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Forest Matters Most for *Hirsutiella zachvatkini* (Schluger, 1948): A Survey of Rodent Infestation in Four Localities within the Mazury Lake District, NE Poland

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Abstract: The family Trombiculidae encompasses numerous and widespread mites, the larvae of which are obligatory parasites of vertebrates. Our research objective was to assess how trombiculid burdens on rodents inhabiting three forested and one open area in NE Poland vary in relation to host identity, sex and body size, and how they are influenced by qualities of the habitat. Trapped rodents ($n = 240$) were measured, weighted and screened for ectoparasites. *Apodemus flavicollis* ($n = 42$) and *Myodes glareolus* ($n = 173$) harbored a total of 4652 *Hirsutiella zachchvatkini* larvae. Statistical tests revealed that both prevalence and abundance were significantly higher in voles (93%, 27 larvae/ind.) than in mice (14%, 0.24 larvae/ind.) but there was no effect of host sex. Among the localities, *H. zachvatkini* was most prevalent (98%) and abundant (41 larvae/ind.) in rodents from the deciduous mixed forest in Tałty compared to fallow land (2.6%, 0.13 larvae/ind.). The highest prevalence and abundance were found in bank voles; in this host species in Tałty, prevalence reached 100% and the mean abundance was >42 larvae/ind. Significant positive correlations were found between the body mass and body length of voles and the number of attached larvae. Our results confirm the sylvan nature of *H. zachvatkini* and its preference for bank voles inhabiting deciduous forests with rich and humid undergrowth. The lack of a sex bias and the moderate correlation between mite burden and host size are in line with observations already made on other rodent-associated Trombiculidae.

Keywords: abundance; habitat preferences; host-parasite association; host sex preferences; host size preferences; prevalence; Trombiculidae

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1. Introduction

The prostigmatid family Trombiculidae (Ewing, 1949) (Actinotrichida: Parasitengona) groups together around 3013 nominal species of terrestrial mites [1]. Trombiculids, chiggers or harvest mites, represent the so-called 'protelean parasites', in which only the larva is parasitic. Larvae feed on vertebrates once in their lifetime. The host groups most heavily infested by trombiculids and associated with the largest number of species are small mammals—mainly rodents, soricomorphs and bats [2]. During the parasitic phase the host's epidermis and dermis are recurringly externally digested and subsequently ingested in liquefied form through a feeding canal (i.e., stylostome) [3]. The latter provides a route through which bacterial pathogens can exploit trombiculids as reservoir/vector hosts in facilitating their circulation in the environment [4]. Subsequent soil-associated developmental stages are calyptostatic (protonymph and tritonymph) and predatory (deutonymph and adultus). The differences between larvae and post-larval instars in their ecology are reflected in conspicuous heteromorphism [5,6].

The requirements of active-post larval forms with respect to the acquisition of food resources and the selection of reproductive sites are considered by many workers as crucial factors determining habitat selection by species, as well as the broad spectrum of hosts for larvae [2,5]. In consequence, trombiculid infestation rates are highest on those mammals which enter microhabitats suitable for adults and in which host-questing larvae may be encountered, thus enabling attachment. This view is supported by observations made in Central Europe: bank voles and mice co-occurring in the same habitat are characterized by distinct burdens of conspecific Trombiculidae [7–9] and, moreover, are subject to seasonal parasitism by larvae. A two-year study of *Hirsutiella zachvatkini* (Schluger, 1948) associated with *Apodemus* spp. mice and *Myodes* sp. voles, has revealed that infestation of the hosts begins in September. Attached larvae engorge but remain on rodents throughout the winter and gradually leave hosts in late spring. Prolonged attachment to mammals during the coldest season allows larvae to overwinter under optimal thermal conditions and results in post-larval instars emerging at a warmer time of the year, favoring foraging and reproduction of the free-living stages [9].

Other factors that influence the extent of parasitism by trombiculids on rodents are host sex and body size. Marked variation in the extent of sex-bias in trombiculid parasitism has been reported between studies. Sex bias appears to depend largely on the host and trombiculid species involved and ranges from distinct female or male bias to no significant difference between the sexes [10–15]. A more unequivocal trend pertains to the host's body size. Kuo et al. [11] found that across different rodent species, larger animals, e.g., *Bandicota indica* (Bechstein, 1800) and *Rattus losea* (Swinhoe, 1871), were more heavily infested by larvae than the smaller *Apodemus agrarius* (Pallas, 1771) individuals. Ludwig et al. [10] also reported that the infestation rate increased with the host's body size. Lv et al. [14] mentioned a moderate positive correlation between infestation by *Leptotrombidium deliense* (Walch, 1922) and the body mass and length of its hosts whereas Wulandhari et al. [15] concluded that the rodent's BMI was a significant factor affecting trombiculid abundance.

