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Cranial lesions caused by helminth parasites and morphological traits in the European polecat *Mustela putorius*

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Summary

We investigated the occurrence of cranial lesions caused by helminth parasites in the European polecat *Mustela putorius* in a museum collection in Slovakia. Selected traits of polecat body morphology and condition were studied with respect to the prevalence of the parasites, and number and extent of helminth-caused lesions in crania of 183 adult polecats. The trematode *Troglotrema acutum* was identified as the parasitic agent (prevalence 62.6 %), with a strong relationship between number of lesions and extent of cranial damage (surface of cranium). Prevalence of infection did not differ significantly between sexes (68.9 % in females, 51.1 % in males). Females with lesions by *Troglotrema acutum* have significantly greater braincase breadth than uninfected ones. Among males, infected individuals were generally significantly heavier and larger than uninfected individuals. Therefore, contrary to assumptions, it seems that parasites did not influence significantly body measurements of the animals, and differences are probably rather a result of an intra-sample age variation among infected polecats (older individuals are bigger, more probably infected and damage is more pronounced).

Keywords: age effect; cranial damage; prevalence; sex differences; Slovakia; trematode; *Troglotrema acutum*

Introduction

Parasite loads can correlate negatively with life history traits in mammals. A negative relationship (i.e. parasite presence associated with reduced reproductive success – "*parasite influence hypothesis*") implies that growth and reproduction of an animal may be limited by parasitic infection (Goater & Holmes, 1997; Møller, 1997; Santi *et al.*, 2006). Potential examples of parasites exerting great influence on the life history and morphology of their mammalian hosts are nematodes and trematodes found in paranasal sinuses of mustelids, especially several nematode species from the genus *Skrjabingylus* and the trematode *Troglotrema acutum*. Their presence causes damage to the cranium, including the braincase, and therefore affects the intellectual and locomotory abilities of infected animals (Vogel & Voelker, 1978; Koubek *et al.*, 2004; Kierdorf *et al.*, 2006). However, it is presently not clear how serious is the influence of these cranial parasites on the body condition, development, and morphological parameters of the host. Furthermore, it is unknown whether there exists a difference in parasite load between sexes. For instance, in the weasel *Mustela nivalis*, King (1977) and Schmidt (1993) did not find significant differences in morphological traits between individuals parasitized by nematodes and/or trematodes and non-parasitized animals. On the other hand, in American mink *Mustela vison* Santi *et al.* (2006) found significant differences in body condition index between individuals infected by *Skrjabingylus nasicola* and non-infected animals. However, the parasite load effect was no longer significant after controlling for the effect of an age. Bowman and Tamlin (2007) observed reduced braincase volume and changed skull shape of parasitized male mink compared with unparasitized ones.
Hansson (1969) suggested that sexual differences in parasite load among mustelids could occur as a result of differences in a cranial size, bone thickness and testosterone level. This view was partially confirmed by King (1991) in a study on the stoat *Mustela erminea*.
The present study investigates the relationship of morphology, body condition, and infection with cranial parasites in the European polecat, *Mustela putorius*. The species, a member of the Mustelidae family, can be found throughout most of Europe. Polecats prefer lowlands and foothills, live in farmland, dales and on wooded river banks, and in Central Europe they often occur near human settlements (Blanford, 1987). The polecat is widespread in Slovakia. Presently, the species can be hunted only during winter (Adamec, 2003), but in the past it was hunted all year

round. Because the European Polecat is hunted throughout most of its range, it is relatively easy to obtain skull material (Hansson, 1968; Anisimova, 2002; Koubek *et al.*, 2004; Kierdorf *et al.*, 2006).

In this paper, we present the results of an investigation on the occurrence of the cranial parasite *Troglotrema acutum* in the European Polecat. The main objectives of our study are:

1) to provide general data on the occurrence of the parasite based on presence of skull lesions;
2) to test the relationship between number of lesions and extent of cranial damage;
3) to test whether differences in parasite presence and cranial damage exist between male and female polecats;
4) to compare morphological traits and body condition index between infected and non-infected individuals.

