Helminths of mustelids with overlapping ecological niches: Eurasian otter *Lutra lutra* (Linnaeus, 1758), American mink *Neovison vison* Schreber, 1777, and European polecat *Mustela putorius* Linnaeus, 1758

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**Summary**
This study presents the helminthological data on three mustelid species with overlapping ecological niches in Lithuania. In general, 14 helminth species or higher taxa were reported from all mustelids: *Isthmiophora melis*, *Strigea strigis* metacercariae, *Pseudamphistomum truncatum*, *Alaria alata* mesocercariae, *Phyllodistomum folium*, *Opisthorchis felineus*, *Metametorchis skrjabini*, *Mesocestoides* sp., *Taenia martis*, *Aonchotheca putorii*, *Crenosoma schachmatovae*, *Eucoleus aerophilus*, *Molineus patens*, and Nematoda g. sp. The largest number of helminths was detected in *M. putorius* (11) and *N. vison* (10) from wetlands; 7 helminths were detected in *M. putorius* from forests, and 8 in *N. vison* and 4 in *L. lutra* from water bodies. Habitat-related differences were found in the abundance and prevalence of *E. aerophilus* in *M. putorius*. *M. putorius* has higher indices of infection by *I. melis*, *S. strigis* metacercariae, and *E. aerophilus* compared to *N. vison* in wetlands. Differences in the abundance and prevalence of *P. truncatum* among *N. vison* and *L. lutra* in water bodies have been observed. Helminths detected in *N. vison* in the present study are native European parasites.

**Keywords:** *Lutra lutra*; *Neovison vison*; *Mustela putorius*; helminths

**Introduction**

The Eurasian otter *Lutra lutra* (Linnaeus, 1758) (subfamily Lutrinae), the American mink *Neovison vison* Schreber, 1777 and the European polecat *Mustela putorius* Linnaeus, 1758 (subfamily Mustelinae) are mammals belonging to the family Mustelidae, with different affinity to the aquatic environment.

It is thought, that *L. lutra* originated in Asia and spread into Europe at the latest Pleistocene and early Holocene (Willemsen, 1992). Due to the loss of the riparian habitat, water pollution, polychlorinated biphenyls (PCBs) concentrations, hunting, declining food resources and road traffic accidents, *L. lutra* population declined in all of its distribution during the 20th century (MacDonald & Mason, 1988; Lodé, 1993b; Roos et al., 2015). Today *L. lutra* is listed as "Near Threatened" though it has one of the widest distributions of all Palearctic mammals (cover Europe, Asia and North Africa) (Roos et al., 2015). *L. lutra* is semi-aquatic mustelid found in a variety of aquatic habitats (Mason & MacDonald, 1986), whose diet consists mainly of aquatic prey (Bonesi et al., 2004). The species has been the subject of several more detailed helminthological studies, mainly in Belarus (Shimalov et al., 2000; Anisimova, 2002), Poland (Górski et al., 2010), Ukraine (Korol et al., 2016), United Kingdom (Fahmy, 1954; Jefferies et al., 1990; Weber, 1991; McCarthy & Hassett, 1993; Sherrard-Smith et al., 2015b), Germany (Schuster et al., 1988), and southwest Europe (Torres et al., 2004). Parasites act as a factor which could have an impact on the otter population dynamics, therefore the knowledge of the parasites may be useful for protecting the species. *E. aerophilus* is semi-aquatic mustelid found in a variety of aquatic habitats (Mason & MacDonald, 1986), whose diet consists mainly of aquatic prey (Bonesi et al., 2004). The species has been the subject of several more detailed helminthological studies, mainly in Belarus (Shimalov et al., 2000; Anisimova, 2002), Poland (Górski et al., 2010), Ukraine (Korol et al., 2016), United Kingdom (Fahmy, 1954; Jefferies et al., 1990; Weber, 1991; McCarthy & Hassett, 1993; Sherrard-Smith et al., 2015b), Germany (Schuster et al., 1988), and southwest Europe (Torres et al., 2004). Parasites act as a factor which could have an impact on the otter population dynamics, therefore the knowledge of the parasites may be useful for protecting the species.