Of 19 nominal trombiculid species recorded from Poland to date [16,17], the majority have been collected from Cricetidae and Muridae (Rodentia) hosts and some have shown specific tendencies for occurrence in particular habitats [16,18]. Notably, *H. zachvatkini* has been reported recently to be the most widespread trombiculid feeding on woodland inhabiting mice and voles but this species appears to be absent on rodents inhabiting open areas such as grasslands [9, H.M. pers. obs.]. In rare cases, *H. zachvatkini* was accompanied by distinctly less numerous species: *Neotrombicula inopinata* (Oudemans, 1909)—a mite frequently occurring in the highlands, *N. vulgaris* (Schluger, 1955)—found in human-affected habitats and the rarest—*Miyatrombicula muris* (Oudemans, 1910) [18, H.M. pers. obs.].

In the current study, our principal objective was to identify the key extrinsic factors (i.e., environmental, such as characteristics of the collection site and host-associated such as species, sex and size) shaping trombiculid burdens on rodents trapped in four localities differing with regard to vegetation cover and humidity. Based on earlier studies, we predicted that habitat, host species and size would have the most profound effects on the prevalence and abundance of trombiculid infestations, but given the varying and contrasting published findings, we made no prediction about the impact of host sex.

2. Materials and Methods

2.1. Experimental Localities

Rodents were trapped from the 16th until the 23rd of September 2022 in the Mazury Lake District, north-eastern Poland, in four sites. Three woodland sites, referred to as

Urwitałt, Tałty and Pilchy, have been described previously in detail [19] (Figure 1) and are inhabited by the forest species: *Myodes glareolus* (Schreber, 1780), *Apodemus flavicollis* (Melchior, 1834) and *Apodemus sylvaticus* Linnaeus, 1758. Our study also included a fallow land locality near the field station in Urwitałt, in which the open-area species *Microtus* spp. and *A. agrarius* were trapped. [20]. The number of hosts by study site is presented in the Results section.

Figure 1. Experimental areas: (**A**,**B**)—Pilchy (trapping points), (**C**)—Tałty, (**D**,**E**)—Urwitałt forest, (**F**)—Urwitałt fallow land.

2.2. Rodents Trapping and Morphometry

The host animals comprised 240 rodents representing seven species in two families: the striped field mouse *A. agrarius* (*n* = 14; 8♀6♂), the yellow-necked mouse *A. flavicollis* (*n* = 42; 20♀22♂), the wood mouse *A. sylvaticus* (*n* = 1♀), the Eurasian harvest mouse *Micromys minutus* (Pallas, 1771) (*n* = 1♂) (Muridae), the short-tailed field vole *Microtus agrestis* (Linnaeus, 1761) (*n* = 5; 4♀1♂), the common vole *Microtus arvalis* (Pallas, 1778) (*n* = 4; 3♀1♂) and the common bank vole *M. glareolus* (*n* = 173; 76♀97♂) (Cricetidae).

Rodents were captured in live traps 25 × 10 × 10 cm, which were set along 2–3 transects (forest roads/paths) at each forest site. Each transect measured approximately 200–400 m and the traps were set 10–20 m apart. Mostly, two traps were set at each trapping point. The bait was a mixture of grains and sliced fresh fruit (apples). Traps were checked every morning (8–9 am) and evening (6–7 pm). Trapping lasted for approximately 3–4 nights at each site. Rodents were transported to Warsaw University's field station in Urwitałt and were euthanized by cervical dislocation following deep anesthesia with isoflurane (Vetpharma, Barcelona, Spain). Rodents were weighed and sexed on the basis of urogenital distance. Juvenile animals were distinguished from adults by sexual maturity and body weight/dimensions, as previously described [21]. Selected rodent body measurements were taken: body length from nose to anus, head length, head width, tail length and body weight. Blood samples from the heart, intestines, internal organs, fur, tail and ears were collected from all the necropsied animals and will constitute material for subsequent publications.