Materials and methods

Study site and general description of the procedure

The polecats were collected in NE Slovakia, near the town of Bardejov (49º03´N - 49º27´N; 20º30´E – 21º47´E), in the transition region between the Eastern and Western Carpathians. The extensive farmland of the region has a mosaic structure. In the lower parts of the valleys,agricultural fields are traditionally smaller than in lowlands, with many shelter belts, and groups of bushes and trees. Forests are represented by riparian forests along creeks and rivers and continuous forests on hill tops and mountains. The total percentage of woodland in the region is up to 40 %.

A large number of hunters led by Tibor Weisz, collector of the Šarišské Museum Bardejov (SMB), collected the polecat specimens during the years 1958 – 1978. Polecats were collected during all months, with a peak between November and March. Sex was determined during dissection of gonads. Polecats were aged according to the size and shape of the baculum (Walton, 1968), and cranial traits (Buchalczyk & Ruprecht, 1977). However, because of the small sample size of juveniles, only data obtained from adults were used. Shortly after shooting, the crania were removed from the body, immersed into 5 % ammonia solution for at least 24 h to soften the flesh and then boiled, brushed clean and bleached with hydrogen peroxide. The crania were then washed in water, air-dried and kept in museum boxes.

Measurement of morphological traits

Body mass (g) and body length (mm) were measured directly after shooting on fresh polecats by the main collector T. Weisz. A body condition index was calculated according to Buskirk and Harlow (1989) as the residual value of body mass on body length ($y = 0.852x + 7.835$; $R^2 = 72.5$ %; $P < 0.001$).

The following skull measurements were taken: condylobasal length (CbL), braincase breadth (BcB), zygomatic breadth (ZyB), braincase height (BcH), postorbital breadth (PB), mandible length (MdL), height of the mandibular ramus (HRM. Measurements were made with an electronic ruler (accuracy of 0.05 mm) according to Buchalczyk & Ruprecht (1977) and DeMarinis (1995). For males also three baculum (*os penis*) measurements, viz. length, breadth, and distal width (after Walton, 1968) were taken with an electronic ruler (accuracy 0.05 mm).

Cranial lesions and the nematode identification problems

Skulls of the European Polecat stored in the museum collection were checked for the presence of cranial lesions attributable to parasites. Several parasitic species can be found in the paranasal sinuses of mustelids: the trematode *Troglotrema acutum* and nematodes of genus *Skrjabingylus* (Koubek *et al.*, 2004; Kierdorf *et al.*, 2006). However, after detailed inspection, visible damage on the skulls were judged to correspond well with earlier descriptions of the cranial lesions in the European polecat caused by *Troglotrema acutum* (Fig. 1, *cf.* details of skull lesions described and photographed by Koubek *et al.*, 2004; Kierdorf *et al.*, 2006).

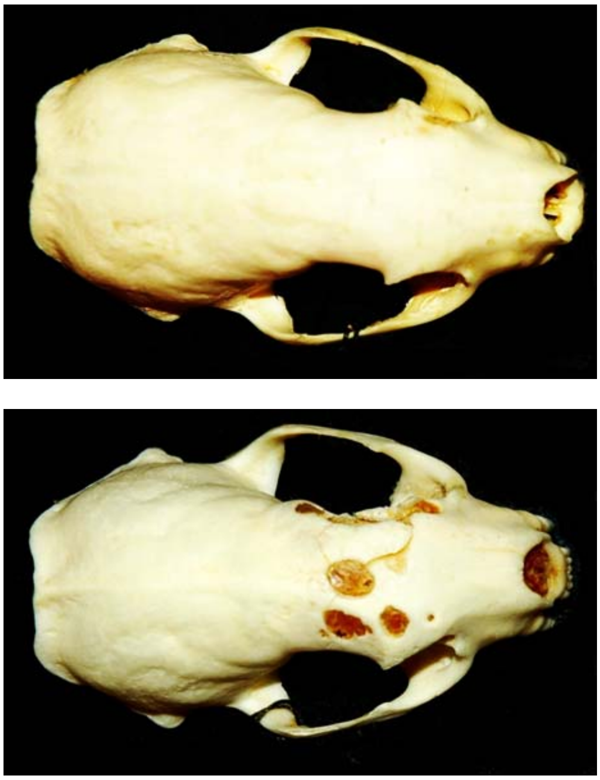

Fig. 1. Examples of the skull of the European Polecat uninfected (upper panel) and infected (lower panel) by *T. acutum*

