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RESEARCH ARTICLE

Auricular mites in Eurasian badgers (*Meles meles*) from Romania with a focus on *Baloghella melis*

Kosta Y. MUMCUOGLU[1,4], Harold SALANT[2], Gad BANETH[2], Yaarit NACHUM-BIALA[2], Andrei Daniel MIHALCA[3], Andreea Daniela IANI[3], Georgiana DEAK[3]

[1]*Parasitology Unit, Department of Microbiology and Molecular Genetics, The Hebrew University-Hadassah Medical School, Jerusalem, Israel*
[2]*Koret School of Veterinary Medicine, The Hebrew University, Rehovot, Israel*
[3]*Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania*
[4]Corresponding author: kostasm@ekmd.huji.ac.il

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ABSTRACT: Out of 55 roadkill mustelid carcasses collected in Romania, 42 (76.4%) belonged to the Eurasian badger, *Meles meles*. The presence of mites in the external auditory canal of these hosts was investigated. In total, 20 (36.36%) of the Eurasian badgers were positive for *Baloghella melis* mites. A total of 1,331 mite specimens were isolated from the infested animals, of which 1,328 (99.8%) were deutonymphs (hypopi) and three (0.2%) were tritonymphs. In two badgers, a male of the ear mite *Otodectes cynotis*, and the larva of an unidentified trombiculid mite were found positive for *B. melis*. Two pooled samples of *B. melis* were sequenced and showed high coverage and identity with the *Glycyphagus hypudaei* complex. To the best of our knowledge *B. melis* was described for the first time in the Eurasian badger in Romania, while *O. cynotis* was described for the first time in the Eurasian badger.

Keywords: Eurasian badger, *Meles meles*, *Baloghella melis*, *Otodectes cynotis*, Romania
Zoobank: https://zoobank.org/1D327E88-9CB2-4144-9106-25C90A449750

INTRODUCTION

The family Mustelidae is one of the most diverse groups within the order Carnivora, comprising 67 species worldwide distributed among several genera. Of these, more than 20 species occur in the Palearctic region. In Romania, the family is represented by 11 species: the Eurasian otter (*Lutra lutra* (L.)), European badger (*Meles meles* (L.)), pine marten (*Martes martes* L.), stone marten (*Martes foina* (Erxleben)), European polecat (*Mustela putorius* L.), steppe polecat (*Mustela eversmanii* (Lesson)), least weasel (*Mustela nivalis* L.), stoat (*Mustela erminea* L.), European mink (*Mustela lutreola* (L.)), marbled polecat (*Vormela peregusna* (Güldenstädt)), and the American mink (*Neogale vison* (Schreber)) (Nowak, 2018). Within the family, there are small to medium-sized species, inhabiting various environments, like weasels, polecats, otters, and badgers (Lanszki and Heltai, 2011). All these animals play important roles in the ecosystem and serve as crucial hosts for a variety of parasites (Veronesi et al., 2023). Among these, the Eurasian badger is one of the most abundant mesocarnivores (Kurek et al., 2022) and inhabits mainly woodlands (Lovell et al., 2022). It is also commonly seen in urban areas or near human settlements (da Silva et al., 1993). Despite the abundance and variety of mustelids, few studies have investigated the ectoparasitic fauna of these species (Veronesi et al., 2023). Among symbiotic arthropods associated with mustelids, parasitic insects and ticks are more commonly reported, but records of mites are scarce (Thompson, 1961; Cox et al., 1999). In small mustelids, there are several studies on mites of the Demodecidae family in polecats and otters (Izdebska et al., 2024).

In badgers, one of the mites found is *Baloghella melis,* Mahunka 1963 (Sarcoptiformes: Glycyphagidae), which was first described in 1963 by Mahunka, who collected them from the nest of a Eurasian badger (Wurst and Pfister, 1990). While studying the external auditory canal of badgers, Fain and Lukoschus (1968) described the heteromorphic deutonymph (hypopus) and tritonymph of this species under the name *Melesodectes auricularis* (Fain and Lukoschus, 1968) (today synonym of *B. melis*), while the male, female, deutonymph, and larval stages of this species were later described by Lukoschus et al. (1971) from the ear canal of *M. meles* collected in Switzerland and the Netherlands.

Considering the abundance and diversity of mustelids in Romania and the wide diversity of parasites infesting badgers, the study aimed to investigate the presence of mites in the external auditory meatus of these hosts and to molecularly characterize the species.

