Sci Parasitol 13(3):101-108, September 2012
ISSN 1582-1366

Ultrastructural study of *Pseudamphistomum truncatum* (Rudolphi, 1819) (Opisthorchidae Trematoda) from the Caspian Seal *(Pusa caspica)* (Phocidae, Mammalia) using Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray Analysis (EDXA)

Richard A. Heckmann[1]✉, Ali Halajian[2, 3]

1 – Department of Biology, MLBM, Brigham Young University, Provo, Utah 84602 USA.

2 – Department of Biodiversity (Zoology), University of Limpopo, Turfloop Campus, Sovenga 0727, Polokwane, South Africa.

3 – Department of Parasitology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Correspondence: Tel. 801-422-2495, Fax 801-422-0090, E-mail richard_heckmann@byu.edu

Abstract. *Pseudamphistomum truncatum* (Rudolphi, 1819) samples were obtained from a Caspian Seal *(Pusa caspica)* which was dead, possibly due to Canine Distemper Virus (CDV). After preparation, several samples were scanned using Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Analysis (EDXA). Key characteristics were displayed with SEM including the oral and ventral (acetabulum) suckers, spined body, oval operculated eggs and truncated posterior region. The oral sucker with the oral cavity is muscular and contains convoluted muscular walls. The acetabulum or ventral sucker is in a depressed area with 3 prominent muscular rings and finger-like internal convolutions. The expanded truncated posterior body region is prominent. Eggs are oval and operculated. The EDXA scans of the body generated data similar for trematodes. The eggs are wavy in appearance and have high levels of calcium and sulfur. This is the first published study of *P. truncatum* using electron optics. This is also the first report of this digene in the seals checked in the southern part of the Caspian Sea, Iranian shores.

Keywords: *Pseudamphistomum truncatum;* Electron Optics; SEM; EDXA; Caspian Seal.

Received 18/07 /2012. Accepted 20/09 /2012.

Introduction

The Caspian Seal *(Pusa caspica = Phoca caspica)* is the host for the trematode, *Pseudamphistomum truncatum*. This mammal can be the definitive host for several parasites. The scientific classification for the host mammal is as follows:

Kingdom: Animalia

Phylum: Chordata
Class: Mammalia
Order: Carnivora
Suborder: Pinnipedia
Family: Phocidae
Genus: *Pusa*
Species: *P. caspica*
Binomial name: *Pusa caspica*

The Caspian Seal *(Pusa caspica)* is one of the smallest members of the earless seal family and unique in that it is found exclusively in the brackish Caspian Sea. They can be found not only along the shorelines, but also on the many rocky islands and floating blocks of ice that dot the Caspian Sea. In winter, and cooler parts of the spring and autumn season, these marine mammals populate the Northern Caspian region. As the ice melts in the warmer season, they can be found on the mouths of the Volga and Ural Rivers, as well as the southern latitudes of the Caspian Sea where cooler waters can be found due to greater depth (Geptnor, 1976; Khuraskin and Zakharova, 2000a; 2000b).

It is unclear as to how these seals became isolated in the landlocked Caspian Sea. One of the most widely known hypotheses argues that the seals reached the Caspian during the Quaternary period from the north when continental ice sheets melted, and that they are descended from the ringed seal. Adults are approximately 1.5 meters (4.9 ft) in length and weigh around 86 kilograms (190 lb); males are generally larger and bulkier than the females. Their dental formula is I 3/2, R 1/1, PC 6/5 (Geptnor, 1976; Khuraskin and Zakhavova, 2000a).

The Caspian seal, *P. caspica,* is endemic to the Caspian Sea. It is distributed throughout the sea from avant-deltas of the Volga and Ural Rivers to the Iranian coasts. Some animals occurred sporadically in the Volga River as far as Volgograd and 200 km upstream the Ural River (Rumyantseu and Khuraskin, 1978; Miyazaki, 2001).

