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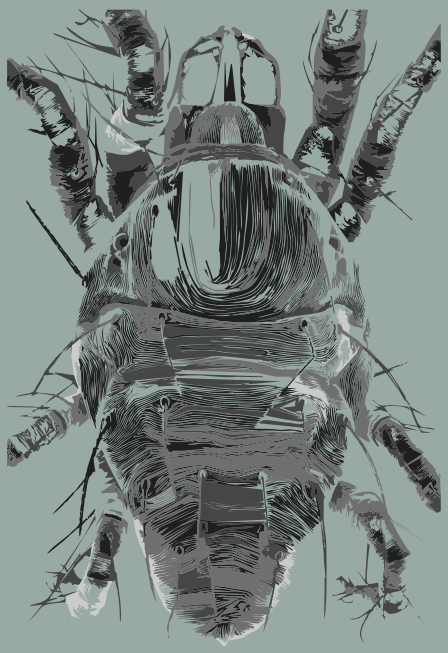

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RESEARCH ARTICLE

Morphological and molecular identification of the hard ticks parasitizing *Tremarctos ornatus* (Carnivora: Ursidae) from paramo of Ecuador

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ABSTRACT: The Andean bear or spectacled bear, *Tremarctos ornatus* (Cuvier), inhabits the Andes and is considered an endangered species due to anthropogenic factors. The aim of this study was to identify the tick species parasitizing the Andean bears in the evergreen shrubland and paramo grassland ecosystem in the Andes Mountain Range of Ecuador. Twenty-six ticks were removed from five Andean bears and morphologically identified as *Amblyomma multipunctum* Neumann, *Ixodes boliviensis* Neumann and *Ixodes montoyanus* Cooley. One specimen of each species was also molecularly analyzed and confirmed by BLAST. This study confirms the presence of *I. boliviensis* parasitizing *T. ornatus* and adds new records of *A. multipunctum* and *I. montoyanus* in its distribution. The parasite-host relationships are new in all cases for Ecuador.

Keywords: Ixodidae, *Amblyomma*, *Ixodes*, Andean bear, paramo, Ecuador.
Zoobank: https://zoobank.org/2598C855-917E-48B3-8E51-0E54AB7B0615

INTRODUCTION

Ticks are a group of Acari that parasite endothermic and ectothermic vertebrates, distributed in various tropical, subtropical and temperate environments (Guglielmone et al., 2020). In Ecuador, 34 species of hard ticks have been reported from the Galapagos Islands, the coastal region of the country, the Amazon Basin, and across an altitudinal gradient encompassing different types of forests and paramos in the Andes Mountain Range (Enríquez et al., 2020; Guglielmone et al., 2021; Paucar et al., 2022; Guglielmone et al., 2023). Other studies show the presence of ticks in anthropogenic and wilderness areas, as well as the pathogens detected in those ticks and the control measures (Labruna et al., 2013; Pesquera et al., 2015; Díaz et al., 2016; Rodriguez-Hidalgo et al., 2017; Gioia et al., 2018; Enríquez et al., 2020; Maya-Delgado et al., 2020; Aguilar-Domínguez et al., 2021; Paucar et al., 2022; Pilatasig, 2022).

The taxonomy and ecology of Ecuadorian ticks remain poorly understood, especially of those species inhabiting remote areas and parasitizing wild vertebrates. One of these wild vertebrates is the Andean or spectacled bear, *Tremarctos ornatus* (Cuvier), which inhabits the Andes, from Venezuela to northern Argentina, at elevations ranging from 800 to over 4500 meters (Sandoval-Guillén and Yánez-Moretta, 2019). In Ecuador, the distribution of this bear occurs in evergreen shrubland and paramo grassland ecosystem (paramo) (Galeas et al., 2013), temperate and subtropical forests between 1000 and 4300 meters, and prefers to inhabit cloud forests (Castellanos, 2010). According to the International Union for Conservation of Nature (IUCN, 2022), the conservation status of *T. ornatus* is Vulnerable (VU) at the global level, but in Ecuador, it is considered as an endangered species (EN) due to the loss and fragmentation of its habitats, the expansion of the agricultural frontier, mining, forest fires and hunting (Tirira, 2017).

The interaction between ticks and Andean bears is poorly understood throughout their entire distribution area and until now only *Ixodes boliviensis* Neumann was reported on this bear in Colombia (Anonymous, 1998; Guglielmone et al., 2021). Recently Carvajal and Castellanos (2021) reported *Amblyomma mixtum* Koch (1 female and 9 "female nymphs") and *Ixodes fuscipes* Koch (13 females) in *T. ornatus* from Ecuador.

The aim of this study was to identify morphologically and molecularly the tick species parasitizing *T. ornatus* of Ecuador and to re-evaluate the identification of ticks reported by Carvajal and Castellanos (2021).

MATERIALS AND METHODS

Ticks collections and morphological re-identification

Ticks identified in this study were removed from Andean bears following the protocol used by Castellanos et al. (2018). Bears examined and released were part of the Research Projects: "Systematics, Ecology, Reproductive Biology and Genomics of the Mammals of Ecuador" and

Table 1. Descriptions of localities of Ecuador.

Province	Locality	Altitude	Number of bears
Pichincha	Papallacta, Cayambe-Coca National Park (CCNP)	3600 m	1
Napo	Cosanga, Antisana National Park (ANP)	2000 m	1
Tungurahua	Patate, Valle Manteles-Leito, Llanganates National Park (LNP)	3190 m	3

"National Mammal Collection Program", which were approved through via Collection Permits: No. MAE-DNB-CM-2019-0126, No. MAAE-ARSFC-2020-0642, and No. MAAE-ARSFC-2021-1644 (Ministry of Environment and Water of Ecuador). The description of the sample localities is shown in Table 1.

All ticks identified by Carvajal and Castellanos (2021) (24 individuals) were again reviewed by one of us (SE) plus two newly collected ticks. Ticks were identified morphologically with a stereo microscope (NIKON model SMZ745T, Tokyo, Japan) (magnifications ×0.67–5), using the taxonomic keys for adult *Amblyomma* (Onofrio et al., 2006a) and *Ixodes* (Onofrio et al., 2006b), and the original descriptions of *Ixodes montoyanus* Cooley (Cooley, 1944; Keirans, 1973) and *Amblyomma multipunctum* Neumann nymphs (Labruna et al., 2013). Tick photographs were taken by a stereomicroscope (NIKON model SMZ745T, Tokyo, Japan) with a camera (Mshot model MS60, Guangzhou, China) and edited with Adobe Photoshop CC 2019.

Molecular identification

Due to the morphological differences found during the re-identification of the material collected from *T. ornatus*, 11 ticks were identified molecularly (nine from the Carvajal and Castellanos collections and two new specimens collected). Individual ticks were bisected longitudinally using sterile scalpels and washed with distilled water to remove ethanol. DNA was extracted using the commercial kit GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) following manufacturer instructions. To compare with other Neotropical ticks, the specimens were analyzed through PCR amplification of a ~460 base pair (bp) fragment of the tick mitochondrial *16S rRNA* gene (Black and Piesman, 1994). Amplicons of the expected size of two *Ixodes* females and one *Amblyomma* nymph identified by Carvajal and Castellanos (2021) were purified, and Sanger sequenced (Macrogen, South Korea). Obtained sequences were edited using MEGA 7 (Kumar et al., 2016), and comparisons with GenBank available sequences were made by the basic local alignment search tool (BLAST) (Altschul et al., 1990).

RESULTS

Morphological and molecular identification

Twenty-six ticks were morphologically identified, corresponding to *A. multipunctum* (11 nymphs), *I. boliviensis* (5 females and 1 male), and *I. montoyanus* (9 females); of these, all ticks identified by Carvajal and Castellanos (2021) were re-evaluated plus two newly collected ticks (Table 2). The principal morphological characteristics to identify *A. multipunctum* nymphs were described by Labruna et al. (2013). Moreover, some characteristics of *I. montoyanus* females were compiled with the works of Cooley (1944), Onofrio et al. (2006b), Apanaskevich and Bermúdez (2017) and Onofrio et al. (2020).

Females of *I. boliviensis* were recognized when compared with the characteristics presented by Onofrio et al. (2006b) because is the more precise work to identify *Ixodes* specimens of South America. In the case of the male, we present here a preliminary description of the unique exemplar collected on one Andean bear in this study. In this sense, our specimen was 3.0 mm long (from the apical part of the hypostome to posterior body margin), a maximum breadth of 1.5 mm; oval idiosoma; dorsal basis of capitulum without cornua, hypostome short, broad, and apically rounded with dentition 4/4 arranged in transversal rows; auriculae small and sharp; scutum with deep punctuations distributed at the margins and in the posterior region; coxa I with two spurs, the internal spur long, extending beyond the middle of coxa II and the external spur triangular small; coxa II with two triangular small spurs; coxae III-IV with a small triangular external spur; genital aperture situated at the level of the third coxa; median plate with deep punctuations evenly distributed, adanal and anal plates with fine punctuations; spiracular plate broadly oval; a ventral region with numerous long white setae (Figs 1-3).

The sequence obtained of one *Amblyomma* nymph (Al-AmuN30) showed 98.30% (404/411 bp) and 98.29% (402/409) identity with *A. multipunctum* from Ecuador (GenBank KC677673 and KJ584366). Regarding *Ixodes* ticks, one sequence (Co-IboH33) showed 99.03% (408/412 bp) identity with *I. boliviensis* (GenBank KM077437), and the sequence obtained from the female of *I. montoyanus* (Al-ImH28) showed 100% (409/409 bp) identity with *Ixodes* sp. AMP-2014 isolate 8C1, both from sequenced reports of Ecuador (GenBank KM077438). Sequences from the *16S rDNA* gene for this study are available in GenBank for *A. multipunctum* (accession number OQ557626); *I. boliviensis* (accession number OQ557627) and *I. montoyanus* (accession number OQ557628). These whole or partially dissected specimens were deposited in the National Reference Collection of Arthropods of Importance in Zoonoses (CONRAZ) of the Zoonosis Research Institute (CIZ), Central University of Ecuador, Quito.

DISCUSSION

Due to the analysis of several factors such as the difficulty of identifying sex in immature stages (larvae and nymphs) of hard ticks (Polanco-Echeverry and Ríos-Osorio, 2016), the presence of an ornate scutum in females of the species *A. mixtum*, its altitudinal distribution (Nava et al., 2014),

Table 2. Morphological and molecular identification of ticks parasitizing Andean bears from Ecuador.

Locality	Sample ID ˣ	Ticks stage	Morphological ID	Molecular ID (GenBank Accession)
CCNP	Da-AmuN01	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN02	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN03	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN04	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN05	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN06	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN07	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN08	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN09	Nymph*	*Amblyomma multipunctum*	
CCNP	Da-AmuN10	Nymph*	*Amblyomma multipunctum*	
ANP	Co-IboH31	Female*	*Ixodes boliviensis*	
ANP	Co-IboH32	Female*	*Ixodes boliviensis*	
ANP	Co-IboH33	Female*	*Ixodes boliviensis*	*Ixodes boliviensis* (OQ557627)
ANP	Co-IboH34	Female*	*Ixodes boliviensis*	
LNP	Al-ImH23	Female*	*Ixodes montoyanus*	
LNP	Al-ImH24	Female*	*Ixodes montoyanus*	
LNP	Al-ImH25	Female*	*Ixodes montoyanus*	
LNP	Al-ImH26	Female*	*Ixodes montoyanus*	
LNP	Al-ImH27	Female*	*Ixodes montoyanus*	
LNP	Al-ImH28	Female*	*Ixodes montoyanus*	*Ixodes montoyanus* (OQ557628)
LNP	Al-ImH29	Female*	*Ixodes montoyanus*	
LNP	Al-AmuN30	Nymph*	*Amblyomma multipunctum*	*Amblyomma multipunctum* (OQ557626)
LNP	De-ImH01	Female*	*Ixodes montoyanus*	
LNP	De-ImH02	Female*	*Ixodes montoyanus*	
LNP	P4-IbolH35	Female⁺	*Ixodes boliviensis*	
LNP	P4-IbolM36	Male⁺	*Ixodes boliviensis*	

ˣEach letter represents a captured Andean bear individual, *Samples identified by Carvajal and Castellanos (2021) and re-identified by the authors, ⁺Samples identified only by the authors.

and its type of hosts (Guglielmone et al., 2021), arose doubts about the identification of Carvajal and Castellanos (2021). For these reasons, it was necessary to review again the identification of these ticks, including the use of molecular analysis.

During this study, the presence of *I. boliviensis* parasitizing *T. ornatus* is confirmed and new records of *A. multipunctum* and *I. montoyanus* are added to its distribution, this increments the parasite-host relationships, which are new in all cases for Ecuador. Consequently, the molecular identifications of *A. multipunctum* nymphs, *I. boliviensis*, and *I. montoyanus* adults, replace the identifications of *A. mixtum* and *I. fuscipes* reported by Carvajal and Castellanos (2021).

It is essential to highlight that *A. mixtum* is an eclectic species with a wide distribution from the southern United States (Texas) to west-northern South America and some Caribbean Islands (Guglielmone et al., 2021). In Ecuador, this species is known from western Ecuador (provinces of El Oro, Guayas, Los Ríos, Manabí and Pichincha, see Nava et al., 2014 and Paucar et al., 2022), in environments such as dry, semiarid, and riparian forests, or savanna lowlands instead of Andean cloud forests or paramo environments (Estrada-Peña et al., 2014; Aguilar-Domínguez et al., 2021). Regarding *I. fuscipes*, its distribution is restricted to three southern Brazilian states (Paraná, Rio Grande do Sul and São Paulo) and Uruguay (Cerro Largo, Florida, Lavalleja, Maldonado, Rocha and Tacuarembó) (Guglielmone et al., 2021). This tick has been widely confused with *Ixodes spinosus* Neumann, while and *Ixodes aragaoi* Fonseca is a synonym of *I. fuscipes* (Guglielmone et al., 2020; Labruna et al., 2020).

On the other hand, *A. multipunctum* occurs in highlands, humid montane forest areas, associated with the distribution of its primary host the mountain tapir, *Tapirus pinchaque* (Roulin) (Labruna et al., 2013; Pesquera et al., 2015; Guglielmone et al., 2021). This tapir species inhabits montane forests and paramos (from 1500 to 4800 m) of Colombia, Ecuador and Peru (Castellanos-Peñafiel et al., 2021) sharing habitat with the spectacled bear; for this reason, *A. multipunctum* might have parasitized both host species, particularly by the immature stages of this tick. The nymph analyzed here, showed more than 98% of identity with the DNA sequences of adults from Ecuador. This is the first host record for *A. multipunctum* nymphs on Andean bears. Regarding *I. boliviensis*, molecular differences between Central America and Southern America populations, indicate the presence of at least two different species (Bermúdez et al., 2021).

Figures 1-3. *Ixodes boliviensis* (male)—**1.** Dorsal view, **2.** Ventral view, **3.** Ventral view of capitulum and idiosoma.

In the present study, there is a preliminary sketch of males and females from Ecuador, which also show some morphological differences regarding the size and dental formula, but without genetic differences. Previously in Ecuador, Pesquera et al. (2015) found one female of *I. boliviensis* on cattle in Papallacta (paramo) at 3300 m of altitude, one of the areas where the exemplars of this study were collected. In addition, Paucar et al. (2022) found females of *I. boliviensis* parasitizing cattle in three farms of Quijos River Valley (Napo province) and Northwest Pichincha province. These areas are in the east and west foothills of the Andes Mountains, respectively at ranging altitudes from 800 to 1600 m. Thus, this geographical and biological information suggests the identification of *I. boliviensis* in Ecuador, although new research is still necessary to define the current status of this species. Also, it will be necessary to carry out exhaustive studies for the formal description of the male of this species.

We report for the first time the presence of *I. montoyanus* on two Andean bears of Ecuadorian paramo. Previously this tick was reported from cattle in the same areas where *I. boliviensis* was found (Paucar et al., 2022). This information contributes to extending the list of hosts of *I. montoyanus* adults because until now just mammals of families Cervidae and Procyonidae were considered as hosts (Guglielmone et al., 2021). It should be noted that a morphological characteristic to identify specimens of *I. montoyanus* is the presence of membranous areas (syncoxa) and the absence of external spur in the coxae I-II. The syncoxae was not mentioned in the original description by Cooley (1944) and Keirans (1973) but is notified by Apanaskevich and Bermúdez (2017) and Onofrio et al. (2020). Due to these structures, females of *I. montoyanus* resemble *Ixodes tapirus* Kohls, *Ixodes lasallei* Méndez & Ortiz, *Ixodes venezuelensis* Kohls, *Ixodes guatemalensis* Kohls, *Ixodes bocatorensis* Apanaskevich & Bermúdez, and *Ixodes catarinensis* Onofrio & Labruna but differs from those species by the long and hooked auriculae, scutal punctuations, size, and form of the porose areas (Apanaskevich et al., 2017; Onofrio et al., 2020). The DNA sequence of *I. montoyanus* is the first sequence verified for this species. With this morphological, biological, and molecular information, we confirm the presence of this tick on the Andean bear from the paramo of Ecuador. Finally, in addition to an adequate taxonomic and morphological review of ticks, many aspects of their distribution and ecology should be assessed.

Authors' contributions

Sandra Enríquez: Conceptualization, morphological identification of the ticks, writing. **María L. Félix:** Molecular analysis, investigation; validation. **Armando Castellanos:** Funding acquisition for fieldwork, field collection of the ticks. **Sergio Bermúdez:** Visualization, writing-original draft, methodology. **José M. Venzal:** Methodology, data curation, resources. All authors participate in the final revision and writing of the manuscript.

Statement of ethics approval

The present study was conducted according to the legal requirements of Ecuador. Ministry of Environment and Water of Ecuador via Collection Permits No. MAE-DNB-CM-2019-0126, No. MAAE-ARSFC-2020-0642 and No. MAAE-ARSFC-2021-1644 authorized ticks capture on Andean bears. All animals were treated carefully and then released into their habitats.

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Conflict of interest

The authors declare that there is no any conflict of interest.

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RESEARCH ARTICLE

Development and life table parameters of the *Phytoseius corniger* Wainstein (Acari: Phytoseiidae) feeding on the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions

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ABSTRACT: Phytoseiid mites are important for biological control of some pest species including phytophagous mites and small insects. The species, *Phytoseius corniger* Wainstein is one of the most abundant predatory mite in fruit orchards and urban green spaces in Razavi Khorasan province, Iran. Biological parameters of this predatory feeding on the two-spotted spider mite *Tetranychus urticae* Koch was studied for the first time under the laboratory conditions (25±2 ºC, 55±5 % RH and 16L: 8D photoperiod). The results indicated that the mean developmental periods for egg, larva, protonymph and deutonymph were 1.12±0.09, 0.73±0.03, 1.01±0.03, and 3.05±0.09 days for females, and 0.95±0.37, 0.67±0.39, 1.05±0.88, and 3.05±0.85 days for males, respectively. The pre-oviposition, oviposition, post-oviposition periods and adult longevity for females were 4.63±0.11, 11.63±0.16, 7.93±0.13 and 24.18±0.21 days, respectively. The females laid an average of 0.48 eggs per day, and 5.60 eggs during their life span. In addition, larvae of *P. corniger* may molt to the protonymph stage without feeding. Consumption rate during nymphal stage was 1.95 prey per day. The highest rate of prey consumption was recorded during the oviposition period, with an average of 3.35 prey items prey per day. Regarding life-history traits, the intrinsic rates of increase (r_m) of this predatory mite 0.064±0.02 (♀♀/♀/day) and its finite rate of increase (λ), net reproduction rate (R_0), generation time (T), and doubling time (DT) were 1.066±0.23 (day^{-1}), 3±0.07 (♀♀/♀/generation), 17.14±0.11 (days), and 10.83±0.65 (days), respectively. Further laboratory and field studies regarding its diet preference and predation capacity are needed.

Keywords: Demographic parameters, predatory mite, *Phytoseius corniger*.
Zoobank: https://zoobank.org/A592E6B4-62A2-441C-BB72-C2AD26BD0676

INTRODUCTION

The family Phytoseiidae (Acari: Mesostigmata) with more than 2800 described species (Demite et al., 2023) have diverse feeding habits, ranging from specializing on spider mites to general consumption of a wide variety of foods, including plant materials (McMurtry and Croft, 1997). According to the latest catalogue of Phytoseiidae fauna of Iran, so far, five species belonging to the genus *Phytoseius* have been reported in the country (Kazemi et al., 2022). Among them, *Phytoseius corniger* Wainstein, 1959 is mostly known in the Palearctic region and reported from India, Iran, Kazakhstan, Kyrgyzstan, Lebanon, Moldova, Pakistan, Serbia, Tajikistan and Turkmenistan (Demite et al., 2023). In Iran, this species has been the most abundant with wide distribution in both fruit orchards (Panahi Laeen et al., 2014) and in urban green spaces in Razavi Khorasan province (Namaghi, 2010). Despite being one of the most common predatory phytoseiids in the region, no study on its basic biological characteristics such as development, reproduction, and predation capacities have been carried out. However, the potential role of the generalist phytoseiids in suppressing spider mite densities has been reported by some researchers (McMurtry and Croft, 1997). According to Pappas et al. (2013) phytoseiid mites of the genus *Phytoseius* are natural enemies of tetranychid and eriophyid mites and they are mostly found on plants with pubescent leaves where they feed on prey, as well as on pollen. Nevertheless, the nutritional ecology and the role of these predators in biological control are only rarely addressed. The present study aimed to obtain preliminary information regarding the biology and life table parameters of *P. corniger* by feeding on the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae).