2.3. Ectoparasites Collection and Identification

Rodents were carefully examined for the presence of ectoparasites. Particular attention was paid to the ears, the area around the eyes and nose, the underside of the muzzle and the paws of the rodents. Ectoparasites (ticks, fleas, mites) were removed using forceps and preserved in 70% methanol. All ticks from a given individual (regardless of species or life stage) were collected into labelled Eppendorf-type tubes. Fleas and mites were kept together, except for mites of the family Trombiculidae, which were collected into separate tubes. The collected specimens were kept at a temperature of +4 °C.

Alcohol-preserved trombiculid larvae were subsequently examined under a stereoscopic microscope (Delta Optical SZ-450T, Poland) with 30× magnification allowing for preliminary separation of mite genera and/or morphotypes. Sub-samples of larvae ($n = 100$), representing different host individuals, sexes and localities were mounted on microscopic slides in Heinze's medium for the purpose of species determination. Special vigilance was given to morphotypes distinguished by legs and idiosoma proportions, as well as by different body setation and coloration. Species recognition was performed under a light microscope (Olympus BX51) paired with a digital camera (ColorView) and Cell D software, based on keys by Kudryashova and Stekolnikov [22,23]. Species determination in the present work was based solely on morphological criteria because a detailed molecular analysis of the mites will be the subject of a separate investigation.

2.4. Parasitological Indices and Statistical Evaluation

The following parasitological indices were analyzed in order to assess trombiculid infestation on rodents from the study sites:

- prevalence—percentage of infested rodents among all screened individuals;
- mean abundance (or relative density of parasites)—mean number of parasites per host, including uninfected animals [24];
- infestation range—minimal and maximal number of parasites infesting a given species.

For the analysis of prevalence, we applied maximum likelihood techniques based on log linear analysis of contingency tables in the PS IMAGO PRO 8 (SPSS v. 28) software package (IBM Corporation). Host species (six species, excluding one individual of *M. minutus*), site of host origin (four sites) and host sex (males and females), were used as the factors in the models, with the presence or absence of trombiculids considered as a binary factor (0, 1). For each level of analysis in turn, beginning with the most complex model, involving all possible main effects and interactions, those combinations that did not contribute significantly to explaining variation in the data were eliminated by a backward selection procedure, beginning with the highest-level interaction [25,26]. A minimum sufficient model (MSM) was then obtained, for which the likelihood ratio of chi-square was not significant, indicating that the model was sufficient in explaining the data. The importance of each term in interactions involving prevalence in the final model was assessed by the probability that its exclusion would affect the model significantly and these values are given in the text. Two models were fitted and tested: first a model for six host species ($n = 239$; excluding a single individual of *M. minutus*) and four sites (three forest sites and one fallow land) and then a model restricted to bank voles *M. glareolus* ($n = 169$) from the three forest sites because the prevalence of infestation was <5% in rodents trapped in fallow land.

To test the hypothesis that trombiculid burdens on hosts increase with increasing size of hosts, we first fitted a GLM that included host size (either weight, or host body length reflected in nose to anal length), and then site (3 levels), sex (2 levels) and interactions between site x size and sex x size. These models were then simplified by a backward selection procedure to generate minimum sufficient models, and the gradients (*ß*) were tested for significant departure from zero. We also provide values for partial eta squared (η^2), indicating the proportion of variance explained (effect size) by relevant factors. Because of the very low number of trombiculids collected from yellow-necked mice ($n = 14$), the low abundance of infestation in this host species (<0.5 larvae/individual) and the very low burdens on the four bank voles trapped on the fallow land, this analysis was carried out on the subgroup of bank voles from the three forest sites ($n = 169$).

3. Results

All the collected trombiculids (including individuals represented by the most prevalent large, yellowish morphotype, as well as larvae that are distinct by their darker coloration and/or smaller body size) were identified as *Hirsutiella zachvatkini* (Figure 2). The study sites are new localities for this species.