Pseudamphistomum truncatum is a parasite common to the Caspian seal as well as other protozoan and helminth parasites. There are several other described species for *Pseudamphistomum. Pseudamphistomum* is a digene that normally is found in the liver and gall bladder. It is part of the Opisthorchidae family which includes *Opisthorchis* Blanchard, 1895, *Metorchis* Looss, 1899 and the Chinese liver fuke *Clonorchis sinensis* Cobbolde, 1875. *C. sinesis* is a major parasite of the liver for humans (Sherrard-Smith et al., 2009). *C. sinesis*is the most important member of this family as it's a zoonotic food-borne liver digene for humans, specially in Southeast Asia (Kang et al., 2008; Sherrard-Smith et al., 2009) where its endemic in several countries (King and Scholz, 2001). Opisthorchid digeneans are typical and common parasites of mammals. *Pseudamphistomum* has three described species namely; *P. truncatum, P. aethiopicum* and and *P. danubiense* that the first is the type species (Yamaguti, 1971). *Pseudamphistomum truncatum* has been reported in different hosts including cat, dog, wolf, red fox, raccoon dog, mink, ferret, otter, shrews, seals (harbor, ringed, harp and Caspian) and human and some fishspecies as intermediate hosts (Schuster et al., 1999; Hawkins et al., 2010). Some of the key characteristics for *P. truncatum* are: spinose, posterior end truncate, anterior end more or less pointed, sucker-like ventro-terminal depression, oral sucker subterminal and muscular, acetabulum or ventral sucker present nearly as large as the oral sucker, body elongate 1300-1550 x 27-475 µm, eggs numerous and oval 26-28 x 13-16 µm. (Yamaguti, 1958; Skryabin, 1950; Scholz, 2008).

One of the parasites found recently in the seal was *Pseudamphistomum truncata* (Rudolphi, 1819). The opisthorchid digenean is a typical and common parasite of wild carnivores. Hawkins et al. (2010) recently reported on this digenean in American Mink *(Mustela vison)* and Eurasian otter *(Lutra lutra)* in Ireland. Simpson et al. (2009) found the bile fluke in Eurasian otters *(Lutra lutra)* in Great Britain. In one study, native Eurasian otter *(Lutra lutra)* and introduced American mink *(Mustela vison)* carcasses collected throughout Ireland were screened for biliary parasites. Carcasses collected throughout Ireland are screened for biliary parasites. Secondary intermediate hosts, cyprinid fish, were also examined for Opistorchiid metacercariae. Twenty-nine mink

and 24 otter gall bladders were screened for biliary parasites. A single mink and three otters were found to be infected with the digenetic trematode *Pseudamphistomum truncatum.* Eighty-nine percent of roach *(Rutilus rutilus)* from the nearby River Shannon were infected with *P. truncatum* metacercariae, confirming the persistence of the parasite. This is the first record of the species in Ireland, and its recent introduction is probably related to the movement and release of cyprinid fishes by anglers (Schuster et al., 1999; Hawkins et al., 2010). As listed in these publications, different fish species are important intermediate hosts for *P. truncatum*. Fish are a major food source for seals and other listed hosts. Fishes can transmit the infection to the final hosts that can be seals, otters, minks and even humans (Sidorovich and Anisimova, 1997; Hawkins et al., 2010).

Materials and methods

Specimens of *Pseudamphistomum truncatum* from *Pusa caspica* (Caspian Seal) were collected in Iran by the second author, from a dead male seal found at the beach of Ramsar City, Mazandaran Province, Northern Iran. After necropsy all the organs were checked carefully. Liver was somewhat pale in some parts. After cutting, numerous digenes were found in the liver tissue and bile ducts. Specimens were cleaned in saline, fixed in 70% ethanol (ethyl alcohol). There are many potential hosts for *P. truncatum* (see discussion and literature cited) around the world. The specimens we obtained for this study were fixed in 70% ethanol (ethyl alcohol). The selected specimens were placed in critical point drying (CPD) baskets then dehydrated using an ethanol series of 95% and 100% for at least 10 minutes per soak followed by the critical point drying (Lee, 1992). Samples were mounted on SEM sample mounts (stubs), gold coated and observed with a scanning electron microscope (XL 30 ESEMFEG; FEI, Hilsboro, Oregon). Digital images of the specimen were obtained using digital imaging software attached to a computer.

For X-ray microanalysis (EDXA), standard methods of SEM preparation were used (Lee, 1992). Coated specimens were examined with an Environmental SEM (XL 30ESEMFEG) equipped with a Phoenix Energy-Dispersive X-ray Analyzer. X-ray spot analysis was performed at 15 KV with a spot size of 5 and results were represented in charts and recorded with digital imaging software attached to the computer.