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N. vison was introduced to Europe at the beginning of 20th century from North America for the purpose of fur farming (Brzeziński & Marzec, 2003). As a result of escapes, deliberate releases, and farm damages by 1990s the feral N. vison population was registered almost in all European countries ( Bonesi & Palazon, 2007; Lesis et al., 2008). Due to competition for food resources and space, N. vison is considered as an invasive species which could have an impact on the decline or disappearance of the European mink Mustela lutreola (Linnaeus, 1761) population in Europe (Maran & Henttonen, 1995). More recently N. vison has been considered as having a negative impact on the populations of L. lutra and M. putorius (MacDonald & Harrington, 2003; Melero et al., 2012). N. vison as well as L. lutra are semi-aquatic mustelids, however N. vison exploits both aquatic and terrestrial prey (Bonesi et al., 2004). The study of parasites related to the N. vison invasion in new regions is important due to possibility of introduction of new parasites to endemic host and transfer of endemic parasites to a new host. The impact of the introduced N. vison on parasite transmission has been studied (e.g., Ivanov & Semenova, 2000; Sherrard-Smith et al., 2015a; Martínez-Rondán et al., 2017).

M. putorius occurs throughout the Western Palearctic (Mitchell-Jones et al., 1999). In the last century its population sharply declined across Europe due to increase in human activities (Baghli et al., 2005). Today the species is listed as Least Concern in the IUCN Red List of Threatened Species (Skumatov et al., 2016). In northern and central Europe M. putorius is known to occupy a variety of habitat types: rivers, marshes, forests, woodland, farms, and villages (Jędrzejewski et al., 1993; Lodé, 1994; Baghli et al., 2005). According to Rondinini et al. (2006) the species is strongly associated with riparian areas in mainland Europe. Based on that, all three mustelid species (L. lutra, N. vison, and M. putorius) could present competition among them, because they exhibit overlap in diet and habitat preference (Lodé, 1993b; Bonesi et al., 2004). No studies of L. lutra helminths from Lithuania have been reported to date. There is also poor documentation of N. vison and M. putorius parasites in this country. Earlier, unidentified nematodes and Isthmiophora melis (Schrank, 1788) Lühe, 1909 were reported in M. putorius (Maldžiūnaitė, 1959; Kazlauskas & Prūsaitė, 1976). Larvae of Trichinella Railliet, 1895 and Alaria alata (Goeze, 1782) were also documented in this host species (Grikiienienė et al., 2001; Senutaitė & Grikiienienė, 2001). Helminths of mustelids, including N. vison and M. putorius were reported by Nugaraitė et al. (2014).

The aim of this study was to explore the helminth communities of three mustelids with overlapping ecological niches: L. lutra, N. vison, and M. putorius.

**Material and Methods**

Carcasses of 6 L. lutra, 59 N. vison, and 27 M. putorius were collected in different localities of Lithuania between 2013 and 2017 (Fig. 1). N. vison and M. putorius were hunted by hunters and collected from car accidents, while L. lutra individuals were collected only from car accidents. Mustelids were assigned to

Fig. 1. Collection sites of Lutra lutra (LL), Neovison vison (NV), and Mustela putorius (MP) in Lithuania. (○ – water bodies; ▲ – forests; ♦ – wetlands). The numbers on the map indicate the numbers of collected animals.
the closest habitat and grouped as follows: (1) forests – *M. putorius* (n = 18); (2) wetlands – *N. vison* (n = 39) and *M. putorius* (n = 8); and (3) water bodies (rivers, lakes, and lagoons) – *N. vison* (n = 20) and *L. lutra* (n = 6). All carcases were stored at -20°C until examination. Mustelids were examined using the method of total helminthological dissection of individual organs (Ivashkin et al., 1971). Frontal sinuses, connective tissue between the muscle fibres, trachea, lungs, heart, liver, gall bladder, kidney, urinary bladder, and entire gastrointestinal tract (stomach, small and large intestines) were analysed. Parasites were collected and stored in 70% ethanol until studied.