Fifty-five randomly selected skulls (with lesions) from infected individuals were chosen to check for a potential relationship between the number of holes created by the parasite *Troglotrema acutum*, and total area of holes of parasitic origin (in mm^2). We assume that these variables can be used as a relatively valid indicator for the severity of the infestation, bearing in mind, however, that they are not direct indicators of the intensity of infection. The results of autopsies of fresh material by Artois *et al.* (1982) show that it is impossible to deduce the infection intensity from the number of perforations (Koubek *et al.*, 2004).

Skulls were photographed with a digital camera (Nikon 200) from different sides and measurements were performed on the images using Image Tool 3.00 software.

Statistical analyses

Statistics were performed using SPSS for Windows and tests are two-tailed. Bonferroni corrections were applied to adjust the *p* values for the increased probability of obtaining statistical significance from multiple tests. 95 % confidential limits were computed additionally for binary data (proportion of parasitized males and females). All other data are presented as means ± SE. Data from all study years were pooled for analyses because of small sample sizes. In total 182 individuals of the European Polecat were measured, but due to various reasons (e.g. partially damaged bones) not all measurements were obtained from all of them. Therefore, sample sizes in particular analysis can slightly vary.

Results

Cranial parasite prevalence and extension of cranial lesions

In the skulls of 182 adult European Polecats, lesions caused by *Troglotrema acutum* were detected in 114 (62.6 %) cases. Number of perforations ranged from 1 to 24 per skull (mean ± SE = 5.7 ± 0.7, and lesions extension from 1.4 to 229.0 mm^3 (mean ± SE = 39.0 ± 7.0) of the cranium. Significant correlation between number of perforations and damaged area was found (r_s = 0.803, n = 55, P < 0.001, Fig. 2).

Table 1. Mean (± SE) morphometric traits of the European Polecat in relation to parasitation by *Troglotrema acutum*. Sample size in parenthesis.
t = test value of *t*-test, U = test value of Mann-Whitney *U*-test

	Uninfected	Infected	Test
	Females		
Body mass (g)	556.8 ± 19.0 (18)	558.5 ± 13.5 (41)	*t* = -0.71, *P* = 0.94
Body length (mm)	339.1 ± 4.3 (18)	345.6 ± 2.8 (42)	*t* = -1.26, *P* = 0.21
Body condition index	-36.4±24.8 (17)	-81.9±24.1(41)	*U* = 289, *P* = 0.31
CbL (mm)	57.76 ± 0.51 (17)	58.72 ± 0.34 (37)	*t* = -1.57, *P* = 0.12
BcB (mm)	26.11 ± 0.40 (17)	27.03 ± 0.14 (34)	*t* = -2.70, *P* = 0.009
ZyB (mm)	34.37 ± 0.35 (17)	33.97 ± 0.36 (33)	*t* = 0.71, *P* = 0.48
BcH (mm)	21.62 ± 0.43 (16)	22.54 ± 0.32(37)	*t* = -1.65, *P* = 0.10
PB (mm)	15.82 ± 0.39 (18)	15.86 ± 0.12 (36)	*t* = -0.12, *P* = 0.90
MdL (mm)	35.10 ± 0.32 (19)	35.46 ± 0.26 (42)	*t* = -0.81, *P* = 0.42
HRm (mm)	16.60 ± 0.20 (19)	16.81 ± 0.15 (42)	*t* = -0.83, *P* = 0.41
	Males		
Body mass (g)	968.4 ± 40.5 (48)	1096.7 ± 33.7 (72)	*t* = -2.42, *P* = 0.017
Body length (mm)	384.1 ± 4.6 (47)	399.2 ± 2.9 (72)	*t* = -2.94, *P* = 0.004*
Body condition index	28.14 ±25.24 (47)	36.49 ±22.39 (72)	*U* = 1556, *P* = 0.43
CbL (mm)	65.31 ± 0.57 (43)	66.11 ± 0.40 (65)	*t* = -1.20, *P* = 0.23
BcB (mm)	30.25 ± 0.21 (41)	29.94 ± 0.27 (65)	*t* = 0.80, *P* = 0.42
ZyB (mm)	39.86 ± 0.52 (36)	40.56 ± 0.38 (58)	*t* = - 1.11, *P* = 0.27
BcH (mm)	24.76 ± 0.19 (43)	24.95 ± 0.17(67)	*t* = -0.74, *P* = 0.46
PB (mm)	16.81 ± 0.14 (45)	17.42 ± 0.11 (62)	*t* = -3.4, *P* = 0.001
MdL (mm)	40.95 ± 0.40 (48)	41.25 ± 0.35 (71)	*t* = -0.56, *P* = 0.57
HRm (mm)	20.23 ± 0.23 (49)	20.50 ± 0.20 (72)	*t* = -0.86, *P* = 0.39
Baculum length (mm)	37.26 ± 0.89 (36)	39.80 ± 0.44 (57)	*t* = -2.84, *P* = 0.006
Baculum breadth (mm)	4.45 ± 0.24 (36)	4.83 ± 0.19 (57)	*t* = -1.25, *P* = 0.21
Distal size of baculum (mm)	4.93 ± 0.12 (35)	5.25 ± 0.07 (57)	*t* = -2.45, *P* = 0.016