MATERIALS AND METHODS

Stock culture of the prey, *Tetranychus urticae*

The prey, *T. urticae* was originally collected from an infested cauliflower plants in vicinity of Mashhad and transferred to Department of Plant Protection at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The stock culture of the prey, *T. urticae* was stablished on potted cauliflower plants (*Brassica oleracea* cv. Romanesco). The plants used in this study, were grown in plastic pots (25 cm diameter x 40 cm depth) filled with a commercial soil of Cocopeat: Perlit (60%: 40%) and kept at a greenhouse conditions set at 25±2°C, 50±10% RH and 16:8 (L: D) h photoperiod. The rearing of cauliflower plants started two months before the experiments.

Stock culture of the predatory mite, *Phytoseius corniger*

The adults of predatory mite were initially obtained from the hackberry tree (*Celtis* sp., Ulmaceae) in Ferdowsi University campus in August 2022 and transferred to rearing units consisting of clean succulent cauliflower leaves, in an

incubator at 25±2 ºC, 55±5 % RH and 16L: 8D hour photoperiod. Clean leaf discs were placed underside facing up on a wet sponge layer in plastic containers (20 x 10 x 5 cm). The borders of leaf discs were surrounded with moistened tissue papers to provide water supply for phytoeiids and to prevent them from escaping. Every day, a mixture of all stages of *T. urticae* from the stock culture were supplied as food.

Biology, predation and life table parameters of *Phytoseius corniger*

This study was conducted using experimental units made of discs of cauliflower leaf discs (2 cm diameter) placed upside down on water saturated sponge in a plastic Petri dish (9 cm in diameter by 1.5 cm in height). The Petri dish was kept permanently open. Leaf discs were bordered with wet tissue paper strips to prevent mites from escaping. A total of 30 females from the stock colony were transferred to the individual arenas with the help of fine camel hair brush and left for 24 h to lay eggs. After egg deposition, females were removed and individual deposited eggs with less than 24 hours old were used for studying the biological attributes of the predator. The newly hatched protonymphs were supplied daily with prey at densities of 10-15 individuals of different motile stages of the two-spotted spider mite. The number of preys supplied to each rearing unit was determined according to preliminary experiments. Each day the number of consumed prey in each rearing unit was recorded and the unconsumed ones were removed and replaced by fresh prey. After the last molting, an adult male, randomly taken from the stock colony, was introduced to each leaf disc containing a newly emerged female. After copulation, males were transferred to stock culture. Mated females were observed to determine the pre-oviposition, oviposition, and post-oviposition periods as well as gathering data on fecundity. Observations on the development were done twice a day and reproduction, survival and prey consumption once a day. Every 4-5 days, the predator was transferred to new arenas, while its eggs were removed daily from the arenas. This procedure was continued until the last predatory mite died. The data obtained on surviving and duration of life stages were recorded and statistically analyzed to work out standard deviation. The fecundity was worked out by recording total number of eggs laid by individual females during their life span. Sex ratio was obtained based on rearing 27 eggs until reaching maturity.

Life table parameters including mean generation time (T), net reproduction rate (R_0), intrinsic rate of increase (r_m), finite rate of increase (λ), doubling time (DT) were estimated based on the age-stage and two-sex life table (Chi, 1988; Chi and Liu, 1985) using the computer program TWOSEX-MSChart (Chi, 2015, version 10.23). To take both sexes into consideration, the age-specific survival rate (l_x) and the age-specific fecundity (m_x) in the age-stage two-sex life table were calculated (Chi and Liu, 1985). Bootstrap method (Huang and Chi, 2012) was used to calculate Standard Errors (SE) of the life table parameters.

RESULTS

Developmental periods

Similar to the most of other phytoseiid species, the life cycle of *Phytoseius corniger* includes egg, larva, protonymph, deutonymph and adult stages. Individuals of the predatory mite, *P. corniger* were able to develop and reproduce on the two-spotted spider mite, but not on honey bee pollen. This is in contrast with our field observation on *P. corniger* on leaves of hackerberry tree (*Celtis* sp.) without any phytophagous mite as possible prey. Also, cannibalism was not noted when there was prey scarcity. The adult females and males of *P. corniger* had an average life span of 29.41±0.43 and 27.32±0.32 days, respectively. After 12-18 hours from the emergence, the adults mated and females start to oviposit after four days (Figure 2). The mean immature developmental time was 5.91 days for females and 5.70 days for males. Unmated females did produce eggs. Duration in days (Mean±SE) of the stages and reproductive parameters of *P. corniger* are given in Table 2.

The adults were shiny at the time of emergence. The males can be distinguished from the female by their more elongated body. The pre-oviposition, oviposition and post-oviposition periods includes 19.12%, 48.07% and 32.79% of female longevity, respectively.

Predation rate

Predation data of *P. corniger* immature and adult stages is given in Table 3. Larvae of *P. corniger* molt to protonymph stage without feeding. The predation rate during nymphal stage was 1.95 prey per day. Adult females consumed more prey than adult males. The highest rate of prey consumption was recorded during the oviposition period, with the female consuming an average of 3.35 prey per day. The higher prey consumption by females compared to males on *T. urticae* has been reported in several cases for other phytoseiid species (e.g., Rasmy et al., 1991; Naher et al., 2005; Khalequzzaman et al., 2007).

Population parameters

Life table parameters are appropriate indexes of population growth under a defined set of conditions that provide a valuable tool to determine the potential of a biocontrol agent under different local and seasonal conditions. The population parameters of *P. corniger* were calculated based on a cohort of 27 individuals. The calculated parameters (Mean±SE) are shown in Table 4. The population of *P. corniger* reared on *T. urticae* in a controlled environment could complete a generation within 17.14±0.08 days. The probability that an individual will survive to age *x* and stage *j* is shown by the parameter s_{xj} (Figure 1). There was clear overlapping in the age-stage survival curves between successive stages, demonstrating the variable developmental rates occurring among *P. corniger* individuals of both sexes. The age-specific survival rate (lx), fecundity (mx) of *P. corniger* are plotted in Figure 2. It was observed that the survival rate (lx) and fecundity (m_x) of *P. corniger* decreased with as the age increased.

Table 1. Duration in days (Mean±SE) of various life stages of *Phytoseius corniger* reared on the two-spotted spider mite, *Tetranychus urticae* under laboratory conditions (25±2 ºC, 55±5 % RH and 16L: 8D h photoperiod).

Developmental stages	Sex[1]	N[2]	Minimum	Maximum	Mean (±SE)
Egg	Female	16	0.83	2	1.12±0.09
	Male	8	0.83	1	0.95±0.365
Larva	Female	16	0.5	1	0.73±0.03
	Male	8	0.62	0.75	0.67± 0.39
Protonymph	Female	16	0.5	1.16	1.01±0.03
	Male	8	1	1.16	1.05±0.88
Deutonymph	Female	16	2.5	4.50	3.05±0.09
	Male	8	2.91	3.25	3.05±0.85
Egg-Adult	Female	16	4.33	8.66	6.495±0.06
	Male	8	5.36	6.16	5.76±0.33
Longevity	Female	16	19.25	27.75	24.18±0.21
	Male	8	20.75	22.75	21.60±0.21

[1]Sex ratio (female: male) was approximately 2: 1 and obtained based on rearing 27 eggs which 24 of them (88.9%) developed successfully to the adult stage. [2]N = Number of specimens observed.

Table 2. Duration in days (Mean±SE) of the stages and egg production of *Phytoseiuus corniger* at 25 ± 2 °C, 55 ± 5% RH, and 16L: 8D h photoperiod, when fed *Tetranychus urticae.*

Stages and reproductive parameters	N[1]	Minimum	Maximum	Average (±SE)
Pre-oviposition period (Days)	16	3.25	6.00	4.62±0.11
Oviposition period (Days)	16	11.00	12.50	11.62 ±0.16
Post-oviposition period (Days)	16	5.00	9.25	7.93±0.13
Number of eggs per female	16	2.70	7.00	5.06±0.38

[1]N = Number of specimens observed.

Table 3. Predation of the predatory phytoseiid mite, *Phytoseius corniger* on *Tetranychus urticae* at laboratory conditions (25±2 ºC, 55±5 % RH and 16L: 8D h photoperiod)

Life stage	Sex	Minimum	Maximum	Mean±SE
Larvae	Female	0.00	0.00	0.00
	Male	0.00	0.00	0.00
Protonymph	Female	1.00	3.00	2±0.191
	Male	0.00	3.00	1.68±0.21
Deutonymph	Female	3.00	8.00	5.85±0.37
	Male	3.00	8.00	5.85±0.34
Pre oviposition		4.00	13.00	8.05±0.64
Oviposition		18.00	46.00	39.35±1.59
Post oviposition		14.00	31.00	25.95±1.22

Table 4. Population parameters (Mean±SE) of *Phytoseius corniger* reared on *Tetranychus urticae* under laboratory conditions (25±2 ºC, 55±5 % RH and 16L: 8D h photoperiod).

Life table parameters	Mean±SE
Net reproductive rate (R_0)	3.00±0.07*
Mean generation time (T)	17.14±0.11
Intrinsic rate of increase (r)	0.06±0.02
Finite rate of increase (λ)	1.07±0.23
Doubling time (DT)	10.83±0.45

Note: R_0, net reproductive rate (♀♀/♀/generation); T, mean generation time (days); r, intrinsic rate of increase (♀♀/♀/day); λ, finite rate of increase (day^{-1}); DT, Doubling time (days); *-the number of observed individuals were 27.

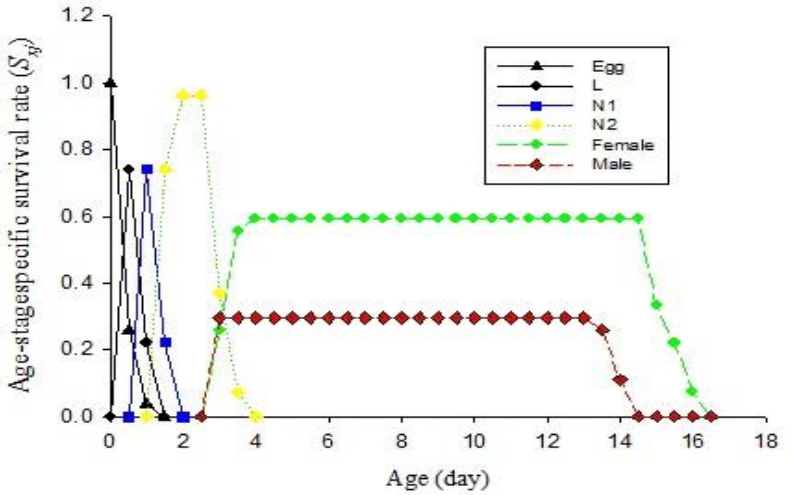

Figure 1. Age-stage specific survival rate (s_{jx}) of the parent cohort of bisexual *Phytoseius corniger* fed on *Tetranychus urticae* under laboratory conditions (25 ± 2 °C, 55 ± 5% of RH, and 16L: 8D h photoperiod). Note: L stands for larva, N1 for protonymph, and N2 for deutonymph, respectively.

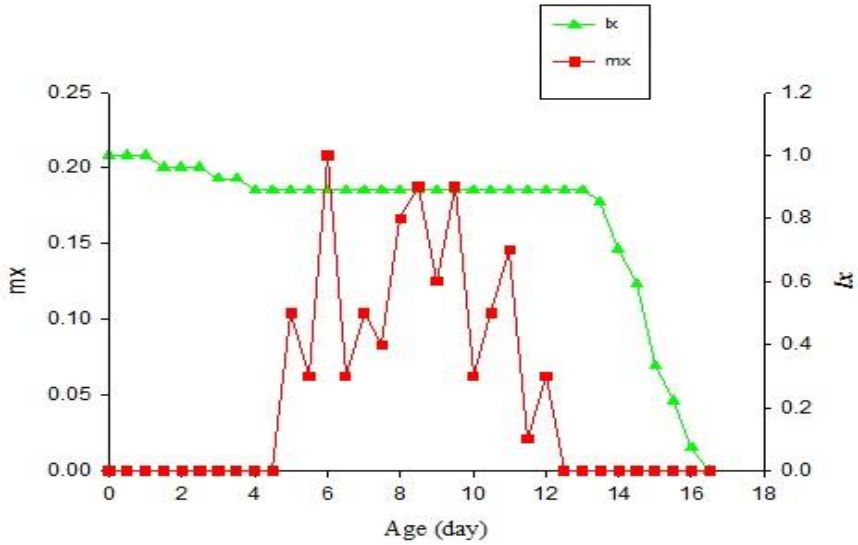

Figure 2. The age-specific survival rate (lx), and fecundity (m_x) of *Phytoseius corniger* fed on *Tetranychus urticae* under laboratory conditions (25 ± 2 °C, 55 ± 5% of RH, and 16L: 8D h photoperiod).

DISCUSSION

Despite the extensive practical use of phytoseiids for biological control of some phytophgous mites, the microhabitat and food preference of vast majority of the species still remains to be unknown (McMurtry et al., 2013). Although, several studies have been reported about the association of *Phytoseius* species with tetranychid, eriophyid, tarsonemid and tenuipalpid mites (Vassiliou et al., 2012; McMurtry et al., 2013), there has been no study on the biology, predation and life table parameters of the *P. corniger* feeding on *T. urticae*. So, the preliminary results obtained from this study are not comparable to the other studies on other species of the genus *Phytoseius*. Among the few works carried out on predation capacity of the species of the genus *Phytoseius*, Duso and Vettorazzo (1999) stated that *Phytoseius finitimus* (Ribaga) is a potentially useful for the control of *Panonychus ulmi* Koch on grape. In laboratory experiments, Pappas et al. (2013) observed that *P. finitimus* may feed and reproduce on larvae of *T. urticae*, as well as on crawlers of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood. Also, the phytoseiid *Phytoseius plumifer* (Canestrini and Fanzago) have been mentioned as predator of tetranychid and eriophyid mites on various crops in Iran (Hajizadeh et al., 2002; Nadimi et al., 2009; Gorji et al., 2012; Khodayari et al., 2013). In a study on the life table parameters of *P. plumifer* on *T. urticae*, Gorji et al. (2012) showed that the net reproduction rate (R_0) was 29.6 females/female and the finite rate of increase (λ) was noted to be the highest at 30°C (1.29 day^{-1}). Results of a study by Al-Azazzy and Alhewairini (2020) suggest that *P. plumifer* has the ability to maintain eriophyid and tetranychid mite densities below damaging levels. In the present study, the net reproductive rate (R_0) of *P. corniger* was 3.00±0.07 which is very lower than those reported by Gorgi et al. (2012) and Al-Azazzy and Alhewairini (2020) for *Phytoseius plumifer*, respectively. It

seems that *P. plumifer* has higher fecundity *P. corniger*. This difference, could be explained by differences in rearing conditions such as a higher temperature and differences in diet preference of these conspecies. It is more likely that other preys such as tydeoid mites maybe more preferred than *T. urticae* by *P. corniger*. The temperature and the type of food are the main factors that affects the population parameters considerably such as development, fecundity and efficiency of predatory mites.

Based on our field observations as well as the laboratory experiments, it is suggested that *P. corniger* is a generalist predator and belongs to the subtype IIIa, since it was found frequently on pubescent leaves of hackerberry tree (*Celtis* sp.) where there were no or only a few tydeoid mites per leaf. Observing 4-5 individuals of *P. corniger* on a leaf of hackerberry tree suggests that this phytoseiid mite might be able to feed on pollen or can utilize plant exudates and honeydew as survival food in the absence of prey, or as complementary food, as has been stated by McMurtry and Croft (1997).

Feeding *P. corniger* on *T. urticae* resulted into an intrinsic rate of increase of 0.18 (females/female/day). The r_m is a value that is one of the basic criteria for evaluating the effectiveness of a biological control agent on their prey. Theoretically, when a predator has a population growth rate higher than its prey, it can regulate the population of its prey. As the present study did not measure the r_m value of the prey, *T. urticae* at the same experimental conditions, it is not possible to judge on the effectiveness of *P. corniger* against *T. urticae* population. Further studies will reveal more details regarding its diet preference and predatory potential of this phytoseiid mite.

Authors' contributions

Leili Baghlani: Investigation, data curation, writing original draft. **Hussein Sadeghi Namaghi:** Supervision, Analyzing data, writing-original draft, review editing. **Lida Fekrat:** Supervision, methodology, analyzing data. The present study is a part of the M.Sc. thesis of the first author.

Statement of ethics approval

Not applicable.

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Conflict of interest

The authors declares that there is no conflict of interest.

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RESEARCH ARTICLE

New records of feather mites (Sarcoptiformes: Astigmata) from some birds in Türkiye

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ABSTRACT: Feather mites (Astigmata: Analgoidea and Pterolichoidea) are commensal ectosymbionts permanently living on avian hosts. The study was based on parasitological examination of 59 bird specimens representing 28 species from the orders Accipitriformes, Apodiformes, Columbiformes, Passeriformes, Pelecaniformes, and Strigiformes collected in Artvin, Samsun, and Sakarya, Türkiye. We recovered 18 feather mite species from the families Avenzoariidae, Eustathiidae, Gabuciniidae, Kramerellidae, Proctophyllodidae and Pterolichidae. Among them, 11 species are recorded for the first time in Türkiye: *Ardeacarus ardeae* (Canestrini, 1878), *Chauliacia securigera* (Robin, 1877), *Eustathia cultrifera* (Robin, 1877), *Gabucinia delibata* (Robin, 1877), *Kramerella aluconis* (Lönnfors, 1937), *K. lunulata* (Haller, 1878), *Michaelia heteropus* (Michael, 1881), *Neochauliacia minuscula* Gaud and Atyeo, 1967, *Proctophyllodes musicus* Vitzthum, 1922, *Pterodectes rutilus* Robin, 1877 and *Scutomegninia phalacrocoracis* Dubinin and Dubinina, 1940.

Keywords: Acari, avian parasites, bird parasites, fauna, first record.
Zoobank: https://zoobank.org/D7E54A9B-B8C4-456F-903C-C1F752225C61

INTRODUCTION

Feather mites (Acari: Astigmata) are small arthropods that live permanently as parasitic or commensal ectosymbionts on birds and can inhabit the wing, tail, and body feathers (plumicoles), cavities of feather quills (syringicoles), feather follicles and skin (dermicoles) of their hosts (Gaud and Atyeo, 1996; Dabert and Mironov, 1999; Proctor, 2003). These mites are currently arranged into two superfamilies (Analgoidea and Pterolichoidea) and altogether include over 2500 described species (Proctor and Owens, 2000; Mironov, 2016).

The effect of feather mites on birds is a phenomenon that varies between parasitism and commensalism. Up to now, studies conducted have shown that feather mites consume as a food such substances as uropygial oil, ceratinous content of rachis, skin residues, and also, by chance, spores, pollen, fungi and other organic materials stuck to feathers (Walter and Proctor, 2013). Unlike arthropods such as ticks, chiggers, chewing lice, fleas and louse flies that parasitize birds, the mouth structure of feather mites is not chewy or bloodsucking, but has a gnawing structure (Proctor and Owens, 2000).

In several past decades, the studies on feather mites in the world increased day by day, but when we look at the acarological literature, there are only a few works on feather mites in Türkiye (Eren and Açıcı, 2022). The first information about feather mites in Türkiye begins with the report of *Diplaegidia columbae* (Buchholz, 1869) (as *Megninia columbae*) and *Falculifer rostratus* (Buchholz, 1869) on the rock pigeons *Columba livia* in the book "Turkey Parasites and Parasitological Publications (In Turkish: Türkiye Parazitleri ve Parazitolojik Yayınları)" (Merdivenci, 1970). In the subsequent years, except for two studies (Gürler et al., 2013; Per and Aktaş, 2018), mostly narrow-scoped studies were conducted and only 45 feather mites have been reported so far from a small number of bird species (Eren and Açıcı, 2022; Eren et al., 2022). The aim of this study is to contribute to the knowledge on feather mite diversity in Türkiye.

MATERIALS AND METHODS

The present study is based on parasitological examination of dead bird carcasses brought to the Parasitology Department of the Faculty of Veterinary Medicine, the Ondokuz Mayıs University (Samsun, Türkiye), in 2022 (as shown in Table 1). Feather mite specimens were collected manually under a stereomicroscope using point tip tweezers and preserved in tubes with 70% ethanol. For identification, a representative number of mites from collected samples were cleared 48 hours with lactophenol (Karatepe and Karatepe, 2015) and then mounted on microscope slides in the Hoyer's medium (Evans, 1992). Although Hoyer's medium possesses cleaning properties, the well-developed integument of the mites can preclude the desired clearing. Therefore, feather mite samples can be more easily identified by clearing them with lactophenol before mounting (see also: Atyeo and Braasch, 1966; Orwig, 1967). Mite species were identified under a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) using corresponding diagnostic keys and careful descriptions (Atyeo and Braasch, 1966; Atyeo and Peterson, 1967; Kwanyuen, 1973; Gaud and Atyeo, 1976, 1982; Mironov, 1989, 1990; Gaud and Barre, 1992; Valim and Hernandes, 2006; Peterson et al., 2007; Han et al., 2016;

Table 1. The identified feather mites and their hosts.