Temporary preparations were used for nematode morphological identification, while trematodes and cestodes were identified using permanent preparations. The identification was based on publications of Kozlov (1977), Sidorovich (1997), Kostadinova & Gibson (2002), Vieira et al. (2012), and Kontrimavičius (1969). Helminthological terms were used according to the recommendations of Bush et al. (1997). The 95% confidence intervals for prevalence were calculated as described by Rojtman & Lobanov (1985). Differences in the abundance and prevalence were tested using the Mann-Whitney U test and the Fisher’s exact test respectively.

**Ethical Approval and/or Informed Consent**

The research related to animals use has been compiled with all the relevant national regulations and institutional policies for the care and use of animals. The study protocol no. 2017.03.22 No. 13. was approved by License of Environmental Protection Agency (EPA).

**Results**

Fourteen species or higher taxa of helminths were found in three species of mustelids from all habitats, i.e., 10 helminths in *N. vison*, 12 in *M. putorius*, and 4 in *L. lutra* (Table 1). The largest number of helminths was detected in *M. putorius* (11) and *N. vison* (10) from wetlands; 7 helminths were detected in *M. putorius* from forests, 8 in *N. vison* and 4 in *L. lutra* from water bodies. *N. vison* and *M. putorius* from wetlands shared 9 helminths; *M. putorius* from forests and wetland shared 6 helminths.

The abundance and prevalence of *Euceles aerophilus* (Creplin, 1839) in *M. putorius* from wetlands was higher than in *M. putorius* from forests (p = 0.003/0.004 respectively). The abundance and prevalence of *Strigea strigis* (Schrank, 1788) metacercariae (p = 0.014/0.019) and *E. aerophilus* (p = 0.030/0.011) in *M. putorius* was higher (p<0.05) than in *N. vison* from wetlands. The abundance of *I. melis* in *M. putorius* was also higher than in *N. vison* (p = 0.010) from wetlands. *N. vison* from different habitats shared 8 helminth species, but differences in the abundance and prevalence of all helminth species were insignificant at p>0.05. The abundance and prevalence of *Pseudamphistomum truncatum* (Rudolphi, 1819) in *L. lutra* from water bodies was higher that in *N. vison* (p = 0.000/0.002). *A. alata* mesocercariae was detected only in mustelids collected from wetlands. All three mustelids shared *I. melis* and *P. truncatum*.

**Discussion**

Of 14 helminths found and discussed in the present study, most are reported in at least one of three species of mustelids in other countries (Table 2), with the exception of *Phyllodistomum folium* (Olfers, 1816) and *Crenosoma schachmatovae* Kontrimavičius, 1969.

*P. folium* was collected from *L. lutra* stomach. *P. folium* is a characteristic parasite of the northern pike *Esox lucius* Linnaeus, 1758, yet it is found in other fish as well (Bykhoverkaya-Pavlovskaya & Kulakova, 1987). The occurrence of *P. folium* in *L. lutra* results from feeding on definitive host-fishes of the fluke. *C. schachmatovae* have been described in stot (Mustela emine Linnaeus, 1758) from Karelia, Russia (Kontrimavičius, 1969). In Lithuania, *C. schachmatovae* have been reported in *N. vison*, *M. putorius*, and stone marten (*Martes foina* (Erxleben, 1777)) (Nugaraitė et al., 2014).

Cestodes of the genus *Mesocestoides* were not determined to the species level due to the poor preservation status of the specimens, however most measurements coincided with those of *Mesocestoides lineatus* (Goeze, 1782). This tapeworm is documented in other countries in *M. putorius*, *N. vison*, and *L. lutra* (Table 2).