Among 61 females 41 (68.9 %, 95 % CL: 55.7 – 80.1), and among 141 males 72 (51.1 %, 95 % CL: 42.5 – 59.6) individuals were infected, and difference between sexes was not significant (χ^2 with Yates correction = 1.14, P = 0.29). Number of lesions per skull, and perforated area on cranium also did not differ between sexes (Mann-Whitney *U*-test, P > 0.75 in both cases).

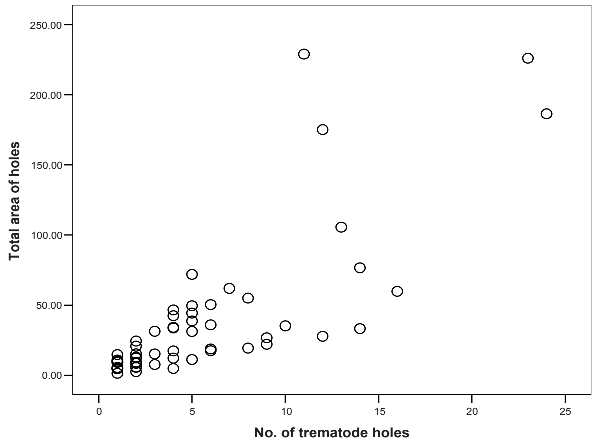

Fig. 2. Relationship between number of parasite holes and area of skull perforations caused by trematodes (mm^2)

Troglotrema acutum infection vs. morphometric traits

Among the analysed morphological traits, only braincase breadth (BcB) differed significantly between non-parasitized and parasitized females, and body mass, body length, as well as baculum length and baculum distal width differed significantly between non-parasitized and parasitized males (in all cases, differences were significant also after sequential Bonferroni correction for multiple testing; Table 1).

Discussion

In analysing museum material, the first problem connects to a diagnosis of cranial parasites based on lesions observed in dry skulls. Two sinus helminths can be found in European polecats (see section Methods): the trematode *Troglotrema acutum* and nematodes of the genus *Skrjabingylus* (Anisimova, 2002; Koubek *et al.*, 2004; Kierdorf *et al.*, 2006). Distinction between signs of these two parasites may be difficult in practice (Koubek *et al.*, 2004; Kierdorf *et al.*, 2006). However, taking into account the appearance of skull perforations, their shape, size and location in the polecat crania, it is suggested that at least the majority of the skull lesions can be ascribed to the trematode *Troglotrema acutum*.