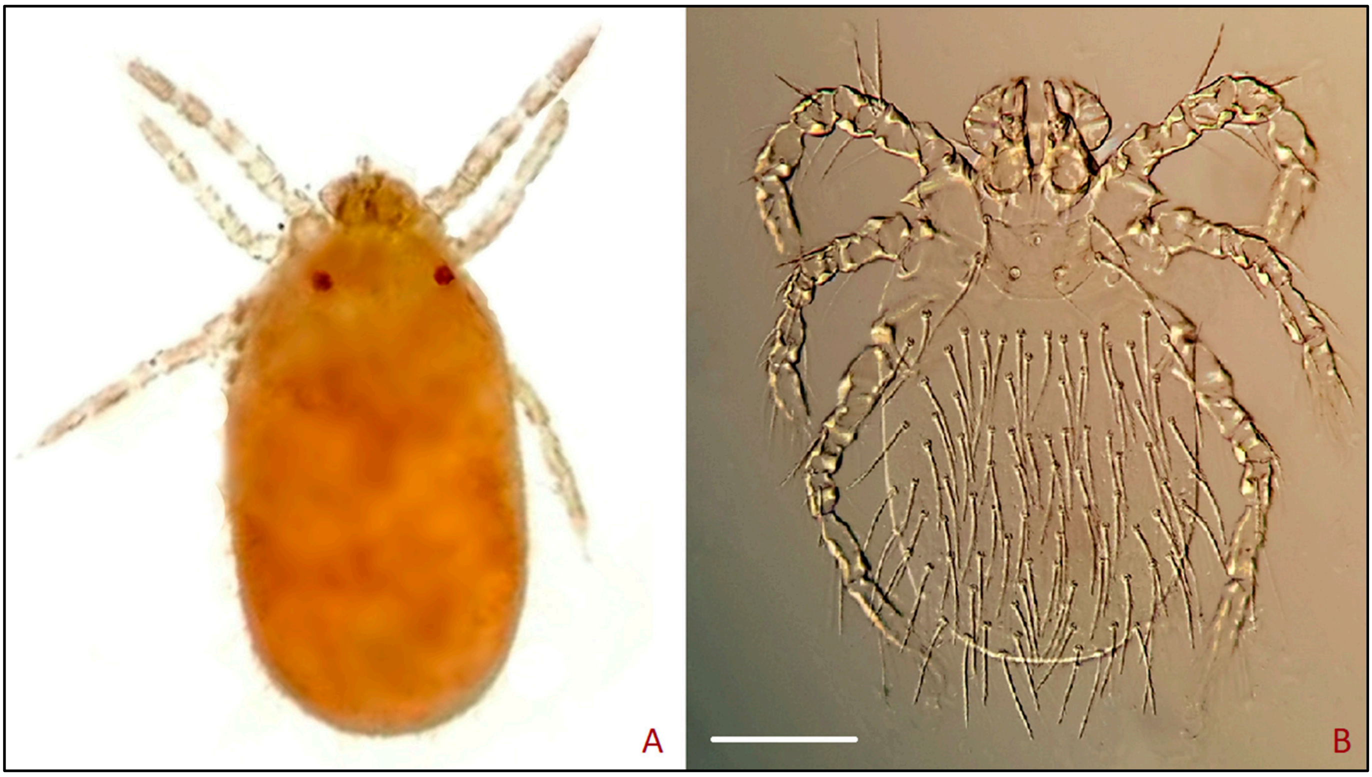

Figure 2. *Hirsutiella zachvatkini*: (**A**)—newly mounted individual with natural body coloring and red ocelli, (**B**)—larval exoskeleton. Scale bar: 100 µm.

The overall prevalence of *H. zachvatkini* in the rodent community was 69.2%. Prevalence and abundance of infestation by host species and trapping site, are presented in Table 1. Bank voles constituted the majority of examined hosts (173/240 = 72.1%) and harbored the majority of collected larvae ($n = 4638$; infestation range: 1–100). Of the seven host species, only *M. glareolus* and *A. flavicollis* individuals were infested with Trombiculidae. Thus, the prevalence of infestation differed significantly between host species (host species x prevalence: $\chi^2_5 = 62.3$, $p < 0.001$) and was more than six times higher in bank voles compared with yellow-necked mice (Table 1).

Table 1. Prevalence and abundance of chigger larvae by site and host species; P—prevalence, A ± SE—mean abundance ± standard error, nd—not done: rodents examined alive, parasites not collected.