Habitat-related differences were found in the abundance and prevalence of *E. aerophilus* in *M. putorius*. *E. aerophilus* has both a direct (by ingestion of larvated eggs) and indirect (by ingestion of oligochaetes containing larvae) life cycle (Anderson, 2000). The diet of *M. putorius* has not been studied in Lithuania, however according to other authors *M. putorius* does not feed on earthworms (Hammershøj et al., 2004; Malecha & Antczak, 2013). Therefore, the direct life cycle is likely the main mechanism of transmission of *E. aerophilus* to the host. Conditions for the survival of eggs are better in wetlands, consequently, wetlands offer a better environment for the life cycle realization.

In wetlands *M. putorius* and *N. vison* shared 9 helminths and, compared to *N. vison*, *M. putorius* was more parasitized by *I. melis*, *S. strigis* metacercariae, and *E. aerophilus*. Sharing the dens between *N. vison* and *M. putorius* may increase the risk of infection with the same species of helminths. Both mustelids do not have specialized diets, are generalist predators and can easily exploit different food resources (Bartoszewicz & Zalewski, 2003; Malecha & Antczak, 2013). According to Lodé (1993a) in wetlands *N. vison* mainly preys on fish and birds, whereas *M. putorius* consumes more rodents and amphibians. Amphibians, as an important component of the diet of *M. putorius*, were also suggested by other authors (Jędrezejewski et al., 1993; Hammershøj et al., 2004). So, the difference in diet could be a reason why *M. putorius* were more parasitized with helminths in whose life cycle amphibians play a role (i.e., *I. melis*, *A. alata*, and *S. strigis*). Transmission of A.
alata and S. strigis usually occurs through amphibians (Kontrimavičius, 1969; Shultz & Gvozdev, 1972), while I. melis is transmitted through both amphibians and freshwater fishes (Dönges, 1964; Radev et al., 2009). Reptiles, birds and small mammals can serve as paratenic hosts for A. alata (Kontrimavičius, 1969). For these two species, mustelids are paratenic hosts (Kontrimavičius, 1969) and are likely infected when they eat second intermediate hosts or, for A. alata, second intermediate or paratenic hosts. Differences in the abundance and prevalence of P. truncatum among N. vison and L. lutra in water bodies have been observed. The life cycle of P. truncatum include two intermediate hosts; the first intermediate host is freshwater Bithynia snails and second is the Cyprinidae fish species (Hawkins et al., 2010). Fish is the major prey category in the diet of L. lutra (biomass 75.28 %) in Europe (Krawczyk et al., 2016). Despite that the diets of L. lutra and N. vison overlap (Bonesi et al., 2004) and both mustelids feed on intermediate hosts of P. truncatum, L. lutra is likely to consume more fishes than N. vison.

M. putorius and N. vison from wetlands harboured the richest helminth communities compared with other habitats. Such differences may be related to the differences in the composition and abundance of intermediate hosts among different habitats and the conditions for surviving of free-living stages of parasites which are better in wet environment.