Bird species	Bird family	Number of examined birds	Number of infected birds	Mite species
Strigiformes				
Athene noctua	Strigidae	1	1	*Kramerella lunulata*
Strix aluco		3	1	*Kramerella aluconis*
Tyto alba	Tytonidae	1	1	*Proctophyllodes troncatus**
Apodiformes				
Apus apus	Apodidae	4	2	*Chauliacia securigera* *Eustathia cultrifera* *Neochauliacia minuscula*
Charadriiformes				
Chroicocephalus ridibundus	Laridae	1	1	*Zachvatkinia larica*
Scolopax rusticola	Scolopacidae	1	1	*Proctophyllodes scolopacinus*
Pelecaniformes				
Ardea alba	Ardeidae	1	1	*Ardeacarus ardeae*
Ardea cinerea		1	1	*Scutomegninia phalacrocoracis**
Suliformes				
Phalacrocorax carbo	Phalacrocoracidae	1	1	*Michaelia heteropus*
Columbiformes				
Columba livia	Columbidae	7	2	*Diplaegidia columbae* *Falculifer rostratus*
Streptopelia decaocto		1	1	*Falculifer rostratus*
Passeriformes				
Corvus cornix	Corvidae	3	1	*Gabucinia delibata*
Delichon urbicum	Hirundinidae	1	1	*Pterodectes rutilus*
Phoenicurus ochruros	Muscicapidae	1	1	*Proctophyllodes mesocaulus*
Sylvia atricapilla	Sylviidae	1	1	*Proctophyllodes sylviae*
Turdus merula	Turdidae	2	1	*Proctophyllodes musicus*

Number (n) of examined and no infected birds: *Buteo buteo* (n: 4), *Buteo rufinus* (n: 5) and *Pernis apivorus* (n: 1) from the order Accipitriformes; *Larus michahellis* (n: 3) from the order Charadriiformes; *Ardea purpurea* (n: 1) and *Ciconia ciconia* (n: 7) from the order Pelecaniformes; *Rallus aquaticus* (n: 1) from the order Gruiformes; *Spilopelia senegalensis* (n: 1) from the order Columbiformes; *Erithacus rubecula* (n: 1), *Parus major* (n: 1), *Passer domesticus* (n: 2) and *Turdus philomelos* (n: 1) from the order Passeriformes.
*Contamination.

Negm and Hassan, 2019). Mite specimens were photographed with a microscope integrated camera (Mshot Mdx4-t, Guangzhou, China). The scale bars on all the mite images are given in as micrometers (µm). In addition, all remaining specimens of examined feather mite samples are preserved in Eppendorf tubes with 70% ethanol in the Parasitology laboratory museum where the study has been carried out.

RESULTS AND DISCUSSION

Superfamily Analgoidea Trouessart and Mégnin, 1884

Family Analgidae Trouessart and Mégnin, 1884

Subfamily Megniniinae Gaud and Atyeo, 1982

Genus *Diplaegidia* Hull, 1932

***Diplaegidia columbae* (Buchholz, 1869)**

Materials examined. 4 males and 4 females from flight feathers of the rock pigeon, *Columba livia* Gmelin, 1789 (Columbiformes: Columbidae), Artvin, Türkiye, 14 June 2022, coll. G. Eren; 4 males and 4 females from the same host species, Samsun, Türkiye, 22 February 2022, coll. M. Öztürk.

Remarks. The genus *Diplaegidia* has included seven species so far, and all of them are associated with birds of the order Columbiformes (Černý, 1975; Gaud, 1976). *Diplaegidia columbae*, which we report in this study, has been reported several times from the rock pigeons, *Columba livia*, and its domestic form, *Columba livia domestica*, in previous studies in Türkiye (Merdivenci, 1970). In addition to the studies in Türkiye, *D. columbae*, has been reported so far on birds of the genera *Columba* Linnaeus, 1758 and *Streptopelia* Bonaparte, 1855 in Africa (Gaud 1976), *Zenaida* Bonaparte, 1838 in North and South America (Goulart et al., 2011; Grossi and Proctor, 2021) and also on the *Columba livia* in Europa (Rózsa 1990). The feather mite, *D. columbae*, is a potential source of allergens for domestic pigeon breeders or people who have close contact with pigeons (Fernández-Caldas et al., 2020).

Family Avenzoariidae Oudemans, 1905

Subfamily Bonnetellinae Atyeo and Gaud, 1981

Genus *Scutomegninia* Dubinin, 1951

***Scutomegninia phalacrocoracis* Dubinin and Dubinina, 1940**

Materials examined. 1 male and 1 tritonymph from flight feathers of the grey heron, *Ardea cinerea* Linnaeus, 1758 (Pelecaniformes: Ardeidae), Samsun, Türkiye, 7 February 2022, coll. M. Öztürk (as shown in Fig. 1).

Remarks. The genus *Scutomegninia* includes fourteen described species associated with birds of the order Pelecaniformes (Anhingidae, Phalacrocoracidae, Sulidae Pelecanidae, and Threskiornithidae) (Mironov, 2000). In fact, *Scutomegninia phalacrocoracis* is associated with the great cormorant, *Phalacrocorax carbo*, and its finding on the grey heron in this study is certainly a case of accidental contamination. As a matter of fact, *S. phalacrocoracis* has been reported a few on cormorant *Phalacrocorax* hosts in African, Asia, and Europe (Atyeo and Peterson, 1967; Mironov, 1990).

Genus *Zachvatkinia* Dubinin, 1949

***Zachvatkinia larica* Mironov, 1989**

Materials examined. 2 males and 1 tritonymph from flight feathers of the black-headed gull, *Chroicocephalus ridibundus* (Linnaeus, 1766) (Charadriiformes: Laridae), Samsun, Türkiye, 1 January 2022, coll. M. Öztürk.

Remarks. The genus *Zachvatkinia* currently includes eighteen species that are associated with birds belonging to the order Charadriiformes (Dromadidae, Laridae, Stercorariidae and Sternidae) and Procellariiformes (Diomedeidae, Hydrobatidae, Oceanitidae, and Procellariidae) (Negm et al., 2013). In studies conducted to date, *Zachvatkinia larica* has been reported from over 20 species and subspecies of gulls worldwide (Asia, Europe, and America) (Mironov, 1989; Han et al., 2016). In Türkiye, this mite species has been reported so far only from the yellow-legged gull, *Larus michahellis* Naumann, JF, 1840 (Eren et al., 2022).

Family Proctophyllodidae Mégnin and Trouessart, 1884

Subfamily Pterodectinae Park and Atyeo, 1971

Genus *Pterodectes* Robin, 1877

***Pterodectes rutilus* Robin, 1877**

Materials examined. 4 males from flight feathers of the common house martin, *Delichon urbicum* (Linnaeus, 1758) (Passeriformes: Hirundinidae), Samsun, Türkiye, 21 June 2022, coll. M. Öztürk (as shown in Fig. 2).

Remarks. The genus *Pterodectes* is monotypic, and its only representative, *Pterodectes rutilus*, is a cosmopolitan mite occurring on swallows (Hirundinidae) worldwide (Asia, Africa, Europe, and South America). Although this mite species most commonly occurs on the barn swallow, *Hirundo rustica* Linnaeus, 1758, in the studies conducted so far, it has also been reported on the following of hirundinids: *Atticora melanoleuca* (Wied, 1820), *D. urbicum* (Linnaeus, 1758), *H. nigrita* Gray, 1845, *Riparia riparia* (Linnaeus, 1758), *R. paludicola* (Vieillot, 1817), and *Stelgydopteryx ruficollis* (Vieillot, 1817) (Valim and Hernandes, 2008). In our study, *P. rutilus* from *D. urbicum* has been reported for the first time in Türkiye.

Subfamily Proctophyllodinae Mégnin and Trouessart, 1884

Genus *Proctophyllodes* Robin, 1877

Remarks. The genus *Proctophyllodes* with 184 described species is most species-rich genus among all feather mite families (Mironov, 2012; 2019; Pedroso and Hernandes, 2021). Mites of this genus are mainly associated with birds of the order Passeriformes, with a few species from birds of the orders Apodiformes, Charadriiformes and Piciformes (Atyeo and Braasch, 1966; Mironov and Hallan, 2022). In studies previously conducted in Türkiye, ten species of this genus, *Proctophyllodes cetti* Badek, Mironov and Dabert, 2008, *P. clavatus* Fritsch, 1961, *P. doleophyes* Gaud, 1957, *P. lusciniae* Burdejnaja and Kivganov, 2009a, *P. mesocaulus* Mac-Fira and Cristea, 1968, *P. rubeculinus* (Koch, 1941), *P. scolopacinus* Vitzthum, 1929, *P. stylifer* (Buckholz 1869), *P. sylviae* Gaud, 1957 and *P. troncatus* Mégnin, 1877, have been reported so far (Eren and Açıcı, 2022).

***Proctophyllodes mesocaulus* Mack-Fira and Cristea-Nastasescu, 1968**

Materials examined. 4 males and 4 females from flight feathers of the black redstart, *Phoenicurus ochruros* (Gmelin, 1774) (Passeriformes: Muscicapidae), Artvin, Türkiye, 21 January 2022, coll. G. Eren.

Remarks. The feather mite *Proctophyllodes mesocaulus* was previously known only from the common redstart, *Phoenicurus phoenicurus* (Linnaeus, 1758), in Europe (Romania) (Mack-Firă and Cristea-Năstăsescu, 1968; Mironov et al., 2022). The finding of *Proctophyllodes mesocaulus* on *Ph. ochruros* is a new host record for this mite.

***Proctophyllodes musicus* Vitzthum, 1922**

Materials examined. 4 males and 4 females from flight feathers of the common blackbird, *Turdus merula* Linnaeus, 1758 (Passeriformes: Turdidae), Artvin, Türkiye, 22 June 2022, coll. G. Eren (as shown in Fig. 2).

Remarks. The feather mite *Proctophyllodes musicus* is restricted to thrushes of the genus *Turdus* (Turdidae) and it has been reported from birds of this genus in Africa, Asia, and Europe (Atyeo and Braasch, 1966). *Proctophyllodes musicus* on the common blackbird, *Turdus merula*, is reported herein for the first time in Türkiye.

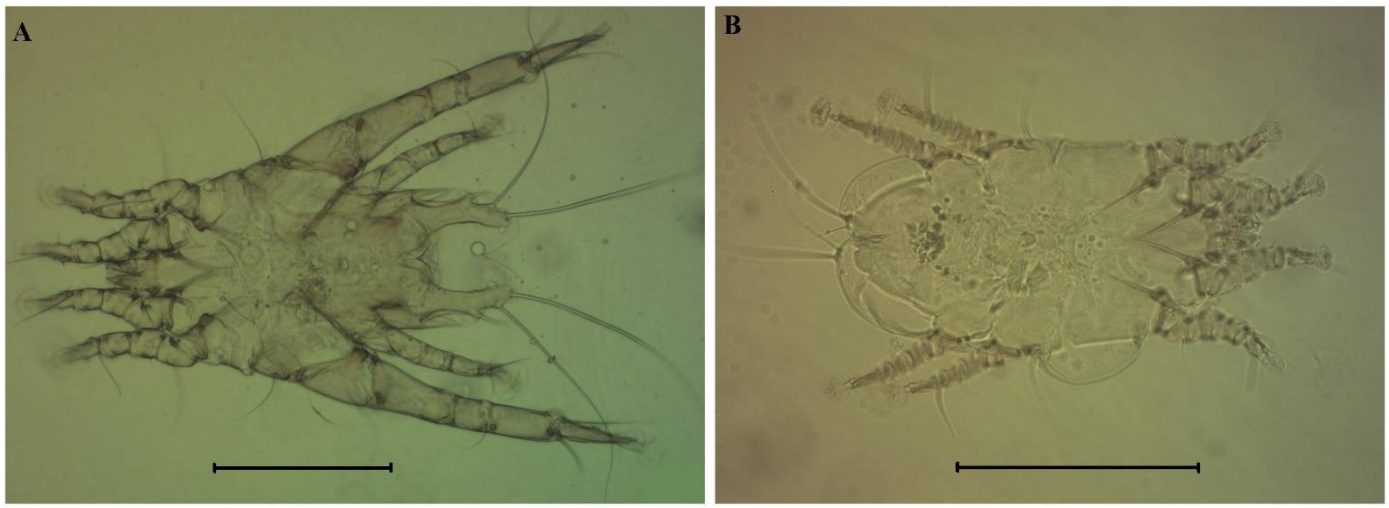

Figure 1. *Scutomegninia phalacrocoracis*—**A.** Male, **B.** Tritonymph (scale bars: 200).

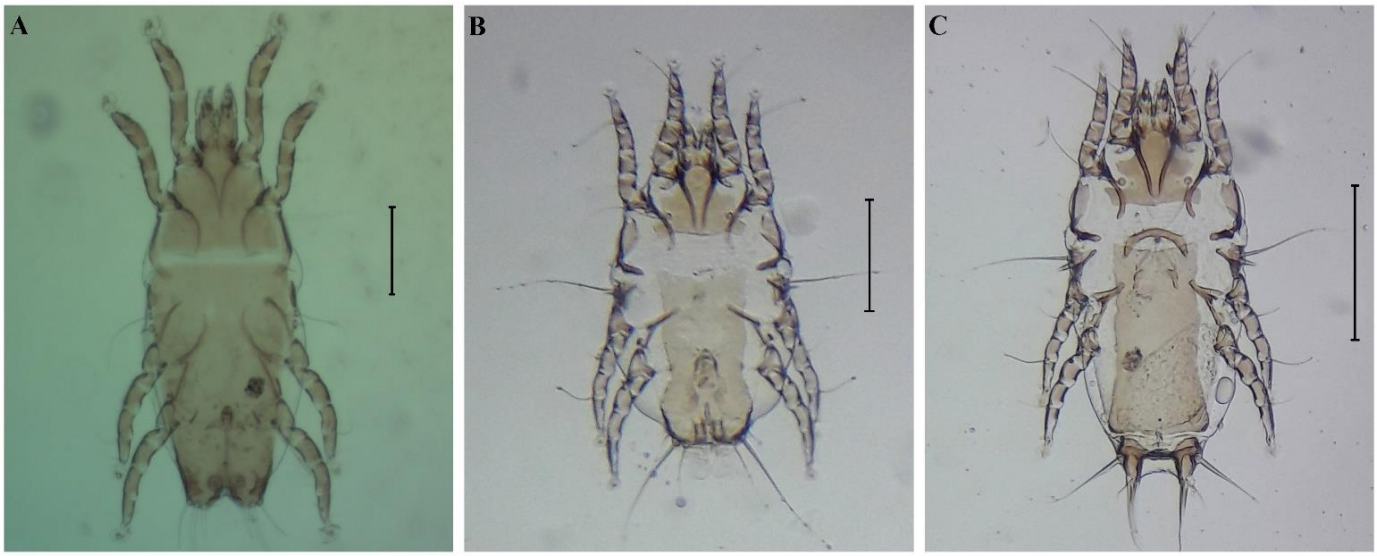

Figure 2. Mites of the family Proctophyllodidae—**A.** *Pterodectes rutilus*, male (scale bar: 100), **B-C.** *Proctophyllodes musicus*, male (scale bar: 200) and female (scale bar: 100).

***Proctophyllodes scolopacinus* (Koch, 1842)**

Materials examined. 4 males and 4 females adult from flight feathers the Eurasian woodcock, *Scolopax rusticola* Linnaeus, 1758 (Charadriiformes: Scolopacidae), Samsun, Türkiye, 27 January 2022, coll. M. Öztürk.

Remarks. The feather mite *Proctophyllodes scolopacinus* is associated with the Eurasian woodcock, *S. rusticola*, in Europe and Asia, and the American woodcock, *Scolopax minor*, in North America and (Atyeo and Braasch, 1966). In studies previously conducted in Türkiye, *P. scolopacinus* from the Eurasian woodcock, *S. rusticola* has been reported (Gürler et al., 2013).

***Proctophyllodes sylviae* Gaud, 1957**

Materials examined. 4 males and 4 females from flight feathers of the Eurasian blackcap, *Sylvia atricapilla*; (Passeriformes: Sylviidae), Samsun, Türkiye, 30 May 2022, coll. M. Öztürk.

Remarks. The feather mite *Proctophyllodes sylviae* is associated with the Eurasian blackcap, *Sylvia atricapilla*, in Europa and Africa (Gaud, 1957; Atyeo and Braasch, 1966). In studies previously conducted in Türkiye, *P. sylviae* from the common reed warbler *Acrocephalus scirpaceus*, the Cetti's warbler *Cettia cetti*, the common chiffchaff *Phylloscopus collybita*, the Eurasian blackcap *Sylvia atricapilla*, the garden warbler *S. borin*, the common whitethroat *S. communis*, the lesser whitethroat *S. curruca*, and the Sardinian warbler *S. melanocephala* has been reported (Gürler et al., 2013; Per and Aktaş, 2018).

***Proctophyllodes troncatus* Robin, 1877**

Materials examined. 1 male and 1 tritonymph from flight feathers the barn owl, *Tyto alba* (Strigiformes: Tytonidae), Samsun, Türkiye, 1 January 2022, coll. by M. Öztürk.

Remarks. The feather mite *Proctophyllodes troncatus* is associated with the sparrows of the genus *Passer* (Passeridae) in worldwide (Europa, Asia, Africa, and America) (Atyeo and Braasch, 1966; Gaud and Atyeo, 1976), and the finding on the barn owl, *Tyto alba*, in this study is most probably a case of contamination most probabaly caused by the interaction between prey and predator. In studies previously conducted in Türkiye, *P.*

troncatus from the house sparrow *Passer domesticus* and the Spanish sparrow *P. hispaniolensis* has been reported (Gürler et al., 2013).

Superfamily Pterolichoidea Trouessart and Mégnin, 1984

Family Eustathiidae Oudemans, 1905

Remarks. The family Eustathiidae is restricted to swifts (Apodiformes: Apodidae and Hemiprocnidae). Up to now, this family has included 63 described species in 16 genera (Kwanyuen, 1973; Peterson et al., 1980). In studies conducted so far, three aforementioned eusththiid species, *Eustathia cultrifera*, *Chauliacia securigera*, *Neochauliacia minuscula*, and also *Thysanocercus cypseli* (Canestrini and Berlese, 1881) (Analgoidea: Thysanocercidae) are known to be common on *Apus apus* (Kwanyuen, 1973; Peterson et al., 1980; Gaud and Peterson, 1987).

Genus *Chauliacia* Oudemans, 1905

***Chauliacia securigera* (Robin, 1877)**

Materials examined. 2 males from flight feathers of the common swift, *Apus apus* (Linnaeus, 1758) (Apodiformes: Apodidae), Samsun, Türkiye, 10 May 2022, coll. M. Öztürk (as shown in Fig. 3).

Remarks. The genus *Chauliacia* includes six described species associated with birds of the order Apodiformes in the Old World (Europe, Africa, and Asia) and the New World (North America and South America). In the previous studies conducted in world, *C. securigera* has been reported on the common swift *Apus apus*, the white-rumped swift *A. caffer*, the Horus swift *A. horus*, the Pacific swift *A. pacificus*, the pallid swift *A. pallidus*, the plain swift *A. unicolor*, and the African palm swift *Cypsiurus parvus* (Kwanyuen, 1973; Peterson et al., 1980). In the present study, three eustathiid species detected on *A. apus*, are new records for feather mite fauna in Türkiye.

Genus *Eustathia* Oudemans, 1905

***Eustathia cultrifera* (Robin, 1877)**

Materials examined. 2 males from flight feathers of the common swift, *Apus apus* (Linnaeus, 1758) (Apodiformes: Apodidae), Samsun, Türkiye, 10 May 2022, coll. M. Öztürk (as shown in Fig. 3).

Remarks. The genus *Eustathia* includes nine described species associated with birds of the order Apodiformes in the Old World (Europe, Africa, and Asia) and the New World (North America and South America). In the previous studies conducted in world, *Eustathia cultrifera* has been reported on the African black swift *Apus barbatus*, the common swift *Apus apus*, the little swift *A. affinis*, the white-rumped swift *A. cafferhas*, the Horus swift *A. horus*, the Pacific swift *A. pacificus*, and alpine swift *Tachymarptis* (*Apus*) *melba* been reported (Kwanyuen, 1973; Peterson et al., 1980).

Genus: *Neochauliacia* Gaud & Atyeo, 1967

***Neochauliacia minuscula* Gaud and Atyeo, 1967**

Materials examined. 2 males from flight feathers of the common swift, *Apus apus* (Linnaeus, 1758) (Apodiformes: Apodidae), Samsun, Türkiye, 10 May 2022, coll. M. Öztürk (as shown in Fig. 3).

Remarks. The genus *Neochauliacia* includes sixteen described species associated with birds of the order Apodiformes in the Old World (Europe, Africa, and Asia) and the New World (North America and South America). In the previous studies conducted in world, *N. minuscula* has been reported on the common swift *Apus apus*, the African black swift *A. barbatus*, the pallid swift *A. pallidusthe*, and alpine swift *Tachymarptis* (*Apus*) *melba* has been reported (Kwanyuen, 1973; Peterson et al., 1980).

Family Falculiferidae Oudemans, 1905

Genus *Falculifer* Railliet, 1896

***Falculifer rostratus* (Buchholz, 1869)**

Materials examined. 4 males and 4 females from flight feathers of the rock pigeon *Columba livia* Gmelin, 1789 (Columbiformes: Columbidae), Artvin, Türkiye, 14 June 2022, coll. G. Eren; 2 males and 2 females from the same host species, Samsun, Türkiye, 22 February 2022, coll. M. Öztürk; 4 males and 4 females from flight feathers of the Eurasian collared dove, *Streptopelia decaocto* (Frivaldszky, 1838) (Columbiformes: Columbidae), Sakarya, Türkiye, 20 July 2022, coll. G. Eren.

Remarks. The genus *Falculifer* includes ten described species associated with birds of the order Columbiformes in the Old World (Europe, Africa, and Asia) and the New World (North America, South America, and the West Indies) (Trouessart, 1898; Gaud, 1976; Gaud and Atyeo, 1976; Gaud and Barré, 1992). In the previous studies conducted in Türkiye, *Falculifer rostratus* has been reported on the rock pigeons, *Columba livia*, and its domestic form, *C. l. domestica* (Eren and Açıcı, 2022). The Eurasian collared dove *Streptopelia decaocto* is a new host record for *Falculifer rostratus* in Türkiye, although formerly it was reported from doves *Streptopelia* spp. and pigeons *Columba* spp. in the Africa (Egypt and Morocco), America (Brazil, Chile, and United States of America), Asia (China, India, Korea, and Russia), Europe (Bulgaria, France, Greece and Italy) (Gaud and Atyeo, 1976).