Results

Figures 1 to 8 are the results of the fine structure study of *Pseudamphistomum truncatum.* Figure 1 depicts the ventral surface of the digenean trematode with a truncated body covered with spines. The anterior end is pointed with a subterminal and muscular oral sucker (figure 2). The oral region is prominent (figure 2) with the oral cavity and pre-pharynx region. There is a mid-body depression with the ventral sucker or acetabulum (figures 1, 3, and 4). The acetabulum (ventral sucker) is circular with a well defined cavity and 3 rings of muscle (figures 1, 3 and 4). The inner area of the ventral sucker has a series of finger like ridges (20-25 in number) which will aid the fluke in host attachment (figure 4). The posterior part of the *P. truncatum* is characterized by an expanded truncated end with a sucker like depression (figures 1, 5 and 6). The “en face” of the posterior end shows a muscular ring following the spinous body with numerous holes (figure 5). There is a sucker-like depression for the posterior end (figure 6). The dorsal side of the fluke shows the same tapering body shape with a truncated terminal end covered with small spines (figure 7). The eggs are oval with a wavy like appearance, note the opercular end (figure 8).

Results for the X-ray microanalysis (EDXA) are shown in figures 9, 10 and 11. The outer shell of the operculated egg is high in calcium and sulphur ions (figure 9). These ions are critical in forming a hardened shell for the egg. EDXA scans of the body of the trematode are shown by figures 10 and 11 (Anterior and Posterior Body). Carbon, Calcium, and Sulphur are depicted by the scans. Gold (Ag), Palladium and Osmium (Os) peaks are due to specimen preparation. The charts list weight percent of the elements.