In the present study I. melis and P. truncatum were detected in all three mustelid species. Detection of I. melis and P. truncatum in all mustelids is closely associated with their living environment and diet. Introduced N. vison and native L. lutra are semi aquatic
| Helminths      | Host                  | Country / Source                                                                 |
|---------------|-----------------------|----------------------------------------------------------------------------------|
| I. melis      | LL Germany (Schuster et al., 1988), Belarus (Sidorovich & Anisimova, 1999; Shimalov et al., 2000) |
|               | NV Germany (Zschille et al., 2004); France (Torres et al., 2008); Belarus (Shimalov & Shimalov, 2001); Caucasus (Itin & Kravchenko, 2016) |
|               | MP Germany (Kontrimavičius, 1969); Bulgaria (Kostadinova & Gibson, 2002), Hungary (Sugár & Matsušáfi, 1978), Poland (Soltys, 1962; Malczewski, 1964; Kontrimavičius, 1969); Belarus (Shimalov & Shimalov, 2002); Russia (Morozov et al., 1939; Kontrimavičius, 1969); Lithuania (Maldzūnaitė, 1959; Kazlauskas & Prūsaitė, 1976); Former Czechoslovakia (Kontrimavičius, 1969; Mituch, 1972) |
| S. strigis     | MP Belarus (Shimalov & Shimalov, 2002) |
| P. truncatum   | LL United Kingdom (Simpson et al., 2005; Sherrard-Smith et al., 2015b, 2016); Poland (Hildebrand et al., 2011); Belarus (Sidorovich et al., 1997; Shimalov et al., 2000); Germany (Schuster et al., 1988); Ukraine (Korol et al., 2016); Denmark; France; Germany; Sweden (Sherrard-Smith et al., 2016); Ireland (Hawkins et al., 2010) |
|               | NV England and Wales (Sherrard-Smith et al., 2015a, 2016); Ireland (Hawkins et al., 2010); Denmark (Skov et al., 2008); Volga Delta (Ivanov and Semenova, 2000); Caucasus (Itin & Kravchenko, 2016); Belarus (Sidorovich & Anisimova, 1997; Shimalov & Shimalov, 2001) |
|               | MP Belarus (Anisimova, 2002; Shimalov & Shimalov, 2002); Russia (Morozov et al., 1939; Kontrimavičius, 1969) |
| A. alata       | LL Poland (Górski et al., 2010); Belarus (Sidorovich et al., 1997; Shimalov et al., 2000) |
| mesocerciae    | NV Germany (Zschille et al., 2004); Caucasus (Itin & Kravchenko, 2016); Belarus (Shimalov & Shimalov, 2001); Volga Delta (Ivanov and Semenova, 2000) |
|               | MP Germany, Italy (Kontrimavičius, 1969); Belarus (Shimalov and Shimalov, 2002); Russia (Kontrimavičius, 1969; Kruchkova et al., 2008); Lithuania (Grikinienė et al., 2001) |
| O. felineus    | LL Germany (Schuster et al., 1988); Belarus (Shimalov et al., 2000) |
|               | NV Belarus (Shimalov & Shimalov, 2001) |
|               | MP Belarus (Shimalov & Shimalov, 2002) |
| M. skrijabinii | MP Gorky Oblast, Russia (Morozov, 1939) |
| M. lineatus    | LL Belarus (Shimalov et al., 2000) |
|               | NV Germany (Zschille et al., 2004); Caucasus (Itin & Kravchenko, 2016) |
|               | MP Belarus (Anisimova, 2002; Shimalov & Shimalov, 2002); Former Czechoslovakia (Mlčuch, 1972) |
| T. martis      | LL Germany (Schuster et al., 1988) |
|               | NV Iberian Peninsula (Torres et al., 2006); Germany (Zschille et al., 2004) |
|               | MP Belarus (Shimalov & Shimalov, 2002); Germany (Kontrimavičius, 1969); Former Czechoslovakia (Mlčuch, 1972) |
| A. putorii     | LL Poland (Górski et al., 2010); France, Spain, Portugal (Torres et al., 2004); Belarus (Sidorovich et al., 1997; Shimalov et al., 2000); Latvia (Vismanis & Ozolins, 1998) |
|               | NV France (Torres et al., 2008); Iberian Peninsula (Torres et al., 2006); Caucasus (Itin & Kravchenko, 2016); Belarus (Shimalov & Shimalov, 2001); Spain (Martínez-Rondán et al., 2017) |
|               | MP Belarus (Anisimova, 2002; Shimalov & Shimalov, 2002); Russia (Morozov, 1939); Poland (Górski et al., 2006); Iberian Peninsula (Torres et al., 1996); France (Torres et al., 2008); Belgium (Bernard, 1969); Former Czechoslovakia (Mlčuch, 1972) |
| E. aerophilus  | MP Russia (Morozov, 1939; Kontrimavičius, 1969; Kruchkova et al., 2008); Belarus (Shimalov & Shimalov, 2002); France (Torres et al., 2008) |
| M. patens     | NV Germany (Zschille et al., 2004); Iberian Peninsula (Miquel et al., 1993-1994; Torres et al., 2006); France (Torres et al., 2008); Belarus (Shimalov & Shimalov, 2001); Spain (Martínez-Rondán et al., 2017) |
|               | MP Belarus (Shimalov & Shimalov, 2002); Russia (Morozov, 1939; Kontrimavičius, 1969); Iberian Peninsula (Torres et al., 1996); France (Durette-Desset & Pesson, 1987; Torres et al., 2008); Belgium (Bernard, 1969); Switzerland (Memod et al., 1983) |
mustelids which coexist in many riparian habitats. Moreover, some studies suggest *M. putorius* preference for the aquatic environment (Jędrzejewski et al., 1993; Zabala et al., 2005; Rondinini et al., 2006). Diets of these mustelid species overlap and include intermediate hosts of these two flukes. *L. lutra* diet consists mainly of aquatic prey, *N. vison* and *M. putorius* exploit both aquatic and terrestrial prey (Bonesi et al., 2004).