Family Freyanidae Dubinin, 1953

Subfamily Michaelichinae Gaud and Mouchet, 1959

Genus *Michaelia* Trouessart, 1884

***Michaelia heteropus* (Michael, 1881)**

Materials examined. 2 males and 2 females from flight feathers of the great cormorant, *Phalacrocorax carbo* (Linnaeus, 1758) (Pelecaniformes: Phalacrocoracidae), Samsun, Türkiye, 19 January 2022, coll. M. Öztürk (as shown in Fig. 4).

Remarks. The genus *Michaelia* includes five described species, and all of them are associated with

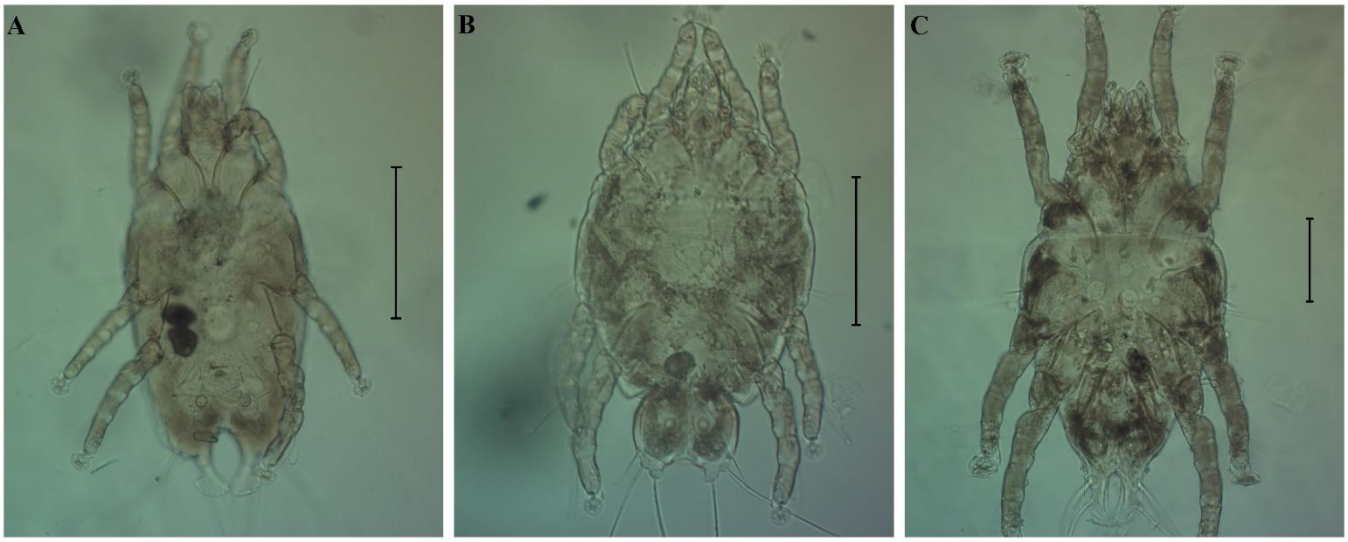

Figure 3. Mites of the family Eustathiidae—**A.** *Chauliacia securigera*, male, **B.** *Neochauliacia minuscula*, male, C. *Eustathia cultrifera*, male (scale bars: 100).

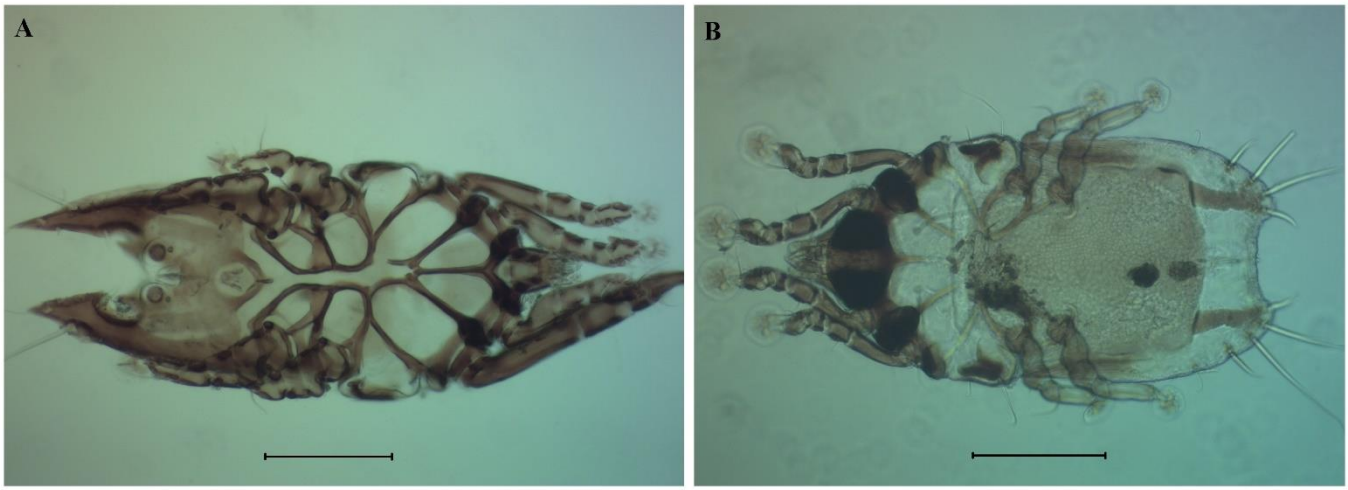

Figure 4. *Michaelia heteropus*—**A.** Male, **B.** Female (scale bars: 200).

cormorants (Phalacrocoracidae) in Africa, America, Asia, and Europe (Dubinin, 1953; Gaud and Atyeo, 1982; Hernandes et al., 2016). In the present study, *Michaelia heteropus* on the great cormorant, *Phalacrocorax carbo*, is reported for the first time in Türkiye.

Family Gabuciniidae Atyeo and Gaud, 1975

Genus *Gabucinia* Oudemans, 1905

***Gabucinia delibata* (Robin, 1877)**

Materials examined. 1 female and 1 tritonymph from flight feathers of the hooded crow, *Corvus cornix* Linnaeus, 1758 (Passeriformes: Corvidae), Samsun, Türkiye, 22 February 2022, coll. M. Öztürk (as shown in Fig. 5).

Remarks. All three presently known species of the genus *Gabucinia* —*Gabucinia delibata* (Robin, 1877), *G. gladiscapulata* Gaud, 1960 and *G. neotropica* Hernandes, 2020— are associated with crows Corvidae (Passeriformes) (Mironov et al., 2014). *Gabucinia delibata*, has been reported so far on hosts belonging to the genera *Corvus*, *Coloeus*, and *Pica* across the Holarctic region (Negm and Hassan, 2019; Hernandes, 2020); in the present study, it is reported on the hooded crow, *Corvus cornix*, for the first time in Türkiye.

Family Kramerellidae Gaud and Mouchet, 1961

Genus *Kramerella* Trouessart, 1916

***Kramerella aluconis* (Lönnfors, 1937)**

Materials examined. 3 males and 3 females from flight feathers of the tawny owl, *Strix aluco* Linnaeus, 1758 (Strigiformes: Strigidae), Artvin, Türkiye, 22 March 2022, coll. G. Eren (as shown in Fig. 6).

***Kramerella lunulata* (Haller, 1878)**

Materials examined. 1 male and 1 tritonymph from flight feathers of the little owl, *Athene noctua* (Scopoli, 1769) (Strigiformes: Strigidae), Samsun, Türkiye, 20 February 2022, coll. M. Öztürk (as shown in Fig. 6).

Remarks. The genus *Kramerella* currently includes 10 species associated with birds of the orders Strigiformes

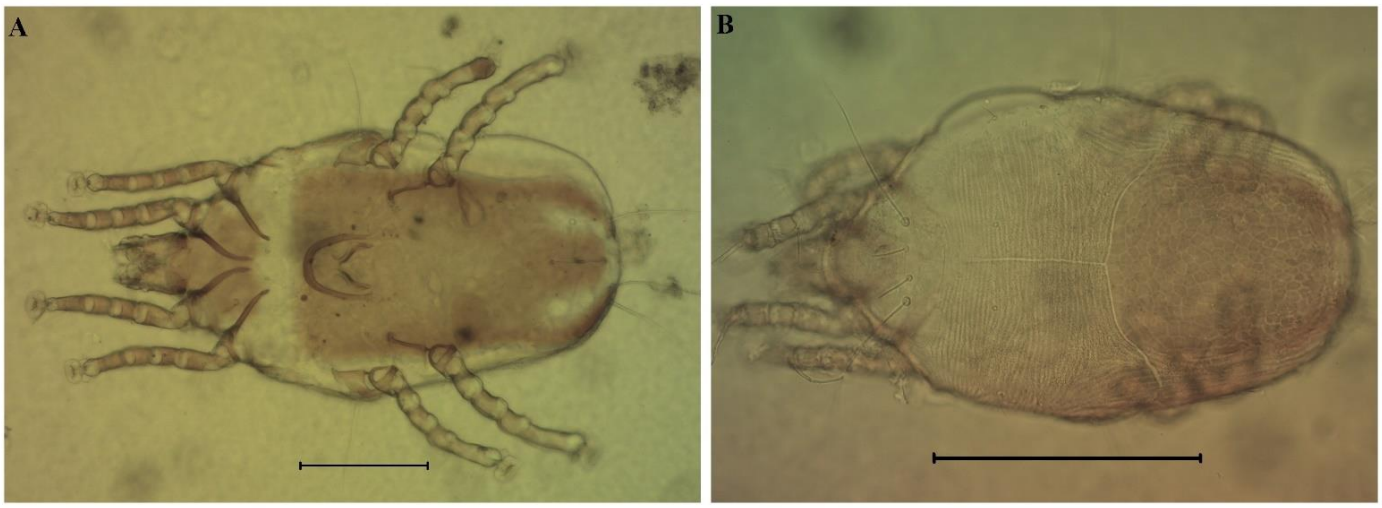

Figure 5. *Gabucinia delibata*—**A.** Female, **B.** Tritonymph (scale bars: 100).

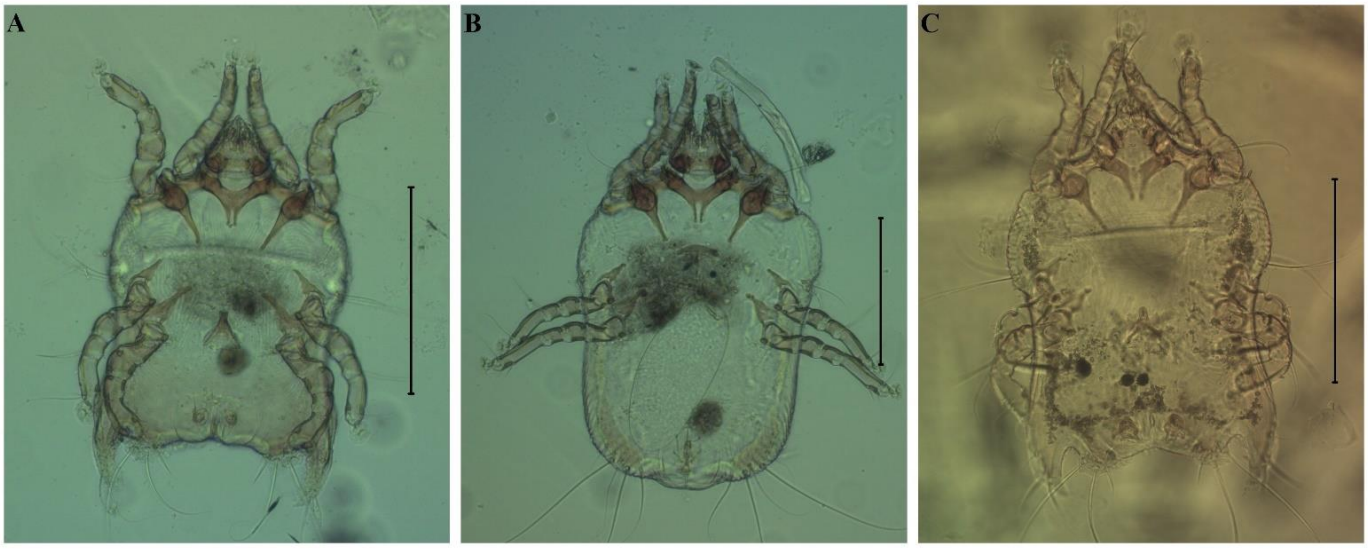

Figure 6. *Kramerella* species—**A-B.** *Kramerella aluconis*, male and female, **C.** *Kramerella lunulata*, male (scale bars: 100).

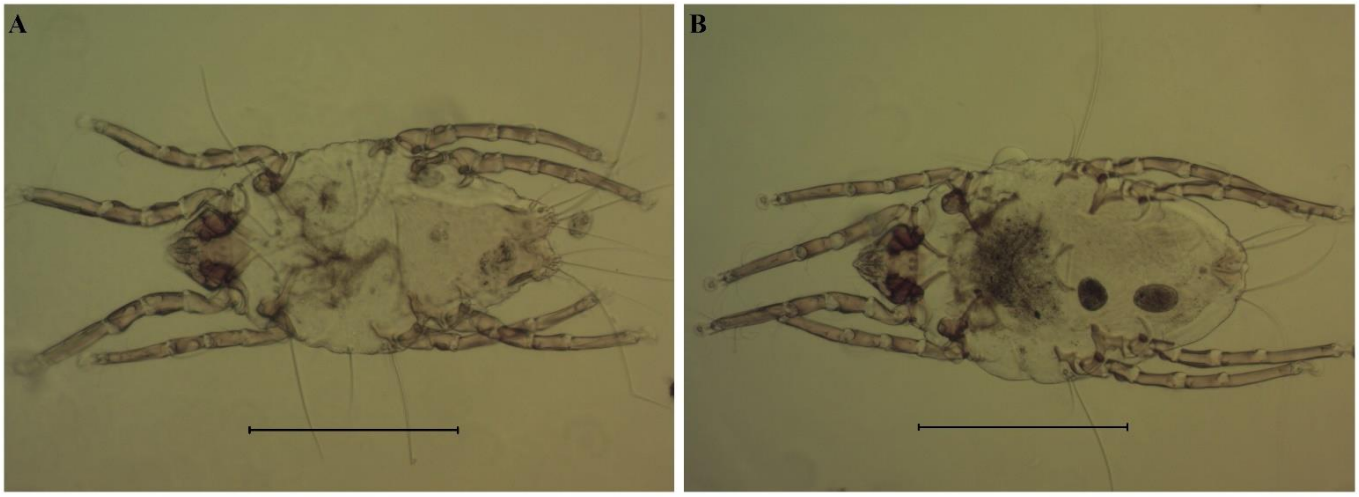

Figure 7. *Ardeacarus ardeae*—**A-B.** Male and female (scale bars: 200).

(Dubinin, 1953; Gaud, 1980; Gaud and Atyeo, 1996; Černý and Wiesner, 1992). In the previous studies in Africa and Eurasia, *Kramerella aluconis* was found on *Strix aluco*, and *K. lunulata* was formally reported from several owl species, *Athene noctua* (Scopoli, 1769), *Bubo lacteus* (Temminck, 1820), *Otus scops* (Linnaeus, 1758), and *Tyto alba* (Scopoli, 1769), as was summarized by Philips (2000). However, all subspecies, formerly recognized in *K. lunulata* and restricted to particular genera of owls (Dubinin, 1953), at present are considered full species (Gaud, 1980). Therefore, the reports of *K. lunulata* on hosts other than owls of the genus *Athene*, quite probabaly represent

records of other *Kramerella* species. *Kramerella aluconis* on *S. aluco* and *K. lunulata* on its type host, *A. noctua*, are reported herein for the first time in Türkiye.

Family Pterolichidae Trouessart and Mégnin, 1884

Subfamily Ardeacarinae Gaud, 1981

Genus *Ardeacarus* Dubinin, 1951

***Ardeacarus ardeae* (Canestrini, 1878)**

Materials examined. 1 male and 1 female from flight feathers of the great egret, *Ardea alba* Linnaeus, 1758 (Pelecaniformes: Ardeidae), Samsun, Türkiye, 2 February 2022, coll. M. Öztürk (as shown in Fig. 7).

Remarks. The genus *Ardeacarus* includes only one species, *Ardeacarus ardeae*, having a cosmopolitan distribution (Africa, Asia, Europe, and America), has been reported so far on herons of the genera *Ardea*, *Bubulcus*, *Butorides*, *Egretta*, *Ixobrychus*, and *Nycticorax* (Pelecaniformes: Ardeidae) (Dubinin, 1956; Černý, 1967; Gaud, 1981; Han et al., 2016). *Ardeacarus ardeae* on the great egret, *Ardea alba*, is reported herein for the first time in Türkiye.

Considering the investigations of the feather mite fauna conducted in Türkiye, it is remarkable that these studies were mainly carried out in coastal provinces of the Black sea, especially Samsun province (Gürler et al., 2013; Per and Aktaş, 2018). Other countries bordering the Black Sea seem to have similar ornithofauna with Türkiye according to checklists provided by the Avibase - World Bird Database (Lepage, 2022), and therefore it would not be wrong to predict that the feather mites faunas of the countries around the Black Sea will also show similarity. The studies carried out so far in these countries reported: about 300 feather mite species in entire Russia and the former USSR (Dubinin, 1953; 1956; Mironov, 1996; Mironov et al., 2022), including 146 species from only passerines in the coastal areas of Russian Black sea (Mironov et al., 2022); over 160 species in Ukraine (Burdejnaja and Kivganov, 2009a-c; 2011a,b; Kivganov and Chernichko, 2007; 2009a,b; 2012); over 150 species in Bulgaria (Kolarova, 2015; Kolarova and Ilieva, 2021), over 30 species in Romania (Constantinescu et al., 2013), and only 2 feather mites in Georgia/Sakartvelo (Bauer, 1939). The diversity of feather mite fauna in Türkiye, including to date 56 species in 34 genera and 15 families, takes the fourth places among the countries having a coast to the Black Sea (Eren and Açıcı, 2022; Eren et al., 2022; present study). De facto, when is compared the number of bird species in ornithoofaunas of the relevant countries according to the eBird database (2022), Türkiye places the second, with 496 bird species (versus Russia: 690; Bulgaria: 389; Georgia: 361; Ukraine: 361; Romania: 324). The main reason for the relatively low number of recovered feather mite fauna in Türkiye, comparing to the richness of its ornithofauna, is the incomparably limited number of parasitological studies of avian hosts conducted in this country. Although the first feather mite report in Türkiye dates back to the 1970s, the number of studies conducted in the past 50 years does not exceed a dozen. With this study, which is a continuation of the feather mite fauna studies in Türkiye, we aimed to contribute to ornitho-parasitological investigations.

Authors' contributions

Gökhan Eren: Conceptualization (supporting), data curation (supporting), formal analysis (lead), visualization (supporting), writing - original draft. **Mehmet Öztürk:** Investigation (equal), writing - review & editing (equal). **Sergey V. Mironov:** Conceptualization (lead), data curation (lead), formal analysis (supporting), investigation (equal), methodology, visualization (lead), supervision, writing - review & editing (equal). **Hatice Özlem Nisbet:** Investigation (equal), writing - review & editing (equal). **Mustafa Açıcı:** Investigation (equal), writing - review & editing (equal).

Statement of ethics approval

Ethical approval is not required as the study material consists of parasite samples collected from dead birds brought to the Parasitology Laboratory, Department of Parasitology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Türkiye.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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RESEARCH ARTICLE

Eustigmaeus absens (Acari: Stigmaeidae) türünden elde edilen kitin ve Ag-dekore edilmiş kitin nanokompozit: İzolasyonu, karakterizasyonu ve antibakteriyel aktivitesi

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ÖZET: *Eustigmaeus absens* Doğan (Acari: Stigmaeidae) akar türünden kimyasal yöntemle 3D kitin elde edildi ve elde edilen kitin üzerine gümüş nanoparçacıkları dekore edildi. Elde edilen kitinler seyreltilmiş toplam yansıma-fourier dönüşümlü infrared spektroskopisi (ATR-FTIR), taramalı elektron mikroskobu (SEM), enerji dağılımlı X-ışını spektrometresi (EDX) ve geçirimli elektron mikroskobu (TEM) analizleriyle karakterize edildi ve antibakteriyel aktiviteleri değerlendirildi. Elde edilen kitinin yapısında karbon (C), azot (N), oksijen (O) elementlerinin yer aldığı, eser miktarda kalsiyum (Ca) elementinin bulunduğu belirlendi. ATR-FTIR analiziyle α-kitin için karakteristik olan amid-I ve amid-II bantları gözlemlendi. SEM görüntüleri kitin yüzeyinin makro gözenekler, mikro gözenekler ve kesik nanoliflerden oluştuğunu açığa çıkardı. TEM analizleri gümüş nanoparçacıkların boyutlarının 6-20 nm arasında değiştiğini gösterdi. Stereo mikroskop ve faz-kontrast donanımlı ışık mikroskobundan alınan görüntülerle organizmanın üç boyutlu yapısını bozmadan kitinin elde edildiğini gösterdi. Ayrıca gümüş nanoparçacıklı kitinin *Escherichia coli* ATCC 25922 ve *Staphylococcus aureus* ATCC 29213 bakterilerine karşı antibakteriyel aktivite sergilediği tespit edildi.