MATERIALS AND METHODS

Hosts

Between October 2021 and May 2024, 55 carcasses of mustelids (Eurasian badgers (n=42); stone marten (n=5); European pine marten (n=1); stoat (n=1); steppe polecat (n=1), and European polecat (n=5) were opportunistically collected as roadkill from various regions of Romania and examined by a complete parasitological necropsy (Rojas et al., 2024). During the process, two cotton ear swabs were collected from each ear of the examined animals and placed in small cryotubes containing 70% ethanol.

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Mites

The swab applicators were removed from the vial, placed in 10 ml of 70% ethanol in a Petri dish, and their surfaces were brushed for one minute with a fine brush to remove any remaining mites. The entire ethanol solution was filtered through a black filter paper (Whatman No. 508), and the filter paper was examined under a stereomicroscope (Motic 3 MZ-168, Hong Kong). The detected mites were removed with a needle. Some of the mites were placed on a drop of tap water on a slide, covered with a coverslip, and examined under light microscopy (Motic BA 310, Hong Kong). For the identification of *B. melis* mites, the publication of Fain and Lukoschus (1968), while for *Otodectes cynotis* (Hering) (Sarcoptiformes: Psoroptidae) the publication of Sweatman (1958) was used. After morphological identification, the mites were transferred to a 3 ml vial with 70% ethyl alcohol to be used later for molecular examinations. The majority of the mites were transferred to a drop of Hoyer's medium, and semipermanent slides were prepared by placing the slides on a warm plate at 50-55°C for one week, and thereafter examined under light microscopy. The majority of the semi-permanent slides of the deuto- and tritonymphs were deposited in the National Natural History Collections of the Hebrew University of Jerusalem (Givat Ram Campus).

DNA extraction and PCR

Ethanol was first removed from two pooled samples each containing 100 (9367L1) and 300 (CJ009367) mites by washing with 1X PBS after which mite derived DNA was extracted using a commercial kit (DNeasy Blood and Tissue kit; Qiagen, Germany) following the manufacturer's protocol after initially performing mechanical disruption of the samples by vortexing them for 20 minutes with an equal volume of glass beads (400-600 nm, Sigma-Aldrich, Missouri, USA), in sterile Eppendorf tubes. PCR was performed targeting the 28S ribosomal RNA gene using primer pairs universal for mites and specific to the D5, D6, and D8 domains, with conditions as used by Zhao et al. (2020). Amplicons were observed using 1.5% agarose gels stained with RedSafe (Intron Biotechnology, Korea). Positive DNA amplicons were sequenced at the Center for Genomic Analyses at the Hebrew University (Jerusalem, Israel) using the BigDye Terminator cycle from Applied Biosystems ABI3700 DNA Analyzer. The ABI Data Collection and Sequence Analysis software (ABI, Carlsbad, CA, USA) was used for analysis. DNA sequences were compared with other sequences deposited in GenBank using the BLASTn website hosted by NCBI (National Institutes of Health, USA; http://www.ncbi.nlm.nih.gov).

RESULTS

Overall, 20 (36.36%) of the Eurasian badgers were positive for *B. melis*. A total of 1,331 specimens of the mite were isolated from the infested animals, of which 1,328 (99.8%) were deutonymphs (hypopi) (Fig. 1), while three (0.2%) were tritonymphs (Fig. 2). Although the majority of mites were found solitary on the surface of the external ear, a large number of mites were also observed embedded in the cerumen (Fig. 3).

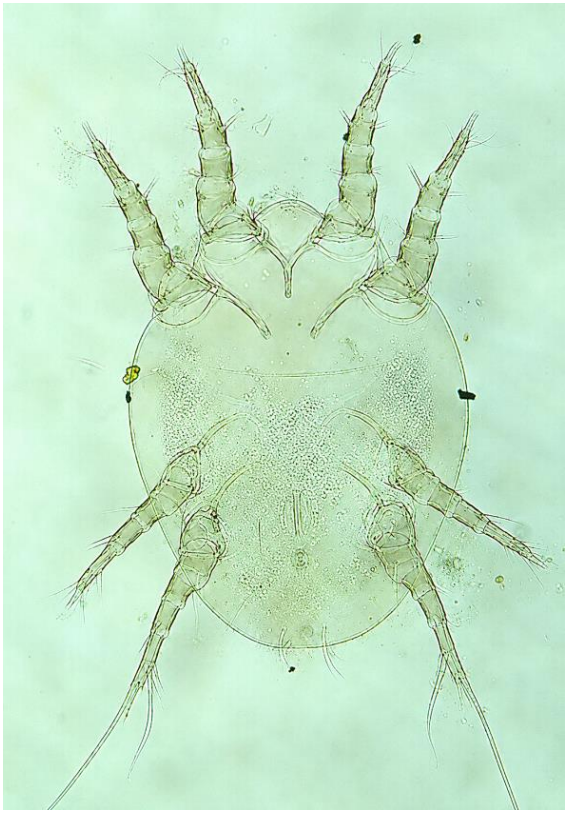

Figure 1. Deutonymph of *Baloghella melis* collected from the external auditory canal of the Eurasian badger. Not drawn to scale.