Host Species	Urwitałt Forest		Tałty Forest		Pilchy Forest		Urwitałt Fallow Land		Total	
	P	A ± SE	P	A ± SE	P	A ± SE	P	A ± SE	P	A ± SE
M. glareolus	59/61 96.7%	25.6 ± 2.57	61/61 100%	42.6 ± 2.93	39/47 83.0%	10.2 ± 1.58	1/4 25%	1.3 ± 1.25	160/173 92.5%	26.8 ± 1.76
A. flavicollis	5/25 20.0%	0.32 ± 0.17	1/2 50%	1.0 ± 1.0	0/30%	0	0/12 0%	0	6/42 14.3%	0.24 ± 0.11
A. sylvaticus	0/1 0%	0	nd	nd	nd	nd	nd	nd	0/1 0%	0
A. agrarius	nd	nd	nd	nd	nd	nd	0/14 0%	0	0/14 0%	0
M. agrestis	nd	nd	nd	nd	nd	nd	0/5 0%	0	0/5 0%	0
M. arvalis	nd	nd	nd	nd	nd	nd	0/4 0%	0	0/4 0%	0
total by site	**64/87 73.6%**	**18.0 ± 2.19**	**62/63 98.4%**	**41.3 ± 2.98**	**39/50 78.0%**	**9.6 ± 1.52**	**1/39 2.6%**	**0.13 ± 0.13**	**166/239 69.5%**	**19.5 ± 1.48**
M. minutus									**166/240 69.2% ***	**19.46 ± 1.498**

* including a single uninfested *Micromys minutus* from the fallow land.

Prevalence of infestation was marginally higher in males than in females (74.0% vs. 64.3%), but this difference was not quite significant (host sex x prevalence: $\chi^2_1 = 2.65$, $p = 0.103$).

The trapping site was the most significant factor affecting the prevalence of trombiculids (site x prevalence: $\chi^2_3 = 33.0$, $p < 0.001$) (Figure 3). The prevalence of infestation was generally high in the three forest sites (74–98%) and very low in rodents from the fallow land (Figure 3A).

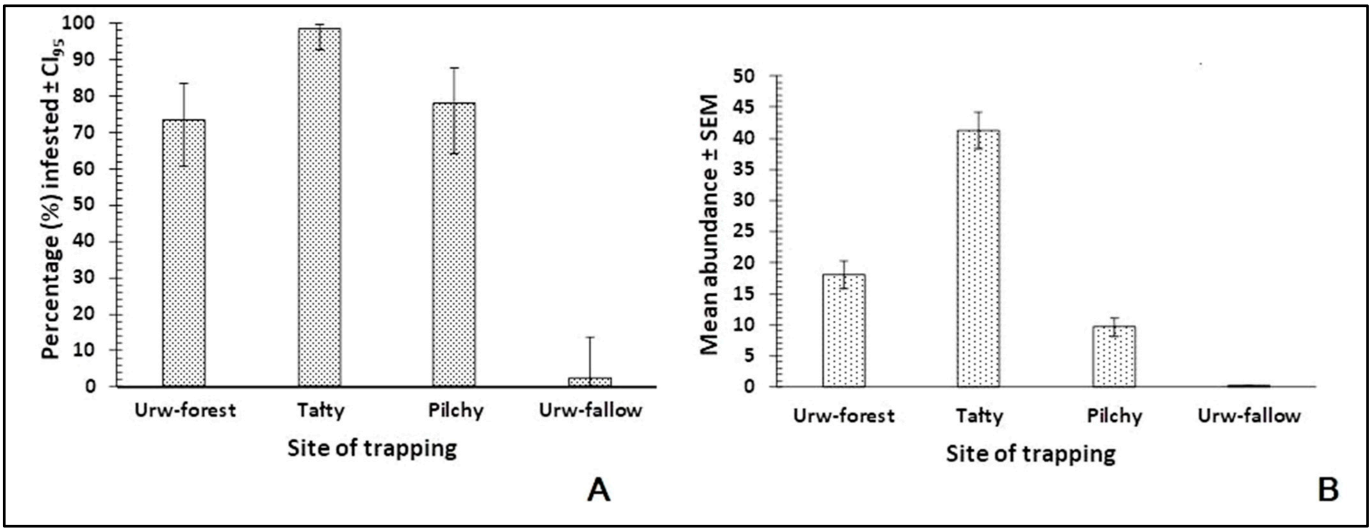

Figure 3. Differences in indices of infestation between localities on all rodents (species combined): (**A**)—prevalence; (**B**)—abundance by site; Urw—Urwitałt.

The highest prevalence was recorded in rodents from Tałty (Table 1) with all bank voles from this site being infested by Trombiculidae larvae (prevalence 100%). Mites were also found on *A. flavicollis* from the Tałty and Urwitałt forest sites (infestation range: 1–4) but were not encountered in mice from the Pilchy forest site or in those from the Urwitałt fallow land (Table 1).