Anahtar Kelimeler: Akar, 3D kitin, gümüş-kitin nanokompozit, SEM, TEM, FTIR, antibakteriyel aktivite.

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Chitin and Ag-decorated chitin nanocomposite obtained from *Eustigmaeus absens* (Acari: Stigmaeidae): Isolation, characterization and antibacterial activity

ABSTRACT: 3D chitin was obtained from the mite species *Eustigmaeus absens* Doğan (Acari: Stigmaeidae) by chemical method and silver nanoparticles were decorated on the obtained chitin. The resulting chitins were characterized by using attenuated total reflectance-Fourier Transform infrared spectroscopy (ATR-FTIR), scanning electron microscope (SEM), energy dispersive X-ray spectrometry (EDX) and transmission electron microscopy (TEM) and their antibacterial activities were evaluated. It was determined that the obtained chitin from *E. absens* contains carbon (C), nitrogen (N), oxygen (O) elements and trace amount of calcium (Ca) element. The characteristic amide-I and amide-II bands for α-chitin were observed by ATR-FTIR analysis. SEM images revealed that the surface of the chitin consists of macropores, micropores and broken nanofibers. TEM analysis showed that the sizes of silver nanoparticles differed between 6-20 nm. Images taken from the stereo microscope and the phase-contrast equipped light microscope showed that the chitin was obtained without disturbing the 3D structure of the organism. In addition, it was defined that silver nanoparticle decorated chitin exhibited antibacterial activities against the bacteria *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213.

Keywords: Mite, 3D chitin, silver-chitin nanocomposite, SEM, TEM, FTIR, antibacterial activity.

GİRİŞ

Kitin, Yunanca zırh anlamına gelen "*kiton*" kelimesinden gelmektedir ve ilk kez Fransız bilim insanı Henri Braconnot tarafından 1811 yılında mantardan keşfedilmiştir. Daha sonra 1823'de Fransız bilim insanı Odier, böcek kutikulasını potasyum hidroksit ile muamele ederek böceklerde kitini keşfetmiştir (Koçer, 2015). Kitin, tabiatta selülozdan sonra en bol bulunan; sert, beyaz, elastik olmayan, azotlu bir biyopolimerdir ve canlıların %70'inden fazlasının yapısında bulunmaktadır (Kumar, 2000). Kitin, yengeç, karides ve ıstakoz gibi birçok organizmanın dış kabuklarından, mantarların ve alglerin hücre duvarlarından, mercan, sünger ve böceklerin ise dış iskeletlerinden izole edilebilmektedir (Çakmak ve Koç Bilican, 2021). Kitin hidrofobik yapıdadır ve güçlü hidrojen bağları içerir. Bu sebeple her türlü çözücüde çözünmez. Mineral asitlerin seyreltik çözeltileriyle birleşen hekzafloroaseton, kloroalkoller ve hekzafloroizopropanol ile %5-8 lityum klorür içeren dimetilasetamit gibi toksik özelliği yüksek olan çözücülerde çözünür (Koçer, 2015). Doğada çok yaygın olarak bulunan ve bir aminopolisakkarit olan kitinin,

ticari amaçlarla selüloz kadar üretildiği düşünülmektedir. Kitin, biyolojik olarak parçalanabilmesi, doğal bir kaynak olması ve çevre kirliliğine sebebiyet vermemesi, hem hayvan hem de bitki dokuları için uygun olması, toksik etkisinin olmaması, biyolojik olarak fonksiyonel bir bileşik olması, molekül yapısının dönüştürülebilmesi nedeniyle endüstriyel alanda aktif bir şekilde kullanılmaktadır (Seyyar ve Demir, 2020). Bu özelliklerin sonucu olarak kozmetikte, atık su arıtımında, ameliyat ipliği imalatında, yenilebilir biyofilm üretiminde, sağlıklı zayıflama haplarında ve kontrollü ilaç salınımında yaygın olarak kullanılmaktadır. Kitin ayrıca antimikrobiyal, antioksidan, antitümör ve antikanser çalışmalarında da yer almaktadır (Kaya vd., 2014a).

Eustigmaeus absens Doğan (Şekil 1) Stigmaeidae familyasında yer almakta ve sadece Türkiye'den bilinmektedir. Trombidiformes takımının Raphignathoidea üst familyasında yer alan bu familya raphignatoidlerin en zengin grubu olup, oldukça geniş bir yayılış göstermektedir. Türlerinin çoğu Nearktik, Palearktik, Oryantal, Afrotropikal ve Avustralya bölgelerinden kaydedilmiştir (Fan ve Zhang, 2005; Doğan ve Doğan, 2020). Stigmaeid akarlar günümüzde 33 cins ve 600'den fazla türle temsil edilmektedir (Fan vd., 2016, 2019; Doğan ve Doğan, 2020). Şu ana kadar Türkiye'den bu familyanın 11 cinsi tespit edilmiş olup, *Eustigmaeus* (Berlese) cinsinden 28 türün kaydı/tanımı verilmiştir (Doğan, 2019; Doğan ve Doğan, 2020).

Şekil 1. *Eustigmaeus absens* (dişi). Dorsal görünüm.

Eustigmaeus absens ilk defa Doğan (2005) tarafından Erzurum'dan tanımlanmış daha sonra Kelkit (Dönel ve Doğan, 2011) ve Harşit Vadilerinden (Dilkaraoğlu vd., 2016) kaydı verilmiştir. Şu ana kadar sadece ülkemizden bilinen ve bazı katalog ve kontrol listelerinde (Doğan, 2007, 2019; Erman vd., 2007; Fan vd., 2016) geçen bu tür, histerozoma plağının 5 çift kıl taşımasıyla cinsin diğer türlerinden ayrılmaktadır. Ayrıca III. bacağın trokanterinde bir kıl bulunması ve sırt plaklarının çokgenimsi desenlere sahip olmasıyla yakın türlerden kolayca ayırt edilebilir (Doğan, 2005). Yaşama alanı olarak daha çok nemli ve çimenli yosunları tercih eden bu türün beslenme şekli bilinmemekle birlikte, cinsin diğer çoğu üyesi gibi predatör olduğu düşünülmektedir.

Bu çalışmada; *Eustigmaeus absens* türünden kimyasal yöntemle kitin elde edildi, elde edilen kitin gümüş nanoparçacıklarla dekore edilerek kitin-nanokompozit sentezlendi. Daha sonra kitinin ve gümüş (Ag)-dekore edilmiş kitin-nanokompozitin yapısal ve morfolojik özellikleri aydınlatıldı ve bunların *Escherichia coli* ATCC 25922 ve *Staphylococcus aureus* ATCC 29213 bakteri türlerine karşı antibakteriyel aktiviteleri araştırıldı. Bu çalışma ile daha önce kitin eldesi ile ilgili üzerinde hiç çalışılmamış olan *E. absens* türünün kitin özellikleri açığa çıkarılarak sonraki çalışmalara ışık tutması amaçlanmıştır.

MATERYAL VE YÖNTEM

Eustigmaeus absens Doğan örneklerinden elde edilen kitin, çalışmanın ana materyalini oluşturmaktadır. Çalışma süresince kullanılan malzemelere aşağıda yer verilmiştir.

Kullanılan kimyasal malzemeler

- Laktik asit, akar örneklerinin ağartılması ve temizlenmesinde %60 saflıkta kullanıldı.
- Hoyer eriyiği; 50 ml saf su, 200 g kloralhidrat, 20 ml gliserin ve 30 g kristal arap zamkı karıştırılarak elde edildi. Akarların preparasyon işleminde kullanıldı.
- Değiştirilmiş Hoyer eriyiği, kitinlerin preparatını yapmak için saf suyla %50 seyreltilmiş Hoyer kullanıldı.
- $AgNO_3$, elde edilen kitinler üzerinde gümüş nanopartiküller büyütmek için 10 mM kullanıldı.
- Etil alkol, akarların elde edilmesi aşamasında %70 saflıkta, muhafaza edilmesinde %95 saflıkta kullanıldı.
- HCl, kitin eldesi aşamasında akarların yapısında bulunan minerallerin uzaklaştırılması için 0,1 M, 0,5 M, 1 M, 1,5 M ve 2 M kullanıldı.
- Kloroform-metanol-su karışımı, organizmanın yapısındaki lipitlerin uzaklaştırılması için kullanıldı.
- NaOH, akarların yapısında bulunan proteinlerin uzaklaştırılması için 0,1 M, 0,5 M, 1 M, 1,5 M ve 2 M kullanıldı.
- Saf su, akarların partiküllerinden arındırılmasında ve elde edilen kitinlerin sıralı işlemler yapılana dek muhafaza edilmesinde kullanıldı.

Kullanılan cihazlar

- Akarların ayıklanması Leica EZ4 stereo mikroskopta yapıldı.

- Preparatları yapılan akar örneklerinin teşhisi için Leica DM500 model ışık mikroskobu kullanıldı.
- Elde edilen kitinler JSON-100 markalı etüvde kurutuldu.
- Tartım işlemleri için Ohaus markalı hassas terazi kullanıldı.
- Elde edilen kitinlerin morfolojik görüntüleri Nikon SMZ25 stereo mikroskoba entegre DS-Ri2 model dijital kamera aracılığı ile alındı.
- Kitinlerin preparatları DM 4000B model faz-kontrast donanımlı ışık mikroskobunda incelendi.
- Elde edilen kitinlerin üzerine gümüş nanopartiküllerin dekore edilmesi için 8 watt gücünde 6 adet UVA lambalı Luzchem LZC-4X fotoreaktör kullanıldı.
- Elde edilen kitinlerin ayrıntılı yüzey morfolojisi hakkında bilgi sağlamak için Quanta FEG 450-FEI alan emisyon kaynaklı taramalı elektron mikroskobu (SEM) ve HITACHI HT-7700 geçirimli elektron mikroskobu (TEM) kullanıldı.
- Kitinlerin element analizi enerji dağılımlı X-Işını spektofotometresiyle (EDX) ile belirlendi.
- Kitinlerin ATR-FTIR spektrumları Thermo Nicolet 6700 spektrometresiyle kaydedildi.

Akarların eldesi, preparasyonu ve teşhisi

Bu çalışmada, daha önce FBA-2019-642 numaralı proje kapsamında Sansa Boğazı'ndan toplanan *Eustigmaeus absens* örnekleri kullanıldı. *E. absens* türünün Sansa Boğazı'nın sınırları içinde, bir akarsu yakınlarında (39°33'30,9''K 40°07'11,4''D) nemli, çimenli ve yosunlu alanlarda yaşadığı belirlendi. Arazide naylon torbalara konularak etiketlenip, laboratuvara getirilen örnekler birleştirilmiş Berlese-Tullgren hunilerinden oluşan ayıklama düzeneğine yerleştirildi. Işık kaynağı en az 7 gün süreyle açık bırakıldı. Berlese-Tullgren hunilerinin alt tarafına yerleştirilen ve içinde %70'lik etil alkol içeren toplama şişelerine biriktirilen organizmalar Petri kaplarına boşaltıldıktan sonra stereo mikroskop altında ayıklandı.

Akarların eldesi ve preparasyonu konusunda Walter ve Krantz (2009)'un eserinden yararlanıldı. Tür teşhisi için örneklerin ağartılması ve temizlenmesinde %60'lık laktik asit çözeltisi kullanıldı. Ağartılmış örneklerin daimî preparatları Hoyer ortamında yapıldı. Teşhis işlemleri Doğan (2005) ve Fan ve Zhang (2005)'e göre gerçekleştirildi. Teşhis işlemleri tamamlandıktan sonra akar örnekleri kitin eldesi için sayılarak, etiketlenip %96'lık etil alkol içinde ve -24°C'de muhafaza edildi.

Kitin eldesi

Kitin eldesi aşamasında organizmanın yapısındaki mineralleri uzaklaştırmak için HCl çözeltisi kullanıldı (Majtán vd., 2007; Liu vd., 2012; Kim vd., 2017; Çakmak ve Koç Bilican, 2021). *Eustigmaeus absens* için uygun HCl çözelti derişimini belirlemek amacıyla, 0,1 M, 0,5 M, 1,0 M, 1,5 M ve 2,0 M HCl çözeltileri hazırlandı. Hazırlanan bu farklı derişimlerdeki asitler 0,5 mL olacak şekilde saklama tüplerine konuldu. Aynı derişime sahip asitlerin farklı sürelerde işleme tabi tutulması amacıyla her bir çözeltiden iki saklama tüpü oluşturuldu. Bir sonraki aşamada, %96'lık etil alkolde muhafaza edilen numuneler, Petri kaplarına dökülüp, partiküllerinden arındırılana kadar saf su ile yıkandı. Yıkanan numuneler sayılıp, her biri eşit sayıda olacak şekilde 10 gruba ayrıldı. Bu işlem sayesinde hazırlanan farklı derişimlerdeki asitler içine eşit sayıda numune konulması sağlandı. Partiküllerinden arındırılan ve sayılan numuneler, içinde farklı derişimlerde HCl çözeltisi bulunan saklama tüplerine konuldu. HCl çözeltisi içindeki numuneler 60°C etüvde 6 ve 8 saat süreyle bekletildi. Etüvden çıkarılan örnekler nötr pH değerine ulaşana kadar saf su ile birkaç kez yıkandı. Her yıkama işleminden sonra pH kâğıdı ile kontrol edilip nötr pH değerine ulaşana kadar bu işlem tekrar edildi. Sonrasında her tüpte kaç numune olduğu sayıldı. Sayma işlemi sayesinde, örneklerin hangi derişimlerde ve hangi sürede zarar gördüğü tespit edildi. Nötr pH değerine ulaşan ve sayılan numuneler, sıradaki aşama uygulanana kadar saf suda 4°C'de buzdolabında muhafaza edildi.

HCl çözeltisinde işlem gören numunelerin, yapısında bulunan proteinlerin giderilmesi için NaOH kullanıldı (Kaya vd., 2014b; Seyyar ve Demir, 2020; Çakmak ve Koç Bilican, 2021). *E. absens* için uygun NaOH derişimini belirlemek amacıyla, 0,1 M, 0,5 M, 1,0 M, 1,5 M ve 2,0 M derişimlerde NaOH çözeltileri hazırlandı. Hazırlanan farklı derişimlerdeki bu bazlar 0,5 mL olacak şekilde saklama tüplerine konuldu. Aynı derişime sahip bazların farklı sürelerde işleme tabi tutulması amacıyla her bir çözeltiden iki saklama tüpü oluşturuldu. Hazırlanan farklı derişimlerdeki bazların her birine saf su ile yıkanıp partiküllerinden arındırılmış *E. absens* numuneleri koyuldu. Numuneler, 80°C etüvde 8 ve 11 saat bekletildi. Etüvden çıkarılan numuneler nötr pH değerine ulaşana kadar saf su ile yıkandı. Sonrasında her tüpte kaç numune olduğu sayıldı. Nötr pH değerine ulaşan ve sayılan numuneler, sıradaki aşama uygulanana kadar saf suda 4°C'de buzdolabında muhafaza edildi.

Eustigmaeus absens'in yapısındaki yağları uzaklaştırmak için kloroform-metanol-su karışımı, hacimce 1:2:4 oranında olacak şekilde hazırlandı (Kaya vd., 2014b). Numuneler, belirlenen orandaki karışıma koyularak, oda sıcaklığında 30 dk. süreyle bekletildi. Karışımdan çıkarılan numuneler nötr pH değerine ulaşana kadar saf su ile yıkandı. Gerekli analizler yapılana kadar saf su içinde 4°C'de buzdolabında muhafaza edildi.

Elde edilen kitinler; FTIR, TEM, SEM ve EDX analizlerinin alınması, ışık ve stereo mikroskopta görüntü alınımı, antibakteriyel aktivite ve kitinlerin dekorasyon işleminin yapımı için kurutulmadan saf su içinde 4°C'de buzdolabında muhafaza edildi. FTIR analizi için kitinler ölçüm alınmadan önce 3 gün 60°C'de kurutuldu.

Kitin üzerine gümüş nanoparçacıklarının dekorasyonu

Buzdolabında saf su içinde muhafaza edilen kitinler, dört farklı Petriye eşit sayıda konuldu. Kitin numunelerinin üzerine, kitinleri kapatacak şekilde 10 mM gümüş nitrat

($AgNO_3$) çözeltisi eklendi (Hong vd., 2006; Guin vd., 2007). Daha sonra 4 Petri kabı fotoreaktörde ultraviyole A (UVA) ışınları (365 nm) altına konuldu. Birinci Petri UVA ışınlarına 30 dk., ikinci Petri 1 saat, üçüncü Petri 2 saat ve dördüncü Petri 3 saat maruz bırakıldı.

Kitinin antibakteriyel aktivitesinin incelenmesi

Antibakteriyel aktivite deneyleri bir Gram-negatif (*Escherichia coli* ATCC 25922) ve bir Gram-pozitif (*Staphylococcus aureus* ATCC 29213) bakteri türü üzerine gerçekleştirildi. Bakteriler Luria-Bertani sıvı besiyeri içerisinde 37 ºC'de, 150 rpm'de 18 saat inkübe edilerek ön kültürleri hazırlandı. Bu kültürlerden OD_{600} değerleri *E. coli* ATCC 25922 için 0,4, *S. aureus* ATCC 29213 için 0,25 olacak şekilde taze besiyeri içerisinde dilüsyonları hazırlandı. Hazırlanan bu dilüsyonlardaki bakteri sayısı yaklaşık olarak 1,5 x 10^8/mL'dir. Kültürün 1 mL'si Eppendorf tüplere aktarılarak santrifüj (13.000 rpm, 2 dk.) edildi. Üst faz uzaklaştırılarak pelet steril fizyolojik tuzlu su (% 0,9 NaCl) ile 1 kez yıkandı. Bakteriler 108 cfu/mL olacak şekilde steril fizyolojik tuzlu su içerisinde sulandırıldı ve böylece bakteriyel çalışma solüsyonları hazırlanmış oldu. Steril inokülasyon çubuğu bakteri çalışma solüsyonlarına daldırıldıktan sonra (yaklaşık 100 µL) bakteriler, Petri kaplarında bulunan LB agar besiyeri üzerine her noktasına eşit olacak şekilde yayıldı. Steril agar delici (cork borer) kullanılarak agar yüzeyinde 7 mm kuyular açıldı. Kuyuların içerisine aktivitesine bakılacak maddelerden 100 µL (100 µM) ilave edildi. Petri kapları 37 ºC'de 24 saatlik inkübasyonun ardından kontrol (K) (çözücü), saf kitin (A) ve gümüş nanoparçacıkları dekore edilmiş kitin (C) maddelerinin bulunduğu kuyuların etrafındaki inhibisyon zonlarının çapları ölçüldü. Ayrıca kültürden seri sulandırma yapılarak canlı hücre sayımı yapıldı. Canlı hücre sayıları üç tekrarın ortalaması alınarak hesaplandı.

Kitin numunelerinin karakterizasyonu

Elde edilen numunelerin yapısal ve morfolojik özellikleri Fourier dönüşümlü kızılötesi (FTIR) spektroskopisi, taramalı elektron mikroskobu-enerji dağılımlı X-ışını analizi (SEM-EDX) ve geçirimli elektron mikroskobu (TEM) teknikleri kullanılarak aydınlatıldı.

Elde edilen kitinler, 3 gün 60°C etüvde kurutulduktan sonra FTIR analizlerinin gerçekleştirilmesi için iki farklı tekniğe tabii tutuldu. İlk teknikte toz haldeki kitinler ile KBr (Potasyum bromür) homojenlik sağlanana kadar öğütüldü. Daha sonra bu karışım yaklaşık 10.000 psi'lik basınç altında sıkıştırılarak saydam bir disk hazırlandı. Elde edilen disk nemden uzak tutularak spektroskopi cihazında ölçümü alındı. Diğer teknikte ise KBr ile pelet hazırlanmadan IR cihazının elmas yüzeyine toz haldeki kitinler sıkıştırılarak ATR tekniği ile ölçüm alındı (Madejová, 2003). Farklı iki teknikten elde edilen analiz sonuçlarına bakıldığında, ATR tekniği ile elde edilen piklerin, pelet hazırlanarak elde edilen piklere göre daha net olduğu görüldü.

Elde edilen kitinlerin SEM ve EDX analizleri gerçekleştirilirken, kitinlerin farklı kısımlarının birbirine yapışmasını engellemek için numuneler kurutulmadan kullanıldı. Analizlerden önce şu hazırlıklar yapıldı: Karbon bant 2-3 mm büyüklüğünde kesilip staba yapıştırıldı. Saf su içinde buzdolabında muhafaza edilen kitinlerden analizleri yapılacak olan numuneler, alkole alındı. Alkole alınan numuneler pipet yardımıyla stab üzerine yerleştirildi. Alkolün saf suya göre daha hızlı buharlaşma özelliği kullanılarak stab üzerinde sıvı birikmesi engellendi. Böylelikle numunelerin karbon banda yapışması sağlandı. SEM, EDX analizlerinden önce numunelerden daha iyi görüntü elde etmek için numuneler altın ile kaplandı. Altın ile kaplanan numuneler SEM cihazına yerleştirildi ve kitinin farklı kısımlarından, farklı büyütmede görüntüler alındı (Kaya vd., 2014b, 2017).

Numunelerin Geçirimli elektron mikroskobu (TEM) görüntüleri, 120 kV'luk bir hızlanma voltajı altında HITACHI HT-7700 elektron mikroskobu ile alındı.

BULGULAR VE TARTIŞMA

Kitinin stereo mikroskop görüntülerinin değerlendirilmesi

Eustigmaeus absens'in kimyasal muamele görmemiş halinin üstten görüntüsü Şekil 2a'da ve yapısındaki minerallerin, proteinlerin ve yağların uzaklaştırılması işlemlerine tabii tutulduktan sonra elde edilen kitininin üstten görüntüsü Şekil 2b'de verilmiştir. Şekil 3'te ise yine *E. absens*'in işlemsiz ve işlemli hallerinin lateral görüntüsü verilmektedir.