Figure 2. Tritonymph of *Baloghella melis* collected from the external auditory canal of the Eurasian badger.

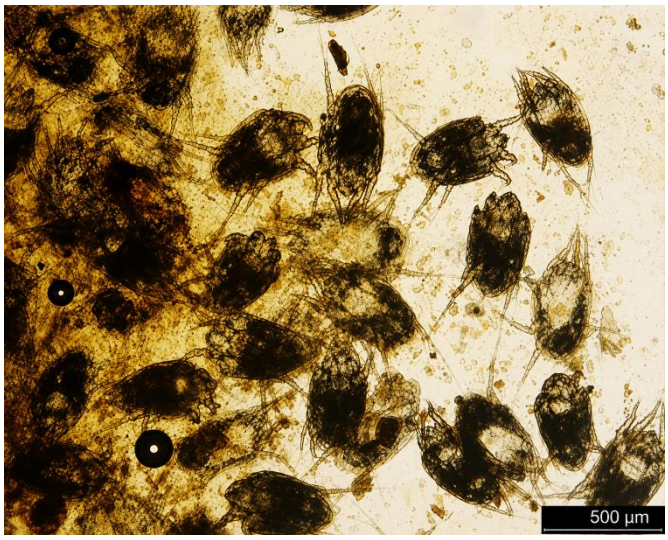

Figure 3. *Baloghella melis* group of deutonymphs in the ear cerumen from the external auditory canal of a Eurasian badger.

The total number of mites isolated from the right ear of infested animals was 339 (mean 17.8) and from the left ear 992 (mean 52.2). The maximum number of mites found in an infested animal was 801, out of which 732 were isolated from the left ear. Four animals had mites in the right ear only, another four had mites in the left ear only, and 12 had mites in both ears. In two samples (positive for mites), eggs of most likely the Muscidae family were found, while in two additional samples, positive for *B. melis,* a male of the ear mite *O. cynotis*, and the larva of an unidentified trombiculid mite were found. In one sample from *M. putorius,* an oribatid mite has been isolated. All the remaining mustelids were negative for auricular arthropods (Fig. 4).

The two pooled samples of *B. melis* were sequenced and showed high cover and identity with the *Glycyphagus hypudaei* Koch (Sarcoptiformes: Glycyphagidae) complex {Sample 9367L1 [(D5; 99% cover, 99.52% identity), (D6; 100% cover, 97% identity), (D8; 99% cover, 96% identity)] and sample CJ009367 [(D6; 99% cover, 91% identity); (D8; 99% cover, 92% identity)]}. All sequences were deposited in GenBank with accession numbers: PQ374122, PQ374123, PQ374124, PQ374125, and PQ409641, respectively.