The two infested rodent species harbored a total of 4652 trombiculid larvae, parasitopes of which were the auricles and external ear canals. The abundance of *H. zachvatkini* also differed significantly between host species (main effect of host species on abundance: $F_{5,\,238} = 3.55$, $p = 0.004$). Mean abundance was almost 100× higher in bank voles than in yellow-necked mice (Table 1) but was similar in male and female rodents (21.4 ± 2.13 and 17.3 ± 2.05 mites/individual, respectively).

There were significant differences in the mean abundance between the sites (main effect of site on abundance: $F_{3,\,238} = 3.29$, $p = 0.022$) (Figure 3). Mean abundance was highest in rodents from Tałty and lowest in rodents from the fallow land near the Urwitałt field station (Figure 3B). The highest mean abundance was noted for bank voles from the Tałty forest site (above 42 mites/individual) (Table 1).

The statistical analysis of abundance of infestation was next repeated but confined to bank voles from the three forest sites ($n = 169$). This confirmed the significant effect of the trapping site ($F_{2,\,166} = 38.3$, $p < 0.001$, $\eta^2 = 0.316$) and the lack of difference in abundance between the sexes ($F_{2,\,165} = 0.41$, $p = 0.52$, $\eta^2 = 0.002$).

We next tested the hypothesis that larger animals should harbour heavier burdens of mite infestation [27,28]. For this, we used two proxies of body size (host weight and host body length from the nose to the anus), and the analysis was confined to bank voles from the three forest sites because this species accounted for most of the sampled rodents and the four bank voles trapped on the grassland sites harboured very low mite burdens (mean = 1.25 mites). Because mammals often show sexual dimorphism in body size, we first tested for differences in weight and body length between the sexes. There was no significant difference in weight (males = 15.8 ± 0.30 g, females = 16.4 ± 0.42 g, $F_{1,\,167} = 1.29$, $p = 0.26$) but females were marginally longer than males (8.7 ± 0.09 cm and 8.5 ± 0.06 cm, respectively, $F_{1,\,166} = 4.14$, $p = 0.044$).

In a full factorial GLM, that included site, sex (categorical factors) and weight (covariate) as explanatory factors (adjusted $R^2 = 0.32$) the interaction of weight with site was not significant ($F_{1,\,161} = 0.27$, $p = 0.77$). Without this interaction, and with site ($F_{2,\,163} = 34.20$, $p < 0.001$, $\eta^2 = 0.296$) taken into account, neither the interaction of weight with sex ($F_{1,\,163} = 0.01$, $p = 0.93$) nor the main effect of sex ($F_{1,\,163} = 0.01$, $p = 0.93$) were significant. However, trombiculid burdens were significantly associated with host weight ($F_{1,\,163} = 7.37$, $p = 0.007$). The relationship between trombiculid burden and host weight was positive (Figure 4A; MSM comprising only site and weight, $ß = 1.209 \pm 0.455$, $t = 2.66$, $p = 0.009$) and accounted for 4.1% of variance ($\eta^2 = 0.041$).

A similar model, but with host body length rather than weight ($R^2 = 0.318$), also indicated that neither the interaction between sex and body length ($F_{1,\,162} = 0.358$, $p = 0.55$), nor the main effect of sex ($F_{1,\,162} = 0.463$, $p = 0.497$) were significant. With site ($F_{2,\,162} = 36.56$, $p < 0.001$, $\eta^2 = 0.311$), sex, and the interaction taken into account, trombiculid burdens were significantly associated with host body length ($F_{1,\,162} = 4.937$, $p = 0.028$), increasing with increasing body length (Figure 4B; MSM comprising only site and body length, $ß = 4.754 \pm 2.192$, $t = 2.168$, $p = 0.032$), but accounted for only 2.8% of variance ($\eta^2 = 0.028$).