The European polecat is known very well as the main definitive host of *Troglotrema acutum* (Koubek *et al.*, 2004; Kierdorf *et al.*, 2006). The first intermediate host of *Troglotrema acutum* is a snail of genus *Bythinella* or maybe other prosobranchs, while anurans act as second intermediate hosts (Koubek *et al.*, 2004; Kierdorf *et al.*, 2006). A polecat becomes infected when it eats a frog or toad (this prey is a rather frequently found dietary item, Anisimova, 2002; Blanford, 1987; Brzeziński & Romanowski, 1997) containing metacercariae (Vogel & Voelker, 1978; Kierdorf *et al.*, 2006).

Unfortunately, it is rather difficult to compare the prevalence of *Troglotrema acutum* infection between various studies. First of all, papers published so far based on the presence of changes in the frontals, which are part of the neurocranium of the skull (see remarks in Koubek *et al.*, 2004) did not distinguish between presence of *Troglotrema acutum* and other potential parasites. Secondly, prevalence data in other studies were not derived from the presence of skull lesions, but based on dissection (Anisimova, 2002) or the analysis of faecal samples (Górski *et al.*, 2006). Therefore, we can compare our findings only with the data obtained by Koubek *et al.* (2004) who obtained them in a way similar to the present study. Doing this, we can conclude that the polecats in Eastern Slovakia were generally more frequently infested than those from the Czech Republic. The main reason for this is probably the fact that only adult individuals of the European polecat were included into our analysis. It is well known that the probability of infection by cranial parasites increases with host age (Hansson, 1968; Addison *et al.*, 1988, King, 1991; Koubek *et al.*, 2004; Santi *et al.*, 2006). This was also supported by our results. We observed that in females, infected individuals had a greater braincase breadth than non-infected ones, and that infected males showed greater body mass and length, as well as baculum length and distal size compared with non-infected ones.

These findings seem to be in contradiction to the "*parasite influence hypothesis*"; however, a possible explanation for this seemingly contradictory observation can be the age differences between the compared groups. Older polecats are generally larger than younger ones and older males have bigger bacula (Walton, 1968; Buchalczyk & Ruprecht, 1977; DeMarinis, 1995). All variables in which we have found significant differences between parasitized and non-parasitized individuals can be attributed to the age of both males and females. During the longer lifespan, it is more probable that animals become infected by parasitic helminths, as well as that they become infected not just once but several times, thus harbouring increasing numbers of adult *T. acutum* in the paranasal sinues, what is leading to more extensive destructions of the cranium. Therefore, we assume that a potential negative influence of the cranial parasites on morphological parameters of infected polecats is masked by the age differences between the compared groups. A similar situation was noted also in study on the weasel in that no differences in morphological traits between uninfested and infested individuals were found (King, 1976). On the other hand, two other studies (King, 1991; Santi *et al.*, 2006) observed a relationship between parasite infestation and body size as well as condition. However, when the authors included also animal age into the analysis, the differences were no longer significant. More recently, Bowman and Tamlin (2007) reported that cranial nematodes caused reduced braincase volume in older male minks.

Interestingly, this study is the first to record that cranial sinus parasite occurrence is positively correlated with damage dimension in the host skull. Also, in an earlier study on the sinus nematode *Skrjabingylus nasicola*, Santi *et al.* (2006) investigated a correlation between parasite prevalence and infection intensity, but their work was based on differences between sites, not between individuals. Hence, it may be assumed that destruction of cranial bones by *Troglotrema acutum* parasites is related to the number and duration of infections of an host during its life.

Also, contrary to the assumptions we have observed no sex difference in parasite occurrence as well as number of perforations. Males and females of the European polecat differ in body size, males being significantly larger in all body measurements. However, they probably have similar foraging habits and life span and therefore, there is probably no difference in infection rates between the sexes (King, 1991; Kierdorf *et al.*, 2006).

In conclusion, our results suggest that the common polecat trematode parasite *Troglotrema acutum* does not affect measured morphological traits of the host and, moreover, there were no significant difference in parasite occurrence between male and female polecats. However, potential effects of infection can by masked by heterogeneity of the group, especially with respect to the age. Therefore further studies and especially controlled experiments are required to identify possible causal relations between infection intensity and morphological traits of infected polecats.

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