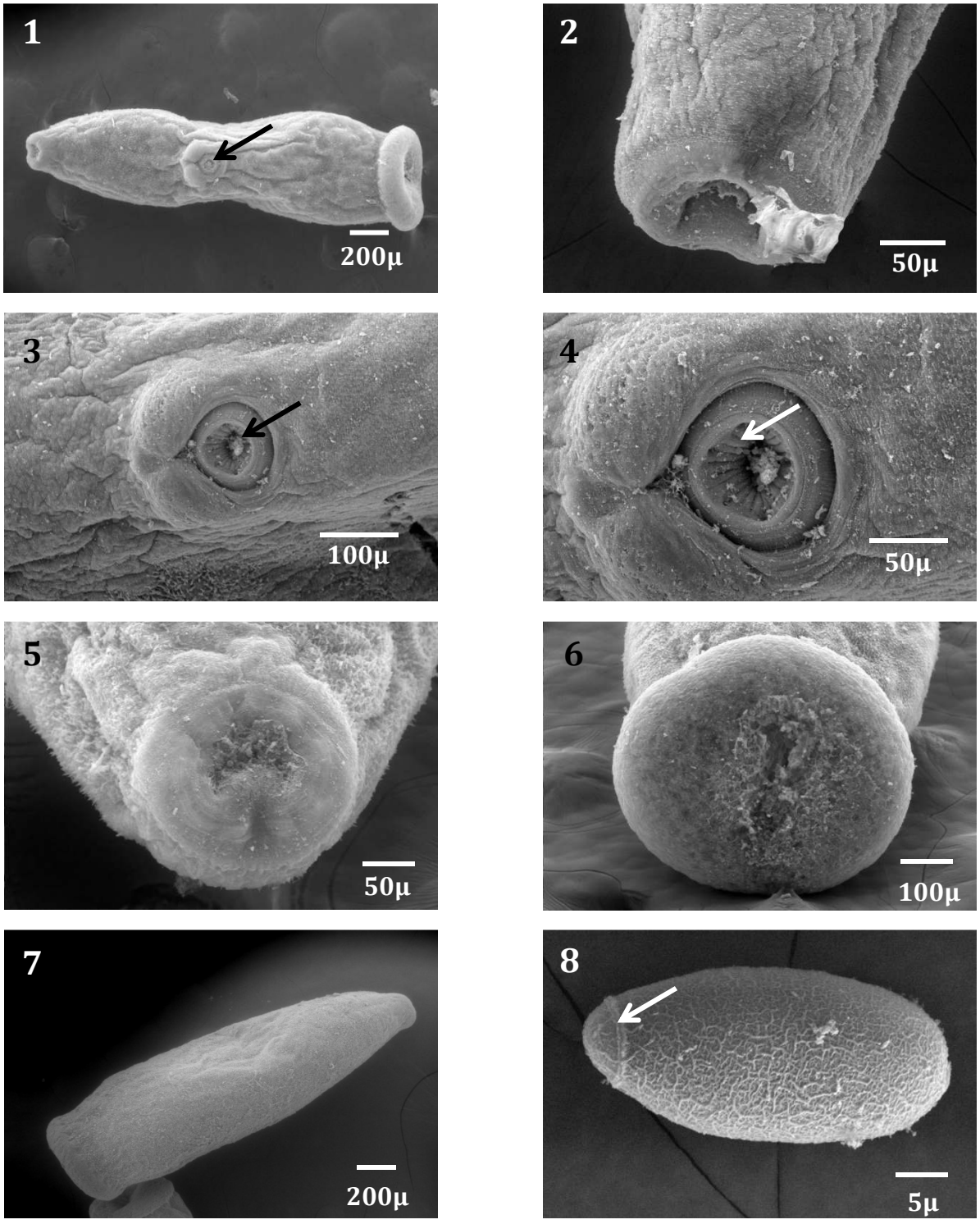
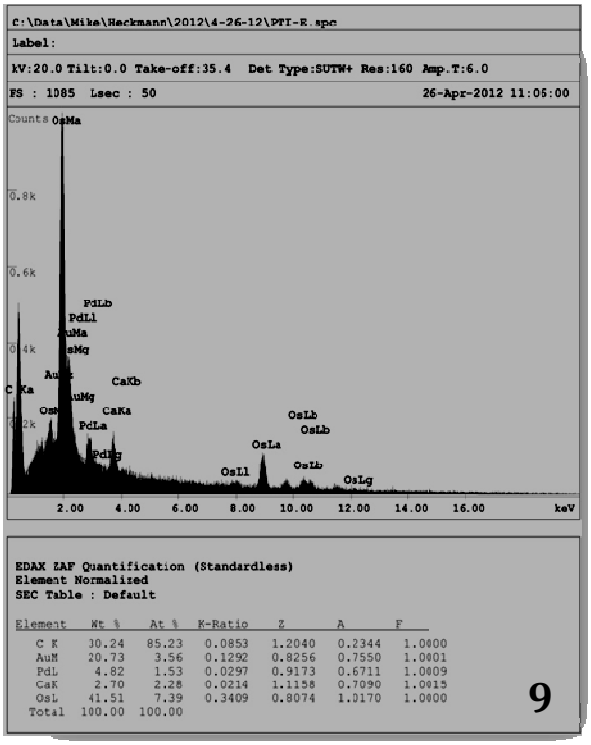
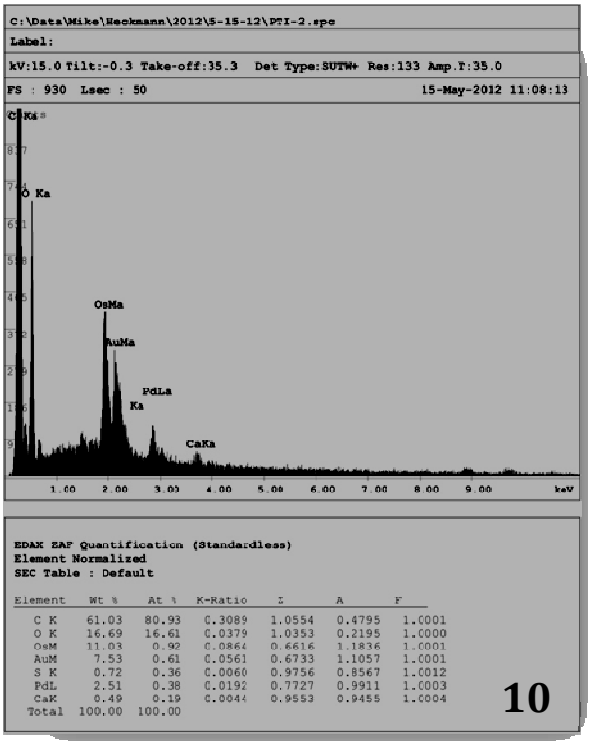


Figure 1. Ventral surface of *Pseudamphistomum truncatum* showing the muscular oral sucker and the acetabulum or ventral sucker (arrows) with a blunt, truncated terminal area. The body is covered with spines. The body is slightly distorted due to fixation and preparation. **Figure 2.** The oral region of *P. truncatum* showing the muscular oral sucker and beginning of the blind digestive system. Note the convolutions and spines on the body surface. The pre-pharynx area is visible. **Figure 3.** Mid body on the ventral side is the acetabulum (arrows) within a concave depression. This is a major attachment structure for the trematode. The muscular rings around the acetabulum are visible. **Figure 4.** Higher magnification of the acetabulum or ventral sucker. With the rings of muscle surrounding the finger-like inner rows (arrow). Modifications for attachment. **Figure 5.** "En face" or terminalis of the body of *P. truncatum* circular truncated end of the body with a concave depression. **Figure 6.** Higher magnification of Figure 5 displaying the concave depression of the terminalis for the trematode. A sucker-like structure. **Figure 7.** The dorsal side of *P. trucatum*. Spines appear along the length of the helminth. An arrow-head like body that tapers from the pointed anterior end to the truncated terminal end. **Figure 8.** The operculated (arrow) egg with a wavy like appearance. Numerous eggs were visible for the specimen.





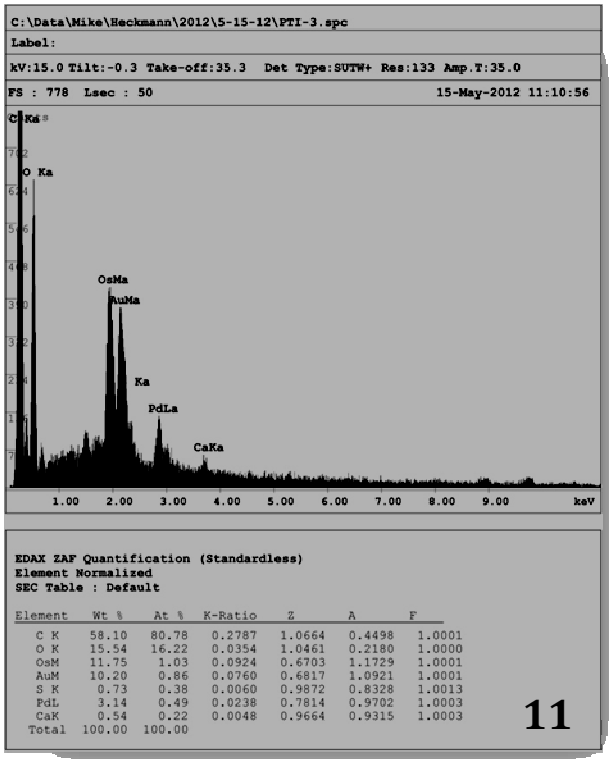


Figure 9. EDXA (Energy Dispersive Analysis for X-Ray) scan of the chemical elements present on the surface of the egg. Elements are listed in weight percent (wt %) on the chart with peak heights on the graph. **Figure 10.** EDXA scan for the anterior region of *P. truncatum*. **Figure 11.** EDXA scan for the posterior region of *P. truncatum.*

Discussion

The second author examined Caspian seals that probably died from Canine Distemper Virus (CDV) near the Caspian Sea. A previous paper had described an acanthocephalon from the same mammal (Amin et al., 2011). The first detailed information on Caspian seal parasites was reported by Shehupakov (1936) who listed 6 species of nematodes, trematodes and acanthocephalans prompting Dogiel (1947; 1962) to declare the Caspian seal a relic species for having lost cestode and arthropod parasites.

The pioneer of parasitological investigation of the Caspian sea was D.M. Landa who in 1929 dissected two seals on the Mangyshlak Peninsula. He wrote that "the seals were infected with cestodes and nematodes" (Landa, 1931). Since 1957 a great deal of parasitological research has been carried out by Y.V. Kurochkin and others (Kurochkin, 1958; 1962; 1964; 1975).

Canine Distemper Virus (CDV) has been verified as the cause of death for the Caspian seals (Forsyth et al., 1998; Kennedy et al., 2000). The disease has been a major problem for mammals and represented the die-off for the seal. *P. truncatum* was obtained from one of the dead seals.

The morphology of *P. truncatum* was studied using electron optics and elemental microanalysis (EDXA). Additional structures were visible with this technique. *Pseudamphistomum truncatum* is a typical and common parasite of wild carnivores as well as other mammals. The most common frequent hosts of this fluke are mustelids, Ermine, *Mustela ermina* (Shimalov and Shimalov, 2001a; 2001b), European polecat, *M. putorius* (Shimalov and Shimalov, 2002a; Anisimova, 2002), American mink *Neovison vison* (Sidorovich and Anisimova, 1997; Shimalov and Shimalov, 2001a; Skov et al., 2008; Hawkins et al., 2010), least weasel, *M. nivalis* (Shimalov and Shimalov, 2001b), European mink, *M. lutreola* (Anisimova, 2004; Torres et al., 2008), Eurasian otter, *Lutra lutra* (Shimalov et al., 2000; Torres et al., 2004; Simpson et al., 2005; Hawkins et al., 2010). It has been noted as the parasite of the red fox, *Vulpes vulpes* (Schuster et al., 1999; Shimalov and Shimalov, 2003), arctic fox *V. lagopus* (Malczewski, 1962), grey wolf, *Canis lupus* (Shimalov and Shimalov, 2000), raccoon dog, *Nycteratus procyonoides* (Shimalov and Shimalov, 2002b), as well as two species of seals, harbor seal *(Phoca vitulina)* and grey seal *(Halichoerus grypus). P. truncatum* is found mainly in the Central and Eastern Europe and in Russia. It has been also observed in Denmark, Ireland, England, Germany and Southern Europe. This study extends hosts to the Caspian seal *(Pusa caspica)* from the Caspian Sea. This is the first report of this digene in a Caspian seal *(Pusa caspica)* from the southern shores of Caspian Sea, as the previous study (on 24 dead seals) found no parasites in the livers (Eslami and Kiai, 2009).

In Poland *P. truncatum* has been recorded twice as a fox parasite (Hildebrand et al., 2011). In the 1950s and 1960s it was found in the wild red foxes near the Baltic Sea Coast and in the Wielkopolska region of Poland on a farm of arctic foxes which had been fed fishes from the Vistula Lagoon (Malczewski, 1962; Gorski et al., 2006; Pojmanska et al., 2007; Gorski et al., 2010).

The Caspian seal, *P. caspica* is a host to a wide array of parasites some of which may contribute to the decrease in numbers of the host. Research should continue on both the protozoan and metazoan parasites of *P. caspica.*

Acknowledgements

The authors extend thanks to Michael Standing and Dr. John Gardner for their professional help and suggestions for the electron optics study (SEM and EDXA). Dr. Keith Crandall, Chair of the Biology Department, BYU provided research funds. Dr. Atif M. El-Naggar, visiting Professor from Ain Shams University Egypt, helped with the plates for the figures. We also acknowledge the favor of Dr. Wilmien Luus-Powell (Head of the Department of Biodiversity, University of Limpopo).

References

Amin O.M., Heckmann R.A., Halajian A., El-Naggar A.M. 2011. The morphology of a unique population of *Corynosoma strumosum* (Acanthocephala, Polymorphidae) from the Caspian seal, *Pusa caspica,* in the land-locked Caspian Sea using SEM, with special notes on histopathology. Acta Parasitol. 56:1-15.

Anisimova E.I. 2002. Comparative analysis of the helminthocenoses of the otter *(Lutra lutra)* and polecat *(Mustela putorius)* in Belarus. Helminthologia 39:87-90.

Anisimova E.I. 2004. Study of the European mink *Mustela lutreola* helminthocenoses in connection with the American mink *M. vison* expansion in Belarus: story of the study and review of the results. Helminthologia 44:193-196.

Dogiel V.A. 1947. Kurs Obshchey Parasitologii (General Parasitology 2nd ed. Uchpedgiz Press, Leningrad, 1-163.

Dogiel V.A. 1962. Obshchey parasitologii (General Parasitology) 2nd ed. Uchpedgiz Press, Leningrad, Leningrad, 1-163.

Dogiel V.A. 1962. Obshchey Parasitologiya (revised and enlarged by Yv, 1, Polyansky and E.M. Khersinym, 1zd. LGV. Leningrad, 3-463.

Eslami A., Kiai B. 2009. A report on the parasitic infections in Caspian seals *(Phoca caspica)* of southern part of Caspian sea. Veterinary Journal of Islamic Azad University, Garmsar branch 5(1):31-33.

Forsyth M.A., Kennedy S., Wilson S., Eybatov T., Barrett T. 1998. Canine Distemper Virus in a Caspian Seal *(Phoca caspica).* Vet. Rec. 143:662-664.

Geptnor V.G. 1976. Mammals of the Soviet Union. 1976. Vol. II (ed. V.G. Geptner). Moscow. Higher School. 717 p. (in Russian).

Górski P., Zalewski A., Lakomy M. 2006. Parasites of carnivorous mammals in Bialowieża Primeval Forest. Wiadomości Parazytologiczne 52:49-53.

Górski P., Zalewski P., Kazimierczak K., Kotomski G. 2010. Coproscopical investigations of the European otter *(Lutra lutra)* from Bialowieża Primeval Forest. Wiadomości Parazytologiczne 56:179-180.

Hawkins C.J., Caffery J.M., Stewart P., Lawton C. 2010. Biliary parasite *Pseudamphistomum truncatum* (Opisthorchiidae) in American mink *(Mustela vison)* and Eurasian otter *(Lutra lutra)* in Ireland. Parasitol. Res. 107:993-997.

Hildebrand J., Popiolek M., Zaleśny G. Piróg A. 2011. A record of *Pseudamphistomum truncatum* (Rudolphi, 1819) (Digenea, Opsithorchidae) in the Eurasian otter *(Lutra lutra* L.) from Poland. Wiadomosci Paraztologiczne 57:151-154.

Kang S., Sultana T., Loktev V.B., Wongratanacheewin S., Sohn W., Eom K.E., Park J. 2008. Molecular identification and phylogenetic analysis of nuclear rDNA sequences among three opistorchid liver fluke species (Opisthorchiidae: Trematoda), Parasitol. Res. 57:191-197.

Kennedy S., Kuiken T., Jepson P.D., Deaville R., Forsyth M., Barrett T., Van de Bildt M.W., Osterhaus A.D., Eybatov T., Duck C., Kydyrmanov A., Mitrofanov I., Wilson S. 2000. Mass Die-Off of Caspian seals caused by Canine Distemper Virus emerging infectious diseases. Emerg. Infect. Dis. 6(6):637-639.

Khuraskin L.S., Zakharova N.A. 2000a. Dynamics and causes of a decline in the commercial catch of the Caspian seal in the 20th century. *In:* Proceedings of the International Conference: Marine Mammals of Golarctic. Arkhengelsk (in Russian), pp. 410-414.

Khuraskin L.S., Zakharova N.A. 2000b. Present conditions of the development of Caspian seal population bioresources. *In:* Proceedings of the International Conference: Marine Mammals of Golarctic. Arkhangelsk (in Russian). pp. 414-418.

King S., Scholz T. 2001. Trematodes of the family Opistorchiidae: a minireview. The Korean Journal of Parasitology 39(3):209-221.

Kurochkin Y.V. 1958. Helminth fauna of the Caspian seal during its breeding season. Rab. Gelminthol. 80. letiyu Akad. KI. Moscow, pp. 188-194 (In Russian).

Kurochkin Y.V. 1962. Helminth fauna of the Caspian seal from its autumn haul-ground. Tr. Astrakh. Zapoved. 6:119-126.

Kurochkin Y.V. 1964. Scientific results of the 315th helminthological expedition of the Union. Tr. Astrakk. Zapoved. 9:8-31.

Kurochkin Y.V. 1975. Parasites of the Caspian seal, *Pusa caspica*. Rapp. P.V. Reún. Cons. Int. Explor. Mer. 119:363-365.

Landa D.M. 1931. Activity of the 2nd (the 89th All-Union) Expedition of the State Research Institute of Microbiology and Epidemiology of the RSFSR in Kazakhstan. Mikrobiol. Parasitol. Vestn. 10:3-4.

Lee P.E. 1992. Scanning Electron Microscopy and X-ray Microanalysis. Prentice Hall, Englewood Cliffs, New Jersey, 458 pp.

Malczewski A. 1962. Helminth parasites of bred foxes and minks in Poland. Acta Parasitol. Polonica 10:213-260.

Miyazaki N. 2001. A review of studies on the ringed, Caspian, and Baikal Seals *(Pusa hispida, P. caspica* and *P. sibirica*). Otsuchi Marine Science 26:1-6.

Pojmańska T., Niewiadomska T., Okulewicz A. 2007. Pasożytnicze helminty Polski. Gatunki. Żywiciele. Biale Palmy. Polish Parasitological Society, Warszawa.

Rumyantsev V.D., Khuraskin L.S. 1978. New data on the mortality of the Caspian seal due to wolves. Page 187 in Congress of the All-Union Theriological Society, 2nd (P.A. Panteleev et al. eds.). Nauka, Moscow, USSR. ZR 116(19):5669.

Scholz T. 2008. Family Opisthorchiidae Loss, 1899. *In:* Keys to the Trematoda.Vol. 3. (Eds. R. Bray, D. Gibson, A. Jones). CAB International and Natural History Museum, London, 9-50.

Schuster R., Bonin J., Staubach Ch., Heidrich R. 1999. Liver fluke (Opisthorchiidae) findings in red foxes *(Vulpes vulpes)* in the eastern part of the Federal State Brandenburg, Germany – A contribution to the epidemiology of opisthorchiidosis. Parasitol. Res. 85:142-146.

Shehupakov I.G. 1936. Parasite fauna of Caspian seal. To the problem of parasite fauna of relics. Uchenye Zapiske Leningradskogo Ordena Lenina Gosudarstvennogo Universiteta im. A.S. Bubnova, Ser. 7, Biol. 3:134-143.

Sherrard-Smith E., Cable J., Chadwick E.A. 2009. Distribution of Eurasian otter biliary parasites, *Pseudamphistomum truncatum* and *Metorchis albidus* (Family Opisthorchiidae), in England and Wales. Parasitology 136:1015-1022.

Shimalov V.V., Shimalov V.T. 2000. Helminth fauna of the wolf *(Canis Lupus* Linnaeus, 1758) in Belorussian Polesie. Parasitol. Res. 86:163-164.

Shimalov V.V., Shimalov V.T., Shimalov A.V. 2000. Helminth fauna of otter *(Lutra lutra* Linnaeus, 1758) in Belorussian Polesie. Parasitol. Res. 86:528.

Shimalov V.V., Shimalov V.T. 2001a. Helminth fauna of the American mink *(Mustela vison* Schreber, 1777) from Belorussian Polesie. Parasitol. Res. 87:886-887.

Shimalov V.V., Shimlov V.T. 2001b. Heminth fauna of the stoat *(Mustela erminea* Linnaeus, 1758) and the weasel *(M. nivalis* Linnaeus, 1758) in Belorussian Polesie. Parasitol. Res. 87:680-681.

Shimalov V.V., Shimalov V.T. 2002a. Helminth fauna of the European polecat *(Mustela putorius* Linnaeus, 1758) in Belorussian Polesie. Parasitol. Res. 88:259-260.

Shimalov V.V., Shimalov V.T. 2002b. Helminth fauna of the raccoon dog *(Nyctereutes procyonoides* Gray, 1834) in Belorussian Polesie. Parasitol. Res. 86:163-164.

Shimalov V.V., Shimalov V.T. 2003. Helminth fauna of the red fox *(Vulpes vulpes* Linnaeus, 1758) in southern Belarus. Parasitol. Res. 89:77-78.

Sidorovich V.E., Anisimova E.I. 1997. Peculiarities of helminthocenosis in the American mink population inhabiting a severly polluted rivers ecosystem (the Svisloch river, Belarus) Helminthologia 34:45-52.

Simpson V.R., Gibbons L.M., Khalil L.F., Williams J.L.R. 2005. Cholecystitis in otters *(Lutra lutra)* and mink *(Mustela vison)* caused by the fluke *Pseudamphistomum truncatum.* Vet. Rec. 157:49-52.

Simpson V.R., Tomlinson A.J., Molenaar F.M. 2009. Prevalence, distribution, and pathological significance of the bile fluke *Pseudamphistomum truncatum* in Eurasian otters *(Lutra lutra)* in Great Britain. Vet. Rec. 164:397-401.

Skov J., Kaina P.W., Jørgensen T.R., Buchmann K. 2008. Molecular and morphometric study of metacercariae and adults of *Pseudamphistomum truncatum* (Opisthorchiidae) from roach *(Rutilus rutilus)* and wild American mink *(Mustela vison).* Vet. Parasitol. 155:209-216.

Skryabin K.I. 1950. Trematody zhivotnykh I cheloveka. Osnovy trematodologii. Vol. IV. Izdatielstvo AN SSSR, Moskova, Leningrad.

Torres J., Feliu C., Fernández-Morán J., Ruíz-Olmo J., Rosoux R., Santos-Reis M., Miquel J., Fons R. 2004. Helminth parsites of the Eurasian otter *Lutra lutra* in southwest Europe. J. Helminthol. 78:353-359.

Torres J., Miquel J., Fournier P., Fournier-Chambrillon C., Liberge M., Fons R., Feliu C. 2008. Helminth communities of the autochthonous mustelids *Mustela lutreola* and *M. putorius* and the introduced *Mustela vison* in south-western France. J. Helminthol. 82:349-355.

Yamaguti S. 1958. Systema Helminthum, Vol. 1, The Digenetic Trematodes. Interscience Publishers Inc. New York. 978 pp.

Yamaguti S. 1971. Synopsis of digenetic trematodes of vertebrates. Parts I, II. Tokyo: Keigaku Publishing Co. pp. 797-798.