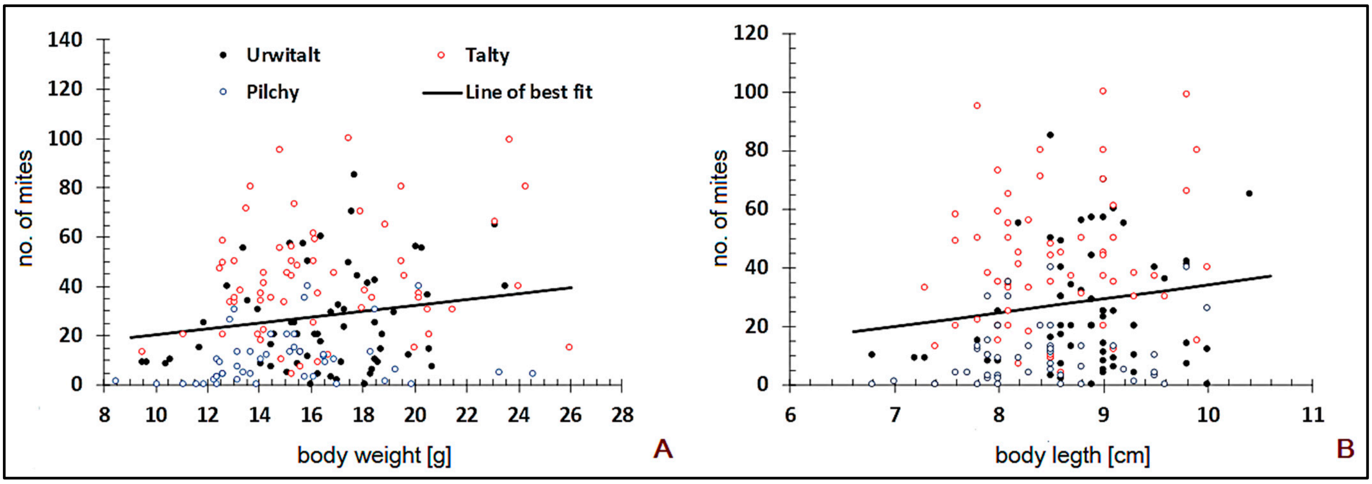

Figure 4. Relationship between trombiculid abundance and host body weight (**A**) and body length (**B**). Actual raw values are shown for bank voles from each of the three sites, and the lines of best fit are from MSM GLMs, with weight (y = 1.209x + 8.049) and body length (y = 4.754x − 13.454) as covariates and adjusted for the significant site differences in trombiculid abundance.

4. Discussion

In the full dataset, we found significant influences of host species and body size along with type of habitat on the prevalence and abundance of *H. zachvatkini* larvae in the rodents from our study sites. The great majority of collected trombiculid larvae originated from the bank voles trapped in forest sites, in comparison to low or zero prevalence/abundance in other rodent species, and especially in individuals trapped in the open habitat (fallow land), including bank voles trapped in the latter site.

Trombiculid abundance did not differ significantly between males and females, although prevalence was marginally higher in males compared to females. Similarly, no sex biases were reported in *Leptotrombidium imphalum* Vercammen-Grandjean and Langston, 1976 parasitizing *R. losea* and *A. agrarius* in abandoned agricultural fields in Taiwan. However, significant male bias was observed in trombiculids (mainly *Leptotrombidium* spp.) associated with Yunnan red-back voles, *Eothenomys miletus* (Thomas, 1914), whereas female bias was recorded in the case of Southeast Asian house rats, *Rattus brunneusculus* (Hodgson, 1845), infested by *L. deliense*, both in China [12,13]. Clearly sexual dimorphism in trombiculid infestation varies extensively between studies and host species and therefore appears to be very much context-dependent.

Controlling for between-site differences, our study has provided strong evidence that trombiculid intensity increases with increasing body size in bank voles. This is consistent with earlier reports of heavier infestations of ectoparasites in larger animals [27,28]. A positive correlation with host body size, despite no significant differences in severity of infestation between sexes, has been reported for the North American chigger—*Eutrombicula alfreddugesi* (Oudemans, 1910) parasitizing hispid cotton rats, *Sigmodon hispidus* Say and Ord, 1825, in the Piedmont habitats of Georgia, USA, and in *L. delinse* collected from Asian house rats *Rattus tanezumi* Temminck, 1844 in China [10,14]. *Leptotrombidium imphalum* larvae also preferred bigger hosts, although in this case the comparison was made across different rodent species of different body sizes [11]. The latter, however, is not supported either in the present work, or by previous observations made in SW Poland [9]. The positive relationship between trombiculid abundance and host body size in our study cannot be attributed to a sexual dimorphism in host size and mite infestation (often in mammals, males are larger) because in our study there was no difference in infestation between the sexes and no significant difference between the weights of male and female voles. Indeed, females were significantly, although only marginally, longer than males. We offer the following possible explanations: larger animals will expose a larger surface for questing stages to adhere to and are likely to be older animals and hence have had a longer period of exposure to parasites. They might also roam further (covering larger areas and penetrate more niches), in search for food (due to higher energy demand) or mating partners, and thus have a higher contact rate with trombiculids in the vegetation and litter. In each case, these characteristics are likely to result in heavier infestations on larger animals.

In the present study, bank voles have clearly been found to be the preferred host for *H. zachvatkini*. This finding is consistent with that of a two-year survey carried out in SW Poland, in a habitat similar to the Tałty deciduous forest but characterised by older stands of trees, which also revealed a significantly higher abundance of *H. zachvatkini* larvae on *M. glareolus* (infestation range: 1–310) compared to sympatric *A. flavicollis* (infestation range: 1–22) [9]. The close association between *H. zachvatkini* and bank voles is even reflected in the phenotypic plasticity of larvae, the trombiculids parasitizing *M. glareolus* having been reported to possess significantly longer legs compared to the parasites of *Apodemus* spp. [8]. However, the two-year study referred to above differs from the current work in that prevalence values for mites were similar in these two rodent species with prevalence being 77.4% in *A. flavicollis* and 75% in *M. glareolus*, compared to our finding of 12.2% in *A. flavicollis* and 92.5% in *M. glareolus*. This discrepancy between the two studies may be attributable to a marked difference in the protocols utilized. Here, we analyzed data from rodents living in four different localities, all assessed at the very beginning of the infestation period (September), whereas the data from the site in SW Poland were derived from a single, rather advantageous habitat for trombiculids (inferred from the maximal recorded numbers of larvae and mean abundance being c. 90 mites/individual in bank voles), which were gathered throughout two entire infestation seasons (September–May). Under optimal conditions and over a longer period of time, larvae can infest available bank voles and yellow-necked mice, and the distinct spatial activity and mobility of the two rodent species [29–31] can explain the significantly different trombiculid burdens observed on animals sampled in SW and NE Poland. An extended study conducted solely in Tałty forest would enable a more accurate comparison of trombiculid prevalence between bank voles and yellow-necked mice from the two sites.

The absence of *H. zachvatkini* larvae on rodents trapped in open fallow land is consistent with previous observations on ectoparasites associated with *M. arvalis* [32,33]. Single cases of infestation in common voles were explained as a consequence of the infested hosts venturing into shrubby and/or woody areas on the peripheries of their usual open grassland habitats [34]. During an ectoparasitic survey of rodents conducted in Germany, Maaz et al. [35] reported on parasitism by *H. zachvatkini* on animals captured exclusively within a large, forested area. The woody site was also characterized by significantly higher trombiculid prevalence and infestation intensity compared to non-wooded habitats.

The avoidance of open habitats by *H. zachvatkini* can be related to humidity and may be partly attributable to climate change because a progressive decrease in soil humidity has been observed recently in Europe [36] and this is likely to be most acute in exposed sites such as the fallow lands in this study. Among our study sites, Tałty forest represents the moistest habitat: mixed forest with a significant share of deciduous trees and hazel (*Corylus avellana* L.) bushes. In contrast, at the Pilchy site, the tree stand is dominated by pines and spruce, with buckthorn (*Frangula alnus* Mill.) bushes, and likely provides the lowest humidity in soil and litter of all three forest sites. Successful laboratory rearing of *H. zachvatkini* requires almost 100% relative humidity, which is essential for efficient reproduction of these mites [37]. A highly or semi-moist environment is preferred by the majority of trombiculid species, some of which increase their abundance after floods and/or heavy rains [37–40]. Open habitats, by comparison, are likely to be drier and therefore, are less favorable for survival and completion of the life cycle of *H. zachvatkini*.

The present work constitutes another contribution that points towards forests as the primary habitat for trombiculids in Central Europe. Long-term research (from spring to autumn) conducted exclusively in non-forested areas is essential to answer the question about other trombiculid species (e.g., *Cheladonta* sp., *Neotrombicula* spp.) [32–34] potentially tolerating lower humidity, with the consequence that in these species, peak host-infestation periods fall in the spring–summer period.

5. Conclusions

Our research indicates that the main factors favoring the occurrence of *H. zachvatkini* are humid deciduous forests and the presence of bank voles. Host sex is not a significant factor in shaping chigger prevalence and abundance; instead, rodent body mass and body length are positively correlated with the number of attached larvae.

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Informed Consent Statement: A.B.: Training for people responsible for planning procedures and experiments and for conducting them, for those performing the procedures, and for persons killing animals used in procedures, 2015, PolLASA, designation no: NR 101/P/2021, expiration date: 31 December 2026.

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