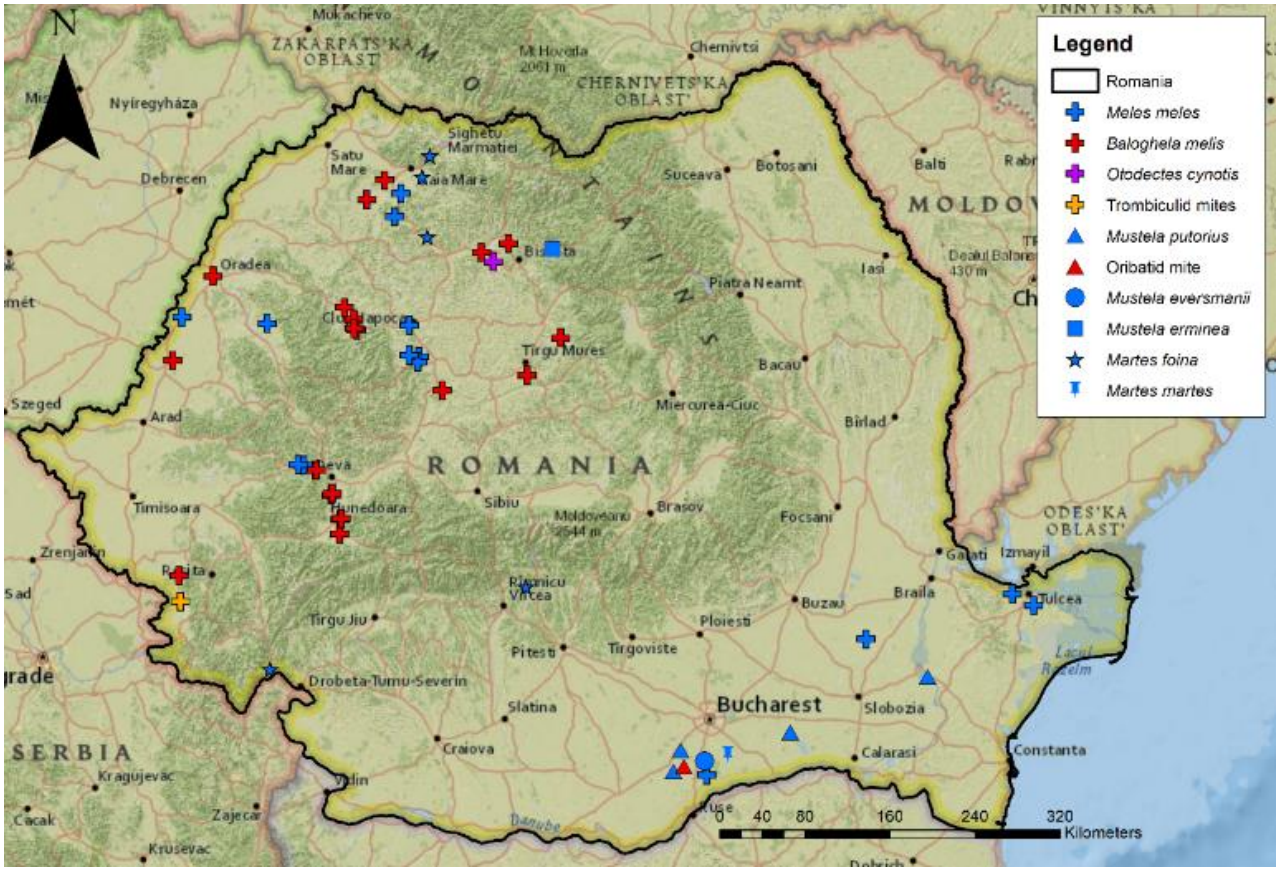

Figure 4. Map showing the shape of Romania, the localizations and species of the investigated animals, and the ear mites identified.

DISCUSSION

This paper presents the first report of *B. melis* in badgers in Romania. These results of epidemiologic and population structure data are in agreement with the observations of Fain and Lukoschus (1968) and Wurst and Pfister (1990), who mainly found the deutonymph (hypopus) stage in the concha of the Eurasian badger. Although the majority of mites were found solitary on the surface of the conch, a large number of mites were also observed embedded in the cerumen, as shown by Wurst and Pfister (1990). The hypopus of this species is characterized by the absence of organs such as sucking plates and claspers on the idiosoma and the absence of functional mouthparts (Fain and Lukoschus, 1968). The hypopus is a specialized developmental stage of several astigmatid mites that ap-

pear between protonymphal and tritonymphal stages, unable to feed and with limited locomotory capabilities. In order to survive adverse environmental conditions, they use other animals (phoresy, phoretic migration) to arrive at places or overwinter until more favorable conditions allow them to continue their development (Griffiths, 1966). The non-feeding hypopus of *B. melis* most likely migrates into the conch of badgers to bridge external living conditions and later leaves to become a tritonymph when conditions become more favorable. Because the hypopus stage does not feed and the developing tritonymph leaves the host, this species is not considered a parasite but rather a commensal mite (Wurst and Pfister, 1990).

To the best of our knowledge, this is the first observation of the ear mite *O. cynotis*, and of an unidentified larva of a trombiculid mite in the Eurasian badger. Infestation by the mange-causing mite*, O. cynotis*, is well-known in domestic dogs, cats, and ferrets with severe external otitis (Kraft et al., 1988; Miller et al., 2006; Legain et al., 2024). Sweatman (1958, 1962) reported *O. cynotis* from ferrets (*Mustela putorius furo*) (today *Mustela furo* L.) on the South Island of New Zealand, while Wilson and Zemke (1985) reported large numbers of *O. cynotis* from a wolverine (*Gulo gulo* (L.)) in Alaska, USA.

Trombiculid mites (chiggers) are known for their larval parasitic stage, which can affect a wide range of vertebrate hosts, including humans (Santibáñez et al., 2015). The presence of a trombiculid larva in a badger may indicate, of course, an incidental finding, but it also reveals the species' possible exposure to areas where trombiculids are active. Although trombiculid infestations are typically transient and may not cause significant pathology and clinical signs, they can result in localized irritation in domestic animals and humans, and in some cases, they are vectors for zoonotic pathogens such as *Orientia tsutsugamushi* (Hayashi) (Rickettsiales: Rickettsiaceae) (the agent of scrub typhus, though this is restricted geographically) (Seo et al., 2021).

Regarding the oribatid mite found in the ear of a European polecat in this study, it can be assumed to be an accidental infestation, while the insect eggs found on two occasions may have been deposited post-mortem by flies inside the external ear.

This study is the first to bring genetic sequence data for *B. melis*. The sequences of the 28S ribosomal RNA gene of *B. melis* showed a high coverage and identity with the confamilial *G. hypudaei* complex. Our findings are in accordance with Zhao et al. (2020) who reported coverage rates of 92-100% and identities of 74-100% of the three 28S ribosomal RNA regions in similar families of mites. Glycyphagidae family includes various species which are phoretic in the hypopus stage in the fur of various mammals (Fain and Lukoschus, 1968; Lukoschus et al., 1971), such as *G. hypudaei* (syn. *Dermacarus hypudaei*), which has been collected from the fur of several North American small mammals including rodents and shrews (Whitaker and French, 1982; Durden and Wilson, 1990; Nims et al., 2004).

Mites such as *B. melis, Acarus siro* L. (Acariformes: Acaridae), *Pergamasus crassipes* (L.) (synonym: *Gamasus crassipes*) (Mesostigmata: Parasitidae), *Pantalozetes pectinatus* (Michael 1885) (Acari: Oribatida) and other arthropods such as Coleoptera, Diptera, Lepidoptera, Siphonaptera, and Ixodidae were found in badger bedding material in the UK (Hancox, 2018). *Demodex melesinus* Hirst, 1921 (Acariformes: Demodecidae) was first described by Hirst in 1921 from Eurasian badgers in the UK, and later redescribed in Poland (Izdebska et al., 2018). The itch mite, *Sarcoptes scabiei* (L.) (Sarcoptiformes: Sarcoptidae) was detected in badgers examined in Norway (Holt and Berg, 1990) and Poland (Kołodziej-Sobocińska et al., 2014). The chewing louse *Trichodectes melis* (Fabricius, 1805) (Phthiraptera, Trichodectidae) the fleas *Paraceras melis* Walker, 1856 (Siphonaptera: Ceratophyllidae) and *Ctenocephalides felis* (Bouché, 1835) (Siphonaptera: Pulicidae) and the ticks *Ixodes hexagonus* (Leach, 1815), *Ixodes canisuga* Johnston, 1849*, Ixodes ricinus* (L.), and *Rhipicephalus sanguineus* sensu lato (Latreille, 1806) (Ixodida: Ixodidae) have been reported on badgers and other mustelid hosts (Butler and Roper, 1996; Cox et al., 1999; Do Linh San et al., 2003; D'Amico et al., 2017; Umur et al., 2019; Sgroi et al., 2021; Deak et al., 2024).

CONCLUSION

To the best of our knowledge, this study provides the first report of *Baloghella melis* in the Eurasian badger from Romania, expanding the known geographic distribution of this species and supporting its strong host-specificity for badgers. Additionally, this study reports for the first time the presence of *Otodectes cynotis* in the Eurasian badger, an important finding considering that this mite is typically associated with domestic carnivores, raising questions about the parasite's host range, transmission dynamics, and pathological effects on wildlife. As mustelids continue to adapt to urban environments, understanding their role in parasite transmission and biology becomes more and more important for both wildlife conservation and public veterinary health.

Authors' contributions

Kosta Y. Mumcuoglu: Conceptualization, formal analysis, methodology, project administration, supervision, validation, visualization, original draft, review & editing. **Harold Salant:** Methodology, software, review & editing. **Gad Baneth:** Methodology, supervision, review & editing. **Yaarit Nachum-Biala:** Methodology, software, validation, review & editing. **Andrei Daniel Mihalca:** Formal analysis, supervision, review & editing. **Andreea Daniela Iani:** Formal analysis, data curation, review & editing. **Georgiana Deak:** Conceptualization, data curation, formal analysis, supervision, validation, visualization, review & editing.

Statement of ethics approval

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Conflict of interest

The authors declare that they have no conflicts of interest.

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