Şekil 2. *Eustigmaeus absens*'in üstten görünümü (a) işlemsiz (b) işlemli.

İşlemsiz *E. absens*'in yapısında; mineral, protein ve yağlar mevcut olduğundan stereo mikroskop görüntüsünün renkli ve vücuttaki ağsı yapıların içlerinin nokta desenli olduğu anlaşılmaktadır. Yapısındaki mineral, yağ ve protein uzaklaştırma işlemlerine tabii tutulduktan sonra içyapısının yok edilmesi sonucu stereo mikroskop görüntüsünün şeffaf olduğu görülmektedir. Görüntülerden anlaşılacağı üzere, yapılan işlemler organizmanın vücut içeriğini yok ederek kitin eldesinin sağlandığını göstermektedir.

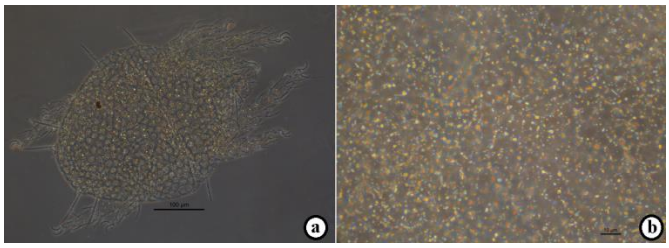
Şekil 3. *Eustigmaeus absens*'in yandan görünümü (a) işlemsiz (b) işlemli.

Kitinin faz-kontrast donanımlı ışık mikroskobu görüntülerinin değerlendirilmesi

Elde edilen kitinin aşırı derecede şeffaf olması nedeniyle ışık mikroskobundaki görüntülerde parlama meydana gelmiştir. Aslında bu durum, elde edilen kitinde kalıntı olmadığını, *E. absens*'in içeriğinin (yapısındaki mineral, yağ ve protein türlerinin) başarılı bir şekilde uzaklaştırıldığını morfolojik olarak ispatlamaktadır. Çalışmalar, *Trachytes pauperior* (Acari: Mesostigmata) türünden elde edilen kitin dışında hiçbir kitinin ışık mikroskobu görüntüsünün alınmadığını göstermektedir (Çakmak ve Koç Bilican, 2021).

Gümüş nanoparçacıkları dekore edilmiş kitinin faz-kontrast donanımlı ışık mikroskobu görüntülerinin değerlendirilmesi

Gümüş nanoparçacıklarının dekore edildiği kitinin, faz-kontrast donanımlı ışık mikroskobundaki görüntüsü Şekil 4'te verilmiştir. Görüntülerdeki sarı, mavi, turuncu ve beyaz renkli noktalar gümüş nanoparçacıklarını göstermektedir. Elde edilen görüntüler gümüş nanoparçacıklarının kitin yüzeyinde homojen bir dağılım sergilediğini göstermektedir.

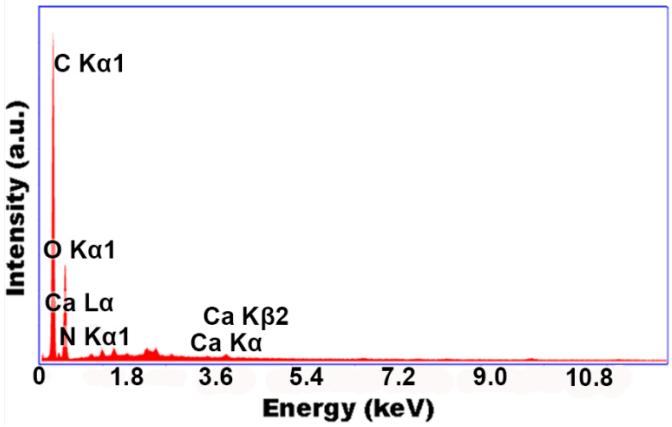


Şekil 4. Gümüş nanoparçacıkları dekore edilmiş kitinin faz-kontrast donanımlı ışık mikroskobu görüntüleri (a) genel görünüm, (b) gümüş nanoparçacıkların yakından görünümü.

Yapılan çalışmalara bakıldığında, daha önce kitin üzerine gümüş nanoparçacıkların dekore edilmediği görülmektedir. Bu çalışma ile kitin üzerine ilk kez gümüş nanoparçacıkları dekore edilmiş ve ışık mikroskobunda görüntüsü alınmıştır.

Kitinin SEM-EDX verilerinin değerlendirilmesi

Eustigmaeus absens'ten elde edilen kitinin EDX spektrumu Şekil 5'te, kütlece ve atomik element yüzdeleri Tablo 1'de verilmiştir. Buna göre elde edilen kitinin yapısında ağırlıklı olarak karbon (C), oksijen (O) ve azot (N) elementlerinin bulunduğu ve eser miktarda kalsiyum (Ca) elementinin yer aldığı görülmektedir. C, N ve O elementlerinin varlığı kitinin protein yapısında olduğunu gösterirken, eser miktarda Ca elementinin dışındaki farklı elementlere rastlanmaması elde edilen kitinin neredeyse %100 saflıkla elde edildiğini göstermektedir. Akarlarda diğer eklembacaklılarda olduğu gibi kutikula tabakası farklı gruplara göre değişik derecelerde sertleşmiştir yani sklerotize olmuştur (sklerotizasyon). Sertleşmede kitin lifleri protein yapısındaki bir matriks içine gömülerek hem sağlam hem de esnek bir karışım oluşturur. Ayrıca bu yapıya kalsiyum ve diğer inorganik tuzların eklenmesi ile sertliğin derecesi artmıştır (Doğan ve Ayyıldız, 2023). Çalışma kapsamında elde edilen kitindeki kalsiyumun varlığı bunu desteklemektedir.



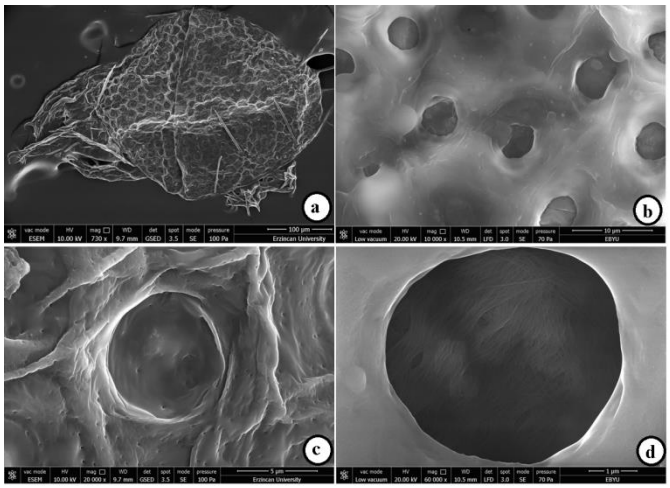
Şekil 5. *Eustigmaeus absens* kitininin EDX spektrumu.

Tablo 1. *Eustigmaeus absens* kitininin element analiz verileri.

	C	N	O	Ca
Kütle (%)	56,04	8,7	35,05	0,21
Atomik (%)	62,35	8,3	29,27	0,07

E. absens'ten elde edilen kitinin SEM görüntüleri Şekil 6'da verilmiştir. Kitinin yüzeyi incelendiğinde, makro gözenekler, mikro gözenekler ve yüzeyin tamamında kesik nanolifler görülmektedir. Makro gözeneklere daha yakından bakıldığında, kesintisiz nanoliflerin bir araya gelerek farklı boyutlarda gözenekler oluşturduğu anlaşılmaktadır.

Literatüre bakıldığında, kitin yüzeyinin canlı gruplarına göre farklılıklar gösterdiği görülmektedir. Mantarlardan elde edilen kitinin yüzey morfolojisinde genellikle nanolifli yapıların, gözeneklerin görülmediği ve yüzey morfolojisinin düz olduğu anlaşılmaktadır (Yen ve Mau, 2007). Karides (Crustacea: Malacostraca) kabuğundan elde edilen kitin yüzeyinin pürüzsüz ve çok sayıda gözeneğe sahip olduğu bilinmektedir (Abdel-Rahman vd., 2015). Öte yandan *Ariolimax califoinicus* (Gastropoda: Stylommatophora) sümüklüböceğinden elde edilen kitinde lifli yüzey morfolojisi görülürken (Montroni vd., 2019), *Hogna radiata* ve *Geolycosa vultuosa* (Arachnida: Araneae) adındaki farklı iki örümcekten elde edilen kitinlerde makro ve mikro gözeneklerle birlikte nanoliflere rastlanmıştır. (Kaya vd., 2014b). Farklı eklembacaklılardan elde edilen kitinlerde de farklı boyutlarda gözenekler, kesik nanolifler ve pürüzsüz lifler görülmüştür (Sajomsang ve Gonil, 2010; Kaya vd., 2017; Seyyar ve Demir, 2020). *Vespa crabro* (Hexapoda: Hymenoptera) yaban arısından elde edilen kitinde ise gözeneklere ve liflere ek olarak balık pulu şeklinde nano lifli yapılar görülmüştür (Kaya vd., 2016). *Ixodes ricinus* (Acari: Ixodida) sert kene türünden elde edilen kitinin yüzey morfolojisinde iç içe karışık lifler gözlenmiştir (Kaya vd., 2015). *Trachytes pauperior* akar türünden elde edilen kitinin yüzey morfolojisinin de nanolifler ve gözeneklere sahip olduğu görülmüştür (Çakmak ve Koç Bilican, 2021).



Şekil 6. *Eustigmaeus absens* kitininin SEM görüntüsü: (a) kitinin genel görüntüsü, (b) makro gözenekler, (c) makro gözenekler ve çevresindeki mikro gözenekler, (d) makro gözeneklerin yakından görünümü.

Akarların yaşam şekli ile derinin yapısı arasında sıkı bir ilişki vardır. Deride katlanmalar, kırışıklıklar, çizgilenmeler, ağsı yapılar, küçük yumrular, tümsekler, çöküntüler ve gözenekler bulunabilir. Çalışma sonucunda elde edilen görüntüler ayrıntılı olarak incelendiğinde; kitinsi lifli yapının kesik kesik olduğu, bazı kısımlarda paralel bazı kısımlarda çapraz dizayn edildiği görülmüştür. Bu durum literatürle uyum içindedir (Sajomsang ve Gonil, 2010; Kaya vd., 2014b; Kaya vd., 2015; Kaya vd., 2017; Seyyar ve Demir, 2020; Çakmak ve Koç Bilican, 2021).

Derideki bazı epidermis hücreleri salgı yapacak şekilde özelleşmiştir. Bunların bağlandığı kanallar, vücut ve bacaklar üzerinde değişik şekillerde olan gözeneklerle sonlanır. Gözenek açıklıkları yuvarlak veya elips şeklinde olabilir (Doğan ve Ayyıldız, 2023). Çalışma kapsamında görüntüleri alınan gözeneklerin yuvarlağa yakın olduğu anlaşılmaktadır. Akarlarda dermal bezlerin işlevleri hala tam olarak bilinmemekle birlikte salgı ürünlerinin semiokimyasal üretimiyle ilişikli olabileceği ve dehidrasyona karşı koruma sağlayabileceği düşünülmektedir (Doğan ve Ayyıldız, 2023).

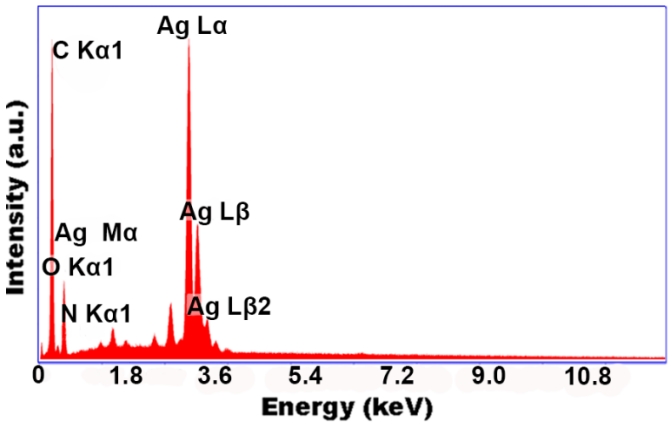
Gümüş nanoparçacıkları dekore edilmiş kitinin SEM-EDX verilerinin değerlendirilmesi

E. absens'ten elde edilen ve üzerinde gümüş nanoparçacıkların dekore edildiği kitinin SEM-EDX spektrumu Şekil 7 ve 8'de, kütlece ve atomik element yüzdeleri Tablo 2'de verilmiştir.

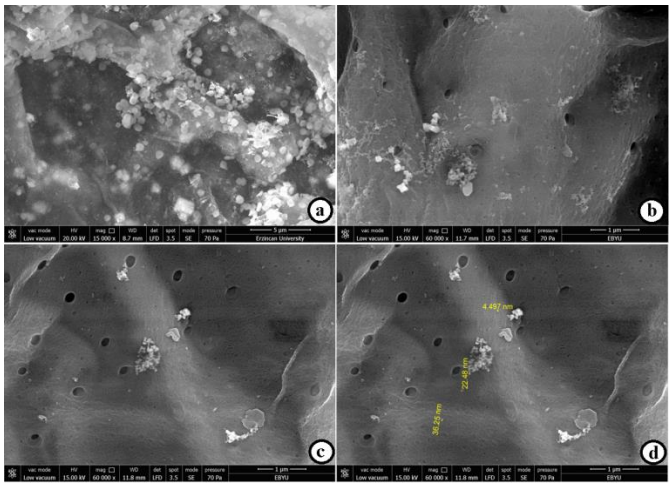
Tablo 2. Gümüş nanoparçacıkları dekore edilmiş kitinin element analiz verileri.

	C	N	O	Ag
Kütle (%)	31,67	4,79	23,17	40,37
Atomik (%)	54,92	7,12	30,17	7,79

Kitinin protein yapısından kaynaklanan C, O ve N elementlerine ilaveten başarılı bir dekorasyon işlemini ispatlayan Ag elementinin varlığı gözlenmiştir. Bu elementlere ilaveten başka elementlerin tespit edilmemesi Ag nanoparçacıklarının saf olarak elde edilen kitinin üzerine dekore edildiğini ispatlamaktadır. Buna ilaveten gümüşün metalik halde olduğu ve farklı tuz yapılarının bulunmadığını göstermektedir.



Şekil 7. Gümüş nanoparçacıkları dekore edilmiş kitinin EDX spektrumu.



Şekil 8. Gümüş nanoparçacıklı kitinin SEM görüntüleri.

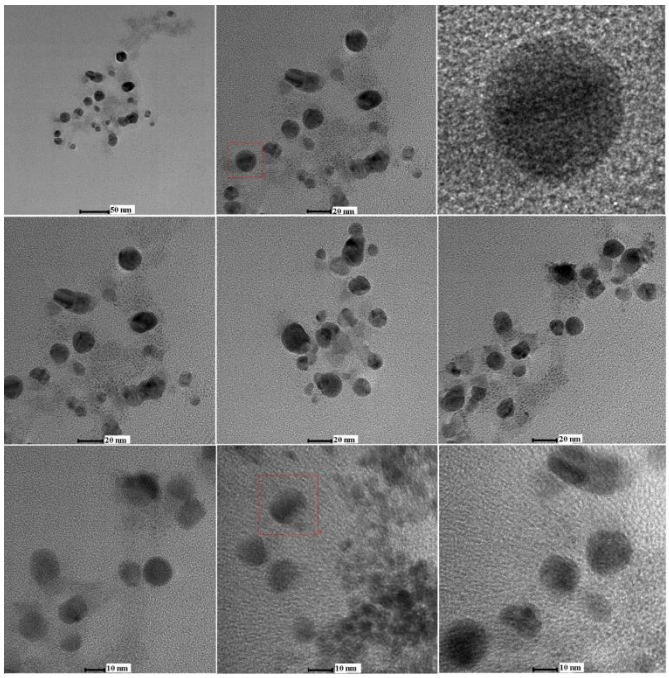
SEM görüntüleri sonucunda kitin üzerine dekore edilen gümüşlerin, kitin yüzeyinin tamamında farklı aralıklarla bulundukları görülmüştür (Şekil 8a-d). Gümüşlerin boyutları ölçüldüğünde, 4 nm ile 37 nm arasında farklılıklar gösterdiği anlaşılmaktadır (Şekil 8d).

Gümüş nanoparçacıklı kitinin TEM verilerinin değerlendirilmesi

E. absens'ten elde edilip üzerine gümüş nanoparçacıkların dekore edildiği kitinin TEM görüntüleri Şekil 9'da verilmiştir.

TEM görüntülerinde görüldüğü gibi numunenin kitin bölümü şeffaf görünümde olup, gümüş nanopartiküllerin ise kitin üzerine dekore olduğu açık bir şekilde anlaşılmaktadır. Gümüş nanoparçacıklarının kitin yüzeyindeki dağılımı bazı bölgelerde kısmi yığılmalar şeklinde olmakla beraber nispeten homojen dağılım sergilediği görülmektedir. Dekore olan gümüş nanoparçacıkları küresel yapıya sahip olup parçacık çapları 6 ile 20 nm arasında değişim göstermektedir. Yapılan çalışmalara bakıldığında elde edilen hiçbir kitinin ve kitin yüzeyine dekore olmuş gümüş nanoparçacıklarının TEM görüntülerinin alınmadığı

görülmüştür. Bu çalışma ile gümüş nanoparçacıkları dekore edilmiş kitinin ilk kez TEM görüntüsü alınmıştır. Kitin yüzeyindeki gümüş nanoparçacıkların boyutunun literatürdeki farklı platformlar yüzeyine dekore olmuş gümüş nanoparçacıklarının boyutlarıyla uyum içerisinde olup, oldukça küçük nanoparçacık çapına sahip olduğu tespit edilmiştir (Kim vd., 2005; Nazeruddin vd., 2014; Xu vd., 2014; Niu vd., 2020). Bilindiği gibi nanoyapıların parçacık boyutu küçüldükçe mikrobiyal aktivitesinde artış gözlenmektedir (Nazeruddin vd., 2014). Çalışmamızdaki gümüş nanoparçacıklarının da küçük boyutta olması antibakteriyel aktivite için avantajlı bir durumdur.



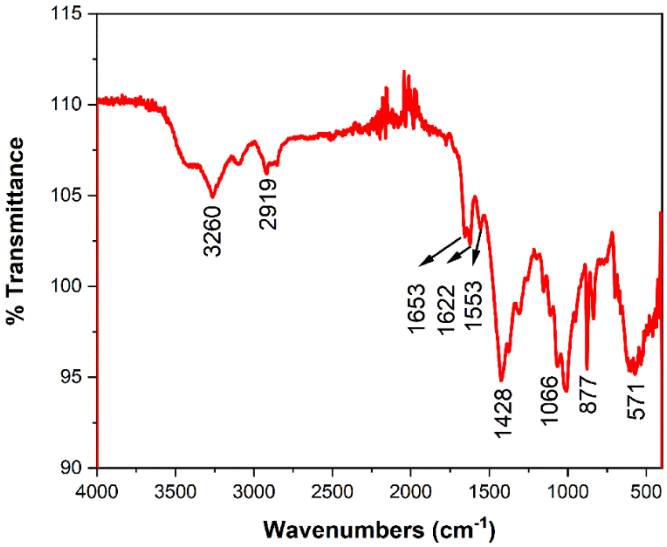
Şekil 9. Gümüş nanoparçacıklı kitinin TEM görüntüleri.

Kitininin ATR-FTIR verilerinin değerlendirilmesi

Eustigmaeus absens türünden elde edilen kitinin ATR-FTIR spektrumu Şekil 10'da, önemli karakteristik titreşim bantları ve dalga sayıları ise Tablo 3'te verilmiştir.

FTIR kitinin protein yapısının moleküler konformasyonunu araştırmak için kullanılan en önemli tekniklerden birisidir (Shao vd., 1999; Nimmen vd., 2008). Moleküler konformasyonun tayininde protein yapısında bulunan amid gruplarına ait amid-I, amid-II ve amid-III bantları olarak adlandırılan ve 1200-1700 cm^{-1} aralığında gelen titreşim pikleri esas alınır. Amid-I bantları C=O gerilmesinden kaynaklanır ve genellikle 1590-1700 cm^{-1} aralığında gözlenir. Amid-I bandının ikili pik halinde görülmesi kitinin alfa formda olduğunu ifade ederken, tek pik halinde görülen amid-I bandı ise kitinin beta formda olduğunu ifade eder (Jang vd., 2004). Amid-II bandı ise N-H ve C-N eğilme titreşimleri ve amid-III bandı ise C-H eğilme titreşimlerinden meydana gelir ve 1460-1590 cm^{-1} aralığında gözlenirler (Koperska vd., 2014).

E. absens kitininin ATR-FTIR spektrumunda 3260, 2919, 1653, 1622, 1553, 1371, 1306, 1066 ve 1010 cm^{-1}'de pikler gözlenmiştir. 3260 cm^{-1}'de gözlemlenen pik protein yapısındaki N-H gerilme titreşiminden kaynaklanırken, 2919 ve 2854 cm^{-1}'de gözlemlenen pikler ise sırasıyla asimetrik ve simetrik alifatik C-H gerilme titreşimine karşılık gelir. 1653 ve 1622 cm^{-1}'de gözlenen IR pikleri amid-I bandını ifade etmektedir. Amid-I bandının ikiye ayrılmış şekilde gözlenmesi kitinin alfa formda olduğunu göstermektedir (Jang vd., 2004). 1553 cm^{-1}'de gözlenen pik amid-II bandının varlığını gösterirken, 1371 cm^{-1} de gözlenen pik C-H eğilmesini, 1306 cm^{-1} amid-III bandının varlığını, 1066 cm^{-1} C-O-C gerilmesini, 1010 cm^{-1} C-O eğilmesini ifade etmektedir.



Şekil 10. *Eustigmaeus absens*'ten elde edilen kitinin ATR-FTIR spektrumu.

Tablo 3. *Eustigmaeus absens*'ten elde edilen kitinin ATR-FTIR titreşim bantları.

Dalga Sayısı (cm^{-1})	Titreşim Türü
3260	N-H gerilme
2919	Alifatik C-H gerilme
1653	Amid-I bandı (C=O gerilme)
1622	Amid-I bandı (C=O gerilme)
1553	Amid-II bandı (N-H eğilme, C-N eğilme)
1371	C-H eğilmesi
1306	Amid-III bandı (C-H eğilme)
1066	C-O-C gerilmesi
1010	C-O eğilmesi

Böcekler (Hexapoda) üzerine yapılan çalışmalar incelendiğinde, *Leptinotarsa decemlineata* (Coleoptera) türünden elde edilen kitinin amid-I bandı 1620-1654 cm^{-1}'de ikili pik halinde ve amid-II bandı 1542 cm^{-1}'de (Kaya vd., 2014a); *Blattella germanica* (Blattodea), *Anoplotrupes stercorosus* (Coleoptera), *Blaps tibialis* (Coleoptera), *Cetonia aurata* (Coleoptera), *Geotrupes stercorarius* (Coleoptera), *Calliphora vicina* (Diptera), *Coreus marginatus* (Hemiptera), *Lygaeus equestris* (Hemiptera), *Pyrrhocoris ap-*

terus (Hemiptera), *Bombus lapidarius* (Hymenoptera), *Formica clara* (Hymenoptera), *Cordulia aenea* (Odonata), *Libellula quadrimaculata* (Odonata) türlerinden elde edilen kitinlerin amid-I bantları 1620-1654 cm^{-1}'de ikili pik halinde ve amid-II bantları 1553 cm^{-1}'de gözlenmiştir (Kaya vd., 2014a; Kaya vd., 2015). Örümceklerden (Araneae) *Hogna radiata* ve *Geolycosa vultuosa'*dan elde edilen kitinlerin amid-I bantları 1619-1654 cm^{-1}'de ikili pik halinde ve amid-II bantları 1542 cm^{-1}'de (Kaya vd., 2014b), *Argiope bruennichi*, *Chaetopelma olivaceum* türlerinden elde edilen kitinlerin amid-I bantları 1620-1654 cm^{-1}'de ikili pik halinde ve amid-II bantları 1553 cm^{-1}'de gözlenmiştir (Kaya vd., 2015). Akarlardan (Acari) *Ixodes ricinus*'dan (Ixodida) elde edilen kitinin amid I bandı 1620-1654 cm^{-1}'de ikili pik halinde ve amid-II bandı 1553 cm^{-1}'de (Kaya vd., 2015) ve *Trachytes pauperior*'dan (Mesostigmata) elde edilen kitinin amid-I bandı 1621-1651 cm^{-1}'de ikili pik halinde ve amid-II bandı 1556 cm^{-1}'de gözlenmiştir (Çakmak ve Koç Bilican, 2021). Buna ilaveten bu çalışmalarda, kitine ait N-H gerilmesi, alifatik C-H gerilmeleri, C-H eğilmeleri, Amid-III bandı, C-O-C gerilmesi ve C-O eğilmesini karşılık gelen IR piklerinin değerlerinin çalışmamızla uyumlu olduğu görülmüştür. Elde edilen kitinlerin hepsinin alfa forma sahip olduğu tespit edilmiştir (Kaya vd., 2014a, b; Kaya vd., 2015; Çakmak ve Koç Bilican, 2021). Tüm bunlar, çalışma kapsamında elde edilen kitinin yüksek saflıkta olduğunu ortaya koymaktadır.

Kitinin asetilasyon derecesinin değerlendirilmesi

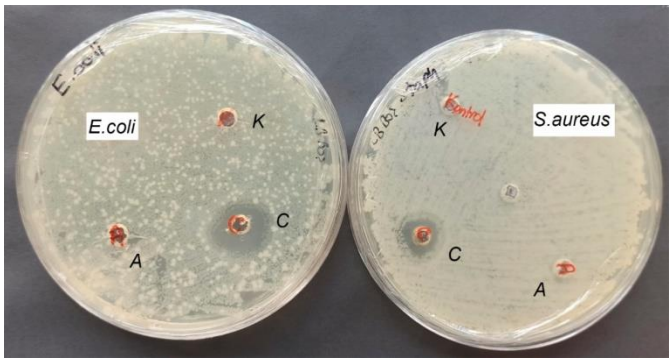
Asetilasyon derecesi (AD), çeşitli organizmalardan elde edilen kitinin saflığını ortaya koyan önemli bir parametredir ve NMR, XRD, elemental analiz ve FTIR analizlerinden hesaplanmaktadır (Majtán vd., 2007; Yen vd., 2009; Kaya vd., 2014a; Kaya vd., 2015). FTIR'dan hesaplanan AD değeri, 1655^{-1} ve 3450^{-1} 'deki IR piklerinin absorbans değerlerinin kullanılması ile aşağıdaki eşitlikten bulunur:

Asetilasyon derecesi (AD): $(A_{1655} / A_{3450}) \times 100$

Bu çalışmada elde edilen kitinin AD değeri, yukarıdaki eşitlik kullanılarak hesaplanmış ve %117 bulunmuştur. Yapılan çalışmalara bakıldığında; *Blattella germanica*'dan (Hexapoda: Blattodea) elde edilen kitinin AD değeri elemental analiz ile hesaplandığında %94,5 bulunurken FTIR ile hesaplandığında %127, *Cetonia aurata*'dan (Hexapoda: Coleoptera) elde edilen kitin AD değeri elemental analiz ile hesaplandığında %70,1 bulunurken FTIR ile hesaplandığında %128, *Coreus marginatus*'tan (Hexapoda: Hemiptera) elde edilen kitinin AD değeri elemental analiz ile hesaplandığında %79,1 bulunurken FTIR ile hesaplandığında %150 bulunmuştur (Kaya vd., 2015). Tüm bunlar, *Eustigmaeus absens*'ten elde edilen kitinin AD değerinin literatürle uyum içinde olduğunu ve elde edilen kitinin yüksek saflık gösterdiğini desteklemektedir. Literatürde, FTIR ile hesaplanan AD değerinin elemental analiz ile hesaplanan AD değerine göre daha yüksek çıktığı ve bu değerin çoğunlukla %100'ün üzerinde olduğu yapılan çalışmalarla ortaya konmuştur (Majtán vd., 2007; Yen vd., 2009; Kaya vd., 2014a; Kaya vd., 2015). Bu durum elemental analiz analizinin kantitatif, FTIR analizinin ise yarı kantitatif olmasından kaynaklanmaktadır.

Kitin ve gümüş nanoparçacıkları dekore edilmiş kitinin antibakteriyel aktivite verilerinin değerlendirilmesi

Petri kaplarındaki inhibisyon zonları incelendiğinde (Şekil 11) kontrol ve saf kitin her iki bakteriye karşı antibakteriyel aktivite göstermemesine rağmen gümüş nanoparçacıkları dekore edilmiş kitine ait kuyularda *E. coli* ATCC 25922 için 15 mm, *S. aureus* ATCC 29213 için 11 mm çapında etkinlik zonları tespit edilmiş olup gümüş nanoparçacıkları dekore edilmiş kitinin her iki mikroorganizmaya karşı antibakteriyel aktivitesinin bulunduğu anlaşılmıştır. Literatürde önerilen olası mekanizma göz önüne alınarak, gümüş nanoparçacıkların inkübasyonu sırasında Ag^+ iyonları ekilen kuyucuklarda kademeli olarak dağılır ve ardından bu Ag^+ iyonları bakteri hücre duvarına yapışır ve metabolik aktiviteyi bozar. Böylece hücre zarının deformasyonuna bağlı olarak zar geçirgenliğinin artmasıyla beraber gerek Ag^+ iyonları gerekse gümüş nanoparçacıkları bakteri hücresine difüze olarak DNA hasarına sebep olurlar (Slavin vd., 2017; Doğan, 2022; Poudel ve Kim, 2023).

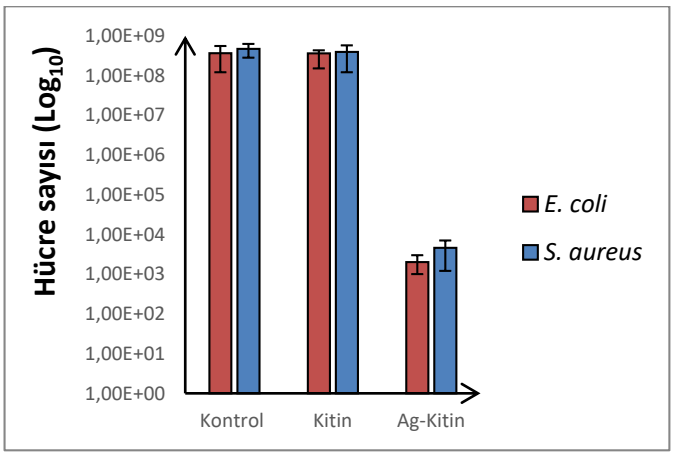


Şekil 11. Saf kitin ve gümüş nanoparçacıkları dekore edilmiş kitinin *E. coli* ATCC 25922 ve *S. aureus* ATCC 29213'e karşı antibakteriyel aktivitelerinin kuyu difüzyon görüntüleri. K (Kontrol), A (Kitin), C (Gümüş nanoparçacıklı kitin.

Gümüş nanoparçacıkları dekore edilmiş kitinin, Gram-negatif (*E. coli* ATCC 25922) bakterinin antibakteriyel aktivitesinin Gram-pozitif (*S. aureus* ATCC 29213) bakteriye karşı olan antibakteriyel aktiviteden daha fazla olduğu görülmüştür. Bu durum Gram-negatif (*E. coli* ATCC 25922) bakterideki negatif yüke sahip yapılar pozitif yüklü Ag^+ iyonlarını elektrostatik olarak çekerek bakteri hücresine diffüzyonlarını ve hücreye alınımlarını arttırarak daha fazla hasara sebep olmalarıyla ilişkilendirilebilir (Slavin vd., 2017; Doğan, 2022).

Maddelerin antibakteriyel aktiviteleri ayrıca sıvı besiyeri ortamında da test edilmiştir. Bunun için yukarıda bahsedildiği gibi bakteri çalışma solüsyonları hazırlanmıştır. Solüsyonların 500 µL'si steril Eppendorf tüpüne aktarılmış ve üstlerine kimyasallar konsantrasyonları 100 µM olacak şekilde ilave edilmiştir. Eppendorf tüpler 37 ºC'de,150 rpm'de 18 saat inkübe edilmiştir. İnkübasyon sonunda kültürden seri sulandırma yapılarak canlı hücre sayımı yapılmıştır. Canlı hücre sayıları üç deneyin ortalaması alınarak hesaplanmıştır. Kontrol (çözücü), saf kitin (A) ve gümüş nanoparçacıkları dekore edilmiş kitin (C) 100µM kontsantrasyonları ile yapılan deneylerde elde edilen canlı bakteri sayısındaki azalma grafikleri Şekil

12'de verilmiştir. Şekil 12'de görüldüğü gibi saf kitin gerek Gram-negatif (*E. coli* ATCC 25922) gerekse Gram-pozitif (*S. aureus* ATCC 29213) bakterisine karşı ihmal edilecek bir antibakteriyel aktivite sergilerken, gümüş nanoparçacıkları dekore edilmiş kitin canlı bakteri sayısında her iki bakteri için logaritmik olarak yaklaşık 105 inhibisyona sebebiyet verdiği anlaşılmıştır. Bu sonuçlara göre gümüş nanoparçacıkları dekore edilmiş kitinin hem *E. coli* ATCC 25922 hem de *S. aureus* ATCC 29213 bakteri türleri üzerine antibakteriyel aktivitesi tespit edilmiştir.



Şekil 12. Saf kitin ve gümüş nanoparçacıkları dekore edilmiş kitinin *E. coli* ATCC 25922 ve *S. aureus* ATCC 29213'e karşı bakteri sayısındaki azalma grafikleri.

Gümüş nanoparçacıklarının antibakteriyel özellikleri geçmişten günümüze çok uzun yıllardır bilinmekte olup literatürde birçok araştırma grubu tarafından da çalışılmıştır (Doğan, 2022). Gümüş nanopraçacıklarının şekilleri ve boyutları bu nanoparçacıkların antibakteriyel özelliklerini önemli ölçüde etkileyen parametrelerdendir. Literatürde verildiği gibi daha küçük boyutlu gümüş nanoparçacıklarının bakterinin membranından geçişinin kolay olması ve Ag^+ iyonlarının ortama salınımının daha kolay olmasından ötürü küçük boyuta sahip gümüş nanoparçacıklar büyük nanoparçacıklara nispeten daha iyi antibakteriyel etki sergilemektedir (Valgas vd., 2007; Kanmani ve Lim, 2013; Acharya vd., 2018; Premkumar vd., 2018; Sharma vd., 2019). Buna ilaveten literatürde yapılan çalışmalarda farklı şekillere sahip olan (küresel, kübik, üçgen, altıgen ve nanoçubuk) gümüş nanoparçacıklarının da farklı antibakteriyel aktivite sergiledikleri tespit edilmiştir (Doğan, 2022). Mevcut çalışmada gümüş nanoparçacıkların boyutunun 6-20 nm arasında farklılık gösterdiği ve küresel şekilde olduğu belirlenmiştir. Gümüş nanoparçacıkların oldukça küçük boyutlanması antibakteriyel aktivitesini artırdığı anlaşılmaktadır.

SONUÇLAR VE ÖNERİLER

Bu çalışma ile ilk defa, bir akar türü olan *Eustigmaeus absens*'in 3 boyutlu yapısı korunarak başarılı bir şekilde kitin elde edilmiş, elde edilen kitin üzerine gümüş nanoparçacıkları dekore edilmiş ve hem kitin hem de gümüş nanoparçacıklı kitinlerin bazı fiziksel ve kimyasal özellikleri; stereo mikroskop, faz-kontrast donanımlı ışık mikroskobu, SEM, EDX, ATR-FTIR ve TEM teknikleri kullanılarak ortaya çıkarılmaya çalışılmıştır. Bu numunelerin ayrıca antibakteriyel aktivitelerinin değerlendirilmesi de yapılmıştır.

Araştırma sonucunda, FTIR ve EDX analizlerinden yola çıkarak; *E. absens*'ten kitin eldesi için en uygun HCl çözelti derişiminin 1,5 M ve HCl çözeltisinde işlem görme süresi 6 saat, NaOH derişimi 1,0 M ve NaOH çözeltisinde işlem görme süresi ise 8 saat olarak belirlenmiştir. Kitin üzerine gümüş nanoparçacıklarının dekorasyonu için SEM ve TEM analizleri sonuçlarından faydalanılarak, 10 mM $AgNO_3$ çözeltisi içindeki kitinlerin UVA ışınları altında bekleme süresi 1 saat olarak belirlenmiştir.

Yapılan FTIR analizi ile elde edilen kitinin alfa formda olduğu ve yapılan diğer çalışmalarla oldukça uyumlu pikler sergilediği gözlemlenmiştir (Zhang vd., 2000; Paulino vd., 2006; Majtán vd., 2007; Sajomsang ve Gonil, 2010; Liu vd., 2012; Kaya vd., 2014a; Kim vd., 2017). Gözlemlenen piklerin keskinliği elde edilen kitinin saf olduğunu kanıtlamaktadır. EDX verileri ile kitinin saf olarak elde edildiği desteklenmiştir. Elde edilen kitinin asetilasyon derecesinin %117 bulunması da kitin saflığını yansıtmaktadır. Yapılan SEM, TEM, stereo mikroskop, faz-kontrast donanımlı ışık mikroskobu analizleri ile kitinin ve gümüş nanoparçacıkları dekore edilmiş kitinin yüzey özellikleri incelenmiş ve elde edilen yüzey morfolojisinin literatürle benzerlik gösterdiği ortaya konmuştur (Yen vd., 2009; Kaya vd., 2015; Ibitoye vd., 2018; Çakmak ve Koç Bilican, 2021). Yapılan antibakteriyel aktivite çalışmaları gümüş nanoparçacıkları dekore edilmiş kitinin hem *E. coli* ATCC 25922 hem *S. aureus* ATCC 29213 bakterilerine karşı etkili olduğunu göstermiştir.

Literatür, kitin eldesinin daha çok makro organizmalardan yapıldığını, akarlardan kitin eldesi ile ilgili sadece dört çalışmanın (Sobotnik vd., 2008; Choi vd., 2016; Kaya vd., 2015; Çakmak ve Koç Bilican, 2021) mevcut olduğunu göstermektedir. Akarların mikro boyutta olması ve elde edilmelerinin zahmetli oluşu akarlar üzerindeki çalışılmaları kaçınılır hale getirmektedir. Bu çalışma ile daha önce üzerinde hiç çalışılmamış bir akar türünden elde edilen kitin verileri bu alanda ve temel bilimler düzeyinde literatür eksiğini bir ölçüde giderecek ve katkı sağlayacak niteliktedir.

Çalışmada kullanılan yöntemler kitinin ve gümüş nanoparçacıklı kitinin antibakteriyel, görüntüleme ve yapısal analizleriyle sınırlıdır. Akarlardan elde edilen kitinin optik, termik ve mekanik özelliklerinin açığa çıkarılması literatüre yeni bilgiler sağlayacaktır. Ayrıca mevcut çalışmada kullanılan akar türünün farklı evrelerinde ve dahil olduğu familyanın (Stigmaeidae) diğer üyelerinde kitin eldesi çalışmaları yapılarak, kitin özelliklerinin daha ayrıntılı ve karşılaştırmalı olarak açığa çıkarılması sağlanabilir.

Kitinin doğal bir kaynak olması, biyolojik olarak parçalanabilmesi, toksik olmaması, antimikrobiyal ve antioksidan oluşu tarım, tıp, gıda endüstrisi, tekstil ve kozmetik gibi alanlarda kullanımını sağlar. Bu çalışma sonucu elde edilen saf ve gümüş nanoparçacıklı kitin bahsi geçen alanlarda kullanılabilir.

Yazar katkıları

Şifanur Uğurlu: Veri toplama, metodoloji, araştırma, görselleştirme, yazma-taslak metin. **Bülent Çağlar:** Denetim, metodoloji, yazma-inceleme ve düzeltme. **Tuğrul Doruk:** Metodoloji, yazma-inceleme ve düzeltme. **Salih Doğan:** Kavramsallaştırma, metodoloji, araştırma, yönetim, danışmanlık, denetim, doğrulama, veri iyileştirme, yazma-inceleme ve düzeltme.

Bu çalışma, ilk yazarın yüksek lisans tezinden üretilmiştir.

Etik onay beyanı

Çalışmanın yürütülmesi ve yayına hazırlanması sürecinde tüm etik kurallara en yüksek standartlarda uyulmuştur; ayrıca bu çalışmada etik kurul onay belgesine gerek yoktur.

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RESEARCH ARTICLE

A massive infestation of the long-legged buzzard, *Buteo rufinus* (Cretzschmar), by *Hyalomma marginatum* Koch (Acari: Ixodidae) ticks in Türkiye

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ABSTRACT: Ticks are obligate blood-sucking ectoparasites of vertebrate animals, including mammals, birds, reptiles, and even amphibians. As a suitable host, birds may carry and spread ticks and serve as reservoirs for some tick-borne pathogens. The present study reports an impressive tick infestation on a long-legged buzzard, *Buteo rufinus* (Cretzschmar), in Türkiye. One hundred fifty-nine engorged nymphal ticks were removed with tweezers from a *B. rufinus* in the Wildlife Rescue Rehabilitation, Training, Practice, and Research Center (AKUREM), Afyon Kocatepe University, Afyonkarahisar province, Türkiye. All ticks were morphologically identified as the *Hyalomma marginatum* group. For accurate species identification, a molecular study on randomly selected two engorged nymphs was performed through Polymerase Chain Reaction (PCR) amplification of a ~460 bp fragment of the mitochondrial 16S rRNA gene. Comparing our mitochondrial 16S rRNA sequences with those from the NCBI Genbank database showed that our ticks have a significant genetic similarity over 99% with *Hyalomma marginatum* Koch. Further, the extracted tick DNAs were also screened for the presence of *Rickettsia*, *Borrelia* and *Bartonella* bacteria targeting the rickettsial citrate synthase (*gltA*, ~750 bp), flagellin B (*flaB*, ~659 bp), NADH dehydrogenase gamma subunit (*nuoG*, ~346) genes, respectively; but samples were negative for these bacteria. To our knowledge, this is the first report of *H. marginatum* infesting *B. rufinus* in Türkiye and the first observation of the massive infestation of *H. marginatum* on *B. rufinus*.

Keywords: Biodiversity, fauna, birds, parasites, vectors, wild animals.
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INTRODUCTION

Ticks (Ixodidae) are ectoparasites that have to suck blood throughout all developmental stages in terrestrial vertebrates, mainly mammals and birds. Today, there are a thousand tick species in three living families: Ixodidae (778 species), Argasidae (221 species) and Nuttalliellidae (1 species). In addition, the extinct families Deinocrotonidae (2 species) and Khimairidae (1 species) were described based on fossil materials from Burmese amber in Northern Myanmar (Dantas-Torres, 2018; Chitimia-Dobler et al., 2022; Guglielmone et al., 2023). Due to its geographical location and climatic characteristics, Türkiye has vegetation, habitats and rich wildlife, allowing a suitable habitat for various tick species. In studies conducted in the country, more than 50 tick species belonging to the Ixodidae and Argasidae families have been reported (Bursalı et al., 2012; Keskin et al., 2014; Keskin and Selçuk, 2021).

Ticks serve as a reservoir of various pathogenic organisms and play an important role in transmitting many pathogens to their hosts, animals, and humans (Jongejan and Uilenberg, 2004; Stafford, 2007; Dantas-Torres et al., 2012). In addition, many ticks may infest a single host animal, cause anaemia and weight loss in animals, and even cause death if they can suck blood excessively (Uilenberg, 1992).

Türkiye has a rich bird fauna, but ectoparasites of birds are unfortunately poorly studied. Although various studies on ticks infesting birds, mainly passerines, have been recently conducted (Keskin et al., 2014; Keskin and Erciyas-Yavuz, 2016, 2019); there is still limited information about the ticks infesting many bird species in Türkiye. In previous reports, more than twenty tick species belonging to the genera *Amblyomma*, *Argas*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Ornithodoros* and *Rhipicephalus* infested on birds have been reported in Türkiye (Bursali et al., 2012; Keskin et al., 2014; Keskin and Erciyas-Yavuz, 2016, 2019; Eren and Açıcı, 2021). One of the tick species infested birds in Türkiye is *Hyalomma marginatum*. The tick species is the primary vector of the Crimean-Congo Hemorrhagic Fever Virus (CCHFV), which is endemic in Africa, southern Europe, the Middle East and Asian countries (Ergönül, 2009). It has high ecological plasticity and can adapt to regions where low or moderate humidity and a long dry season. All active stages of the tick typically live in the steppe, savannah and scrubland hill and valley biotypes in North Africa and Western Asia (Santos-Silva and Vatansever, 2017).

In the present study, we reported the massive infestation of *Hyalomma marginatum* Koch on the long-legged buzzard, *Buteo rufinus* (Cretzschmar), in Türkiye.

MATERIALS AND METHODS

Collection and morphological identification of ticks

One hundred fifty-nine engorged nymphal ticks were removed with tweezers from a *B. rufinus* in the Wildlife Rescue Rehabilitation, Training, Practice, and Research Center (AKUREM), Afyon Kocatepe University, Afyonkarahisar province, Türkiye. Ticks were placed in glass tubes containing 70% ethanol and sent to the Parasitology Research Laboratory, Department of Biology, Tokat Gaziosmanpaşa University, Tokat province of Türkiye. Ticks were morphologically identified using keys by Apanaskevich and Horak (2008) and Estrada-Peña et al. (2017).

A molecular study was performed on randomly selected two engorged nymphs for accurate species identification.

DNA isolation and Polymerase Chain Reaction (PCR)

DNA isolation of ticks was performed by a commercial DNA extraction kit (PureLink™ Genomic DNA Mini Kit, Invitrogen, Carlsbad, CA, USA) with minor modifications, according to the manufacturer's protocol. Briefly, ticks were rinsed in absolute ethanol and dried in a 1.5 ml plastic tube before DNA isolation. After the samples were dried entirely, the tick was mechanically crushed in 180 µl of Digestion Buffer, and 40 µl of Proteinase K was added and incubated overnight at 55 °C. The samples were centrifuged at max speed for 3 minutes, and the supernatant was transferred to a clean tube. 20 µl of RNase A was added to the lysate, mixed well by brief vortexing, and incubated at room temperature for two minutes. 200 µl of Lysis/Binding buffer was added, and then 200 µl of 96% ethanol was added to the lysate. The lysate was transferred to a spin column and centrifuged at 10000 rpm for one minute. The collection tube was discarded, and the spin column was placed in a clean collection tube. 500µl of Wash Buffer 1 was added and centrifuged at 10000 rpm for one minute. The collection tube was discarded, the spin column was placed in a clean collection tube, and 500 µl of Wash Buffer 2 was added and centrifuged at 14000 rpm for three minutes. The column was placed in a sterile 1.5 ml microcentrifuge tube, 50 µl of Elution Buffer was added, and the isolation process was completed by centrifuging at 14000 rpm for 90 seconds. DNA concentration was evaluated using a spectrophotometer (Multiskan Go, Thermo Scientific, Vantaa, Finland) at 260/280 wavelength, and ticks' DNAs were stored at -20 °C until the PCR.

For the molecular identification of ticks, DNAs of randomly selected two engorged nymphs were screened by Polymerase Chain Reaction (PCR) (Bio-Rad T100™ Thermal Cycler, Hercules, CA, USA) amplification of a ~460 bp fragment of the mitochondrial 16S rRNA gene using primers set 16S+1 and 16S-1. The extracted tick DNAs were also examined for the presence of *Rickettsia*, *Borrelia* and *Bartonella* bacteria by PCR using specific primers targeting the rickettsial citrate synthase (*gltA*, ~750 bp), flagellin B (*flaB*, ~658 bp), NADH dehydrogenase gamma subunit (*nuoG*, ~346 bp) genes, respectively. The primer sequences are shown in Table 1.

PCR conditions for all assays were as follows; denaturation at 94 °C for 5 min, then 35 cycles of 40 s at 94 °C, 60 s at 51 °C, and 60 s at 72 °C, followed by 10 min at 72 °C. The PCR reaction mixture (50 µl) contained 25 µl Dream Taq™ PCR Master Mix 2x (Thermo Fisher Scientific, Vilnius, Lithuania), 2 µl forward primer, 2 µl reverse primer, 2 µl of DNA template and 19 µl molecular grade water.

PCR products were verified by electrophoresis in a 1% agarose gel, pre-stained with ethidium bromide and visualized using a gel documentation system (UVP, Upland, CA, USA). Double distilled water was used as a negative control. Purified DNAs were sequenced by Macrogen Inc. (Amsterdam, The Netherlands). For editing raw sequences and generating consensus sequences, BioEdit Sequence Alignment Editor (version 7.0.1.5) was used (Hall, 1999).

RESULTS

In the present study, we collected 159 engorged nymphal ticks from a *B. rufinus* (Fig. 1) in the AKUREM, Afyon Kocatepe University, Afyonkarahisar province. All ticks belonged to the *Hyalomma marginatum* complex. Due to a lack of reliable external characters for differentiating the immature stages of *H. marginatum* complex, the morphological identification of these ticks is currently not possible; therefore, we performed a molecular study on randomly selected two engorged nymphs.

According to BLAST comparisons with the NCBI GenBank database, our sequences (OQ975264-OQ975265) obtained in nymphal *Hyalomma* were 99.78% similar to *Hyalomma marginatum* isolate Hymr1 (KT391060) from Israel, 99.55% *Hyalomma marginatum* isolate HM-Z384US 2017 (MW172439) from Italy, and 99.56% *Hyalomma marginatum* isolate 1 (OL347853) from Türkiye.

For the presence of *Rickettsia*, *Borrelia* and *Bartonella* bacteria, the tick DNAs obtained from two *Hyalomma* specimens were also screened by PCR; but both two samples were negative for these bacteria.

This is the first report *of H. marginatum* infesting *B. rufinus* in Türkiye and the first case of the massive infestation of *H. marginatum* on *B. rufinus*.

DISCUSSION

Wild birds play a significant role in the dispersal of both ticks and various tick-borne diseases such as *Anaplasma phagocytophilum*, *Borrelia* spp., *Rickettsia* spp., *Babesia* spp., and *Neoehrlichia mikurensis* from one region to another region (Hoogstraal et al., 1961, 1963; Dubska et al., 2009; Movilla et al., 2012; Capek et al., 2014; Leblebicioglu et al., 2014; Morozov et al., 2022).

Table 1. The nucleotide sequences of primers, target genes and product size were used for the PCR gene amplification.

Target organism	Target gene	Nucleotide sequence (5' → 3')	Product size (~bp)	References
Ticks	16S rDNA	CTGCTCAATGATTTTTTAAATTGCTGTGG CCGGTCTGAACTCAGATCAAGT	460	Black and Piesman (1994)
Rickettsia	*gltA*	CCTATGGCTATTATGCTTGC ATTGCAAAAAGTACAGTGAACA	750	Roux et al. (1997)
Borrelia	*flaB*	ACATATTCAGATGCAGACAGAGGT GCAATCATAGCCATTGCAGATTGT	658	Barbour et al. (1996)
Bartonella	*nuoG*	GGCGTGATTGTTCTCGTTA CACGACCACGGCTATCAAT	346	Colborn et al. (2010)

Figure 1. The long-legged buzzard, *Buteo rufinus,* presents massive infestation by nymphs of *Hyalomma marginatum* ticks.

Buteo rufinus is a medium-sized and wide-winged predator bird seen in almost every region of our country; they are much more common in the Inner Aegean, Central Anatolia and Eastern Anatolia of Türkiye. The small and medium-sized mammals, birds and reptiles are main foods of *B. rufinus*. The European *B. rufinus* are mainly migratory, but others reside and breed in the Balkans, southern Greece and Türkiye. Also, stragglers, juveniles and subadults migrate to the north and west of the breeding range before autumn (Forsman, 1999).

Like the other birds, *B. rufinus* is a suitable host for the many ectoparasite species. Early studies conducted in Türkiye shown that many parasite species, including ticks [*Haemaphysalis parva* (Neumann), *Hyalomma* spp. and *Rhipicephalus sanguineus* (Latreille)] (Orkun et al., 2014, 2017) and lice [*Colpocephalum nanum* Piaget, *Craspedorrhynchus platystomus* (Burmeister), *Degeeriella fulva* (Giebel), *Kurodaia fulvofasciata* (Piaget) and *Laemobothrion maximum* (Scopoli)], could be infested on *B. rufinus* (Dik and Ozkayhan, 2007; Dik and Kandir, 2021; Dik et al., 2022).

In the present study, we reported *H. marginatum* ticks on *B. rufinus* in Türkiye for the first time. *Hyalomma marginatum* is a two-host tick completed one generation per year in nature. Its immatures mainly feed on wild small mammals and ground-feeding birds, but adults prefer to feed on artiodactyls (Guglielmone et al., 2014). In Türkiye, *H. marginatum* is one of the most common tick species on domestic animals, including cattle, goats, sheep, donkeys and horses. The tick species is responsible for the majority of tick infestations on humans (Bursali et al., 2011; Karaer et al., 2011; Keskin et al., 2015; Karasartova et al., 2018).

In the present study, we also investigated the presence of some pathogenic bacteria, such as *Rickettsia*, *Borrelia* and *Bartonella*, in two tick specimens collected from *B. rufinus*, but these ticks were negative for these bacteria.

Our examination findings suggest that further studies should be conducted to reveal the ectoparasite fauna of the native and migratory populations of *B. rufinus*.

Authors' contributions

Ayşe Sarı: Investigation, resources, writing-original draft. **Emine Hesna Kandır:** Conceptualization, investigation, resources, supervision, visualisation, writing – review & editing. **Bilal Dik:** Conceptualization, investigation, resources, supervision, visualisation, writing – review & editing. **Adem Keskin:** Conceptualization, investigation, resources, supervision, visualisation, writing – original draft, writing – review & editing.

Statement of ethics approval

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Conflict of interest

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RESEARCH ARTICLE

Zihinsel engelli bireylerde *Demodex* akar (Acari: Demodecidae) prevalansı ve yoğunluğu

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ÖZET: Bu çalışma Erzincan ilindeki rehabilitasyon merkezlerinde bakım ve destek hizmeti almakta olan zihinsel engelli bireylerde *D. folliculorum* ve *D. brevis* prevalansı ve yoğunluğunu belirlemek amacıyla yapılmıştır. Çalışmaya Erzincan il merkezinde faaliyet gösteren 7 ayrı rehabilitasyon merkezinde bakım ve destek hizmeti almakta olan 217 zihinsel engelli katılımcı dahil edildi. Örnek materyalleri her bir katılımcının yanak, nazolabial ve çene bölgesinden Standart Yüzeyel Deri Biyopsisi yöntemi ile alındı ve ışık mikroskobunda *Demodex* akar varlığı ve sayısı bakımından incelendi. Çalışmada zihinsel engellilerin %58'inde *D. folliculorum* (ortalama 8,34/cm^2), %25'inde *D. brevis* (ortalama 1,26/cm^2) olmak üzere toplam %61'inde *Demodex* akar (ortalama 9,59/cm^2) tespit edildi. Katılımcılar *Demodex* prevalansı bakımından değerlendirildiğinde *D. folliculorum*'un *D. brevis*'e göre yaklaşık 2,3 kat daha yaygın olduğu belirlendi. Benzer şekilde cm^2'deki ortalama akar sayısı bakımından değerlendirildiğinde *D. folliculorum*'un, *D. brevis*'ten yaklaşık 7 kat daha yoğun olduğu tespit edildi. Diğer taraftan zihinsel engel şiddeti arttıkça *Demodex* akar prevalansının arttığı belirlenirken, *Demodex* yoğunluğunun en fazla orta düzey zihinsel engele sahip katılımcılarda olduğu tespit edildi. Sonuç olarak gerek sağlıklı bireylerde gerek birçok hasta grubunda olduğu gibi zihinsel engelli bireylerde de *D. folliculorum* ve *D. brevis*'in yaygın ve yoğun olduğu tespit edildi. Çalışmamızda elde edilen bulguların dermatolojik şikayetleri olan zihinsel engelli bireylerin klinik değerlendirme sürecinde göz önünde bulundurulmasının faydalı olabileceği düşünüldü.

Keywords: *Demodex*, epidemiyoloji, Erzincan, mental retardasyon.
Zoobank: https://zoobank.org/157C8B64-373E-4B24-852E-4F64D9952533

Prevalence and density of *Demodex* mites (Acari: Demodecidae) in mentally disabled individuals

ABSTRACT: This study was conducted to determine the prevalence and intensity of *D. folliculorum* and *D. brevis* in mentally disabled individuals receiving care and support services in rehabilitation centers in Erzincan province. The study included 217 mentally disabled participants who were receiving care and support services in 7 separate rehabilitation centers operating in the provincial center of Erzincan. Sample materials were taken from the cheek, nasolabial and chin area of each participant by Standard Superficial Skin Biopsy method and examined for the presence and number of *Demodex* mites under a light microscope. In the study, *D. folliculorum* (mean 8.34/cm^2) was detected in 58% of mentally disabled people, *D. brevis* (mean 1.26/cm^2) in 25%, and *Demodex* mites (mean 9.59/cm^2) was detected in 61% of the all participants. When the participants were evaluated in terms of *Demodex* prevalence, it was determined that *D. folliculorum* was approximately 2.3 times more common than *D. brevis*. Similarly, when evaluated in terms of the average number of mites per cm^2, it was found that *D. folliculorum* was about 7 times denser than *D. brevis*. On the other hand, it was determined that the prevalence of *Demodex* mites increased as the severity of intellectual disabilities increased, while it was found that the intensity of *Demodex* was highest in participants with moderate mentally disabled. As a result, it was found that *D. folliculorum* and *D. brevis* were widespread and intense in both healthy individuals and mentally disabled individuals, as well as in many patient groups. It was thought that the findings obtained in our study might be useful to consider mentally disabled individuals with dermatological complaints during the clinical evaluation process.

Keywords: *Demodex*, epidemiology, Erzincan, mental retardation.

GİRİŞ

Demodex akarlar (Acari), Trombidiformes (Reuter, 1909) takımının Demodecidae (Nicolet, 1855) familyasına ait olan eklembacaklı (Arthropoda) mikroskobik organizmalardır. Sadece memelilerde parazit olarak yaşayabilen *Demodex* akarların insanlarda yaşayabilen iki türü tanımlanmıştır: *Demodex folliculorum* Simon, 1842 ve *Demodex brevis* Akbulatova (Desch ve Nutting, 1972; Rufli ve Mumcuoğlu, 1981). Uzun opisthozomaya sahip olan *D. folliculorum* kıl foliküllerinde tek veya gruplar halinde yaşarken, kısa opisthozomaya sahip olan *D. brevis* sebase bezlerde genelde tek olarak yaşamaktadır (Rufli ve Mumcuoglu, 1981). Erginleri yaşadığı habitata uygun olarak puro şeklinde bir vücuda, kitin yapıda bir dış iskelete, delici ağız parçalarına ve terminal ucunda birer çift tırnak bulunan dört çift bacağa sahiptir. Erginlerinin ortalama yaşam süreleri 15 gün kadar olan bu organizmalar 0,3-0,4 mm

vücut büyüklüğe sahiptir. Sahip oldukları delici ağız parçaları ve çeşitli enzimleri sayesinde foliküler epitel hücrelerin içeriği ve sebum ile beslenirler (Desch ve Nutting, 1977; Rufli ve Mumcuoglu, 1981). *Demodex* akarlar sağlıklı kişilerin kıl folikülünde bazen hiçbir patojenik etki yapmadan kalabildiği halde, cilt hijyeninin iyi yapılamadığı hallerde, bağışıklık sisteminin baskılandığı veya zayıfladığı durumlarda fırsatçı patojen olabilmekte akne, roza, perioral dermatit, seboreik dermatit ve blefarit patogenezinde rol oynayabilmektedir (Zeytun, 2017; Zeytun ve Ölmez, 2017; Zeytun vd., 2017; Tilki vd., 2017; Karakurt ve Zeytun, 2018; Sarı vd., 2019; Zeytun ve Yazıcı, 2019, 2022; Zeytun ve Karakurt, 2019).

Genel zekâ işlevlerine göre belirli bir düzeyin altında olma durumu olan zihinsel engellilik, doğumda ya da gelişimsel dönemde kendini göstermektedir Zihinsel engellilik terimi genel olarak zekâ geriliğini (mental retardasyon) tanımlamak için kullanılır. Mental retardasyon, 18 yaşından önce ortaya çıkan gelişimsel bir bozukluktur. Zekâ geriliğinde, zihinsel işlevlerde normallerden önemli derecede gerilik olmasının yanı sıra, günlük yaşamı idame ettirmede gerekli olan uyumsal becerilerde yetersizlik söz konusudur. Uyumsal beceriler; beslenme, giyinme, yıkanma, tuvalet ihtiyacını giderme gibi öz bakım becerileri, ev temizliği gibi ev yaşamı becerileri, dili konuşma ve anlama ile beraber iletişim kurma becerisi, sosyal beceriler, toplumsal yararlılık ve mesleki beceriler gibi beceri alanlarını kapsamaktadır (Kömerik vd., 2012).

Günümüze kadar gerek Türkiye'de gerekse diğer ülkelerde çeşitli dermatolojik (akne vulgaris, rosacea, pityriasis folliculorum, perioral dermatit, seboreik dermatit), oftalmolojik (blefarit ve dandruf gibi) ve diğer hasta gruplarında (diyabet, renal yetmezlik, kanser gibi) ve sağlıklı bireylerde *Demodex* akar prevalansını belirlemeye yönelik çok sayıda epidemiyolojik çalışma yapılmıştır. Bununla birlikte zihinsel engelli bireylerde bu konuda yapılmış herhangi bir çalışma bulunmamaktadır. Bu çalışma Erzincan ilindeki rehabilitasyon merkezlerinde bakım ve destek hizmeti almakta olan zihinsel engelli bireylerde *D. folliculorum* ve *D. brevis* prevalansı ve yoğunluğunu belirlemek amacıyla yapılmıştır.

MATERYAL VE YÖNTEM

Çalışma Erzincan il merkezinde faaliyet gösteren 7 ayrı rehabilitasyon merkezinde yürütüldü. Çalışmaya bu rehabilitasyon merkezlerinde bakım ve destek hizmeti almakta olan 217 zihinsel engelli katılımcı dahil edildi. Katılımcıların zihinsel engel düzeyleri rehabilitasyon merkezlerindeki dosya bilgilerinden alındı. Çalışma için gerekli izinler Erzincan Binali Yıldırım Üniversitesi Etik Kurulu (Karar no: 2015-1/6), Erzincan Aile ve Sosyal Hizmetler İl Müdürlüğü (Karar no: 2015-100724) ve Erzincan İl Milli Eğitim Müdürlüğünden (Karar no: 2015-4444452) alındı ve tüm katılımcılara veya vasilerine Helsinki Deklarasyonu doğrultusunda bilgilendirilmiş onam formu okunarak imzalatıldı.

Örnek materyalleri herbir katılımcının yanak, nazolabial ve çene bölgesinden Standart Yüzeyel Deri Biyopsisi (SYDB) yöntemi ile alındı. Örnek alınacak bölgeler alkol ile temizlenip kurulandı. Temiz bir lam alınarak üzerine bir cm^2'lik alan çizildi. Lamın diğer yüzüne bu alanın ortasına gelecek şekilde bir damla siyanoakrilat (Best, Ankara) damlatılarak örnek alınacak yüzeye hafifçe bastırıldı ve yaklaşık bir dakika sonra yavaşça kaldırıldı. Örnek materyallerinin üzerine katılımcının adı-soyadı ve örneğin alındığı bölge yazıldı.

Örnek materyallerinin üzerine Hoyer eriyiği damlatılarak lamel ile kapatıldı ve preparat haline getirildi. Preparatlar 1 saat içinde ışık mikroskobunda (Leica DM750, İsviçre) 4X, 10X, 40X büyütmelerde incelendi. Akarların tür teşhisi ilgili literatür ışığında (vücut uzunluğuna; opisthozomanın idiozoma'ya olan oranına; opistozomanın terminal kısmının sivri veya yuvarlak oluşuna; gelişim aşamalarına; bacak ve ağız parçalarına vb. bakılarak) yapıldı (Desch ve Nutting, 1972, 1977). Preparatlarda *D. folliculorum* veya *D. brevis*'in larva, nimf veya erginine rastlanılması durumunda örnek materyali *Demodex* bakımından pozitif olarak kabul edildi. 1 cm^2'deki ortalama *Demodex* sayısı; toