1* (597 bp; GenBank KX227385) with two specimens of *D. tetrapterus* from northern fur seals (*Callorhinus ursinus*) from Alaska (Kuzmina et al. 2015) revealed zero and one to two nucleotide differences, respectively, confirming the species identification.

Diphyllbothriid tapeworms have been documented in several mustelid species, although river otters (*Lontra* spp. and *Lutra* spp.) and sea otters are relatively rare hosts, with some reports from northern sea otters. Rausch (1964) reported two sea otters (one each from Amchitka Island and Montague Island, Alaska) and Margolis et al. (1997) reported eight of 66 sea otters examined from Prince William Sound, Alaska, that were

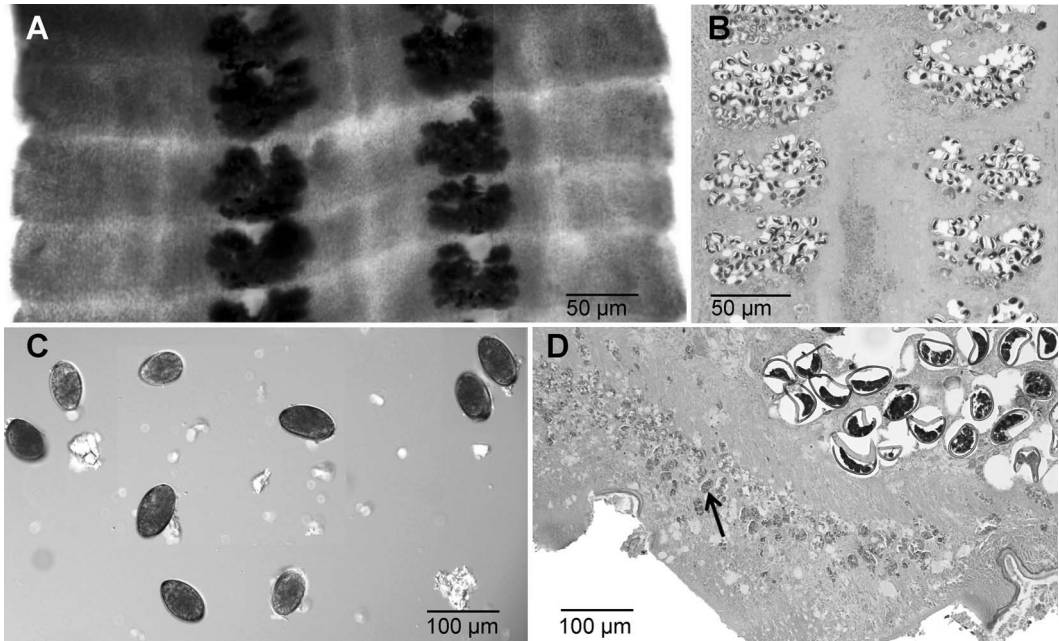


FIGURE 1. Photomicrographs of *Diplogonoporus tetraapterus* recovered from the intestine of a southern sea otter (*Enhydra lutris nereis*). A) Whole mount of gravid proglottids showing double sets of genital organs per proglottid; B) Longitudinal histologic section of gravid proglottids showing double sets of genital organs per proglottid; C) Eggs obtained from gravid segments; D) Higher magnification of longitudinal histological section of gravid proglottids showing uterus with nonembryonated eggs (upper right) and granular calcareous corpuscles (arrow).

infected by *D. tetraapterus*. There also have been reports from the Komandorski and Kuril islands in Russia (Margolis et al. 1997) and several unpublished accounts of tapeworms in northern sea otters. For example, a mortality event in Cordova, Alaska, in the mid-1990s involving several sea otters that had been foraging on fish waste were heavily infected by tapeworms, causing lethal blockages of the intestine in some cases (A. Doroff, pers. comm.). Furthermore, of 176 sea otters examined at the National Wildlife Health Center (1991–2013), two from Washington and four from Alaska were positive for cestodes, two of which were identified as *D. tetraapterus* (R. Cole, pers. comm.).

In California, attempts are made to recover and necropsy every dead southern sea otter that is reported. In more than 40 yr of monitoring, with more than 8,000 carcasses examined, this is the first record of an adult tapeworm from a southern sea otter (Hen-

nessy 1972; Mayer et al. 2003; B. Hatfield, pers. comm.). However, the difference in the frequency of cestode infection between the two closely related sea otter subspecies is not surprising because fish, which are common intermediate hosts for diphyllbothriid cestodes, are frequently consumed by northern sea otters (Watt et al. 2000), but rarely by southern sea otters (Riedman and Estes 1990). The life cycle of *D. tetraapterus* has not been confirmed, but it likely includes marine copepods as the first intermediate hosts and marine fish as second intermediate hosts (Kuchta et al. 2015).

To assess whether fish were a dominant component of the diet of this sea otter, whiskers were collected for stable isotope analysis to evaluate foraging history (Newsome et al. 2009). We predicted that the stable isotope profile would indicate fish consumption, indicated by greater nitrogen isotope ( $\delta^{15}\text{N}$ ) values compared to otters with an

TABLE 1. Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope values from whisker segments collected from a southern sea otter (*Enhydra lutris nereis*; collection SO 7106-14) to determine whether the animal had consumed fish, a source for the cestode *Diplogonoporus tetrapterus* found at necropsy. Isotope values are reported as per mil. Mean  $\delta^{15}\text{N}$  values were consistent with those obtained previously from female sea otters that consumed macroinvertebrates from the same geographic area.

Whisker segment	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
SO-7106-14-A	-12.6	15.2
SO-7106-14-B	-12.8	14.8
SO-7106-14-C	-13.0	15.1
SO-7106-14-D	-13.1	15.3
SO-7106-14-E	-13.0	15.2
SO-7106-14-F	-13.2	15.3
SO-7106-14-G	-13.2	15.2
SO-7106-14-H	-13.2	15.0
SO-7106-14-I	-13.2	15.0
SO-7106-14-J	-13.2	15.4
SO-7106-14-K	-13.2	15.0
SO-7106-14-L	-13.5	14.7
SO-7106-14-M	-13.8	14.2
SO-7106-14-N	-13.4	15.2
SO-7106-14-O	-13.5	15.6
SO-7106-14-P	-13.6	15.9
Mean (SD) this study	-13.2 (0.3)	15.1 (0.4)
Mean (SD) previous study <sup>a</sup>	-12.2 (0.9)	15.4 (1.0)

<sup>a</sup> Mean and SD from 16 other female southern sea otters from Monterey Bay, California (Newsome et al. 2009).

invertebrate-dominated diet. The mean ( $\pm$ SD)  $\delta^{15}\text{N}$  value in parts per thousand (‰) for whiskers from this sea otter was  $15.1 \pm 0.4\text{‰}$  (Table 1), which is similar to mean values for female sea otters from the same geographic area with invertebrate-dominated diets ( $15.4 \pm 1.0\text{‰}$ ; Newsome et al. 2009). Although stable isotopes of whiskers only represent  $\sim 8\text{--}12$  mo of foraging information, this otter exhibited low isotopic variation between adjacent whisker segments, indicating a consistent diet over that time frame. Individual dietary patterns of sea otters generally persist through time (Estes et al. 2003; Tinker et al. 2008; Newsome et al. 2009), so it is unlikely this individual's diet was ever dominated by fish. However, stable isotope analysis may not detect infrequently consumed prey items, so it is possible that fish

was consumed at low levels, resulting in cestode exposure, or that infection occurred through a different exposure route.

Although the route of exposure was not clear in this case, identification of additional cases may ultimately reveal emergence of a novel host-parasite cycle associated with prey redistribution, introduction of novel hosts, or climate change. Spatiotemporal shifts in pathogen exposure have been linked with introduced hosts, and possibly climate change, for other parasites (Miller et al. 2010) affecting southern sea otters. Due to their critical role as keystone species (Estes and Palmisano 1974) and marine sentinels (Jessup et al. 2004), continued disease monitoring for southern sea otters may facilitate identification of emerging health threats to other marine wildlife and people.

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#### LITERATURE CITED

- Estes JA, Palmisano JF. 1974. Sea otters: their role in structuring nearshore communities. *Science* 185:1058–1060.
- Estes JA, Riedman ML, Staedler MM, Tinker MT, Lyon BE. 2003. Individual variation in prey selection by sea otters: Patterns, causes and implications. *J Anim Ecol* 72:144–155.
- Hennessy SL. 1972. *The intestinal parasites and diet analysis of the southern sea otter*. MA Thesis, Biology, California State University Hayward, Hayward, California, 44 pp.
- Hernández-Orts JS, Scholz T, Brabec T, Kuzmina T, Kuchta R. 2015. High morphological plasticity and global geographical distribution of the Pacific broad tapeworm *Adenocephalus pacificus* (syn. *Diphyllobothrium pacificum*): Molecular and morphological survey. *Acta Trop* 149:168–178.
- Jessup DA, Miller M, Ames J, Harris M, Kreuder C, Conrad PA, Mazet JAK. 2004. Southern sea otter as a sentinel of marine ecosystem health. *EcoHealth* 1:239–245.
- Kuchta R, Scholz T, Brabec J, Narduzzi-Wicht B. 2015. *Diphyllobothrium*, *Diplogonoporus* and *Spirometra*. In: *Biology of foodborne parasites*, Xiao L, Ryan U, Feng Y, editors. CRC Press, Boca Raton, Florida, pp. 299–326.
- Kuzmina TA, Hernández-Orts JS, Lyons ET, Spraker TR, Korniyushyn VV, Kuchta R. 2015. The cestode community in northern fur seals (*Callorhinus ursinus*) on St.

- Paul Island, Alaska. *Int J Parasitol: Parasites Wildl* 4: 256–263.
- Margolis L, Groff JM, Johnson SC, McDonald TE, Kent ML, Blaylock RB. 1997. Helminth parasites of sea otters (*Enhydra lutris*) from Prince William Sound, Alaska: Comparisons with other populations of sea otters and comments on the origin of their parasites. *J Helminthol Soc W* 64:161–168.
- Mayer KA, Dailey MD, Miller MA. 2003. Helminth parasites of the southern sea otter *Enhydra lutris nereis* in central California: Abundance, distribution and pathology. *Dis Aquat Organ* 53:77–88.
- Miller MA, Conrad PA, Harris M, Hatfield B, Langlois G, Jessup DA, Magargal SL, Packham AE, Toy-Choutka S, Melli AC, et al. 2010. A protozoal-associated epizootic impacting marine wildlife: Mass-mortality of southern sea otters (*Enhydra lutris nereis*) due to *Sarcocystis neurona* infection. *Vet Parasitol* 172:183–194.
- Newsome SD, Tinker MT, Monson DH, Oftedal OT, Ralls K, Staedler MM, Fogel ML, Estes JA. 2009. Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). *Ecology* 90:961–974.
- Rausch RL. 1964. Studies on the helminth fauna of Alaska XLI. Observations on cestodes of the genus *Diplogonoporus* Lönnerberg, 1862 (Diphyllobothriidae). *Can J Zool* 42:1049–1069.
- Riedman ML, Estes JA. 1990. *The sea otter* (*Enhydra lutris*): Behavior, ecology, and natural history. United States Department of the Interior, Fish and Wildlife Service. Biological Report. Vol. 90 No. 14. XX pp.
- Tinker MT, Bentall GB, Estes JA. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. *Proc Natl Acad Sci U S A* 105:560–565.
- Watt J, Siniff DB, Estes JA. 2000. Inter-decadal patterns of population and dietary change in sea otters at Amchitka Island, Alaska. *Oecologia* 124:289–298.

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