In general, the helminths community of *M. putorius* was richer (12 helminths) compared with *N. vison* (10 helminths), and *L. lutra* (4 helminths). The helminth fauna of *L. lutra* in Europe is probably richer, however results in the present study are derived from small number of animals examined. It is related with *L. lutra* protection status in Europe. Despite the fact that its population is widely distributed across Lithuania (covers 95% of the territory) (Baltrūnaitė et al., 2009), hunting has been prohibited since 1975 (Micievičius, 1993; Baranauskas et al., 1994).

The richer helminth fauna of *M. putorius* is probably related with a wide variety of habitats used by this species.

Introduction of *N. vison* in Lithuania occurred after World War II as the result of releases from fur farms in Kaliningrad Oblast, Russia and Lithuania and introduction of animals from Tatarstan (Russia) (Prūsaitė et al., 1988; Balčiauskas, 1996). The invasion of *N. vison* in new regions may have led to the introduction of new parasites and their transfer to endemic hosts. Helminths of *N. vison* in North America have been reported by numerous authors (e.g., Beaver, 1941; Zabiega, 1996; Foster et al., 2007). A checklist of helminths in *N. vison* from Montana was reported by Barber and Lockard (1973). Some helminth species (i.e., *Metorchis albidos* (Braun, 1893), *Aonchotheca mustelorum* (Cameron & Parnell, 1933) (syn. *Capillaria mustelorum*), and *Baylisascaris devosi* (Sprent, 1952) (syn. *Ascaris devosi*) detected in *N. vison* from Belarus are supposed as species arrived with this animal from its native area, i.e. North America (Anisimova, 2004).

All helminths detected in *N. vison* in our study are native European parasites, which are also common parasites of a wide range of European mustelids and other mammals. All helminth species found in *N. vison* are also found in *M. putorius*. Helminthological studies in other European countries show similar results that *N. vison* is parasitized by native parasites, e.g. in Spain (Torres et al., 2006; Martínez-Rondán et al., 2017) and France (Torres et al., 2008). Invasive *N. vison* could lose their original parasites, because feral populations come from animals raised in fur farms, where parasite cycling is aggravated. Invasive species are likely to accumulate parasites in the environments inhabited by closely related species (Parker & Gilbert, 2007). In our case infection of invasive *N. vison* with native parasites can be facilitated by contacts with taxonomically closely related native mustelids (e.g. *M. putorius*, etc.) and parasites being widespread generalist with a wide host range. It is worth mentioning that the ranges of some parasite species found in our study include invasive and natural range of *N. vison* (i.e., Palearctic and Nearctic). Helminths found by us are known in *N. vison* from North America: *I. melis* (Beaver, 1941), *A. putorii* (Zabiega, 1996; Foster et al., 2007), and *M. patens* (Foster et al., 2007).

From all above we can conclude that all three studied mustelids exchange helminths and have common species. Helminths community structure is influenced by habitat. Our results show that the epidemiological role of invasive *N. vison* is in the maintenance of the life cycles of native parasites.

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Conflict of Interest

Authors state no conflict of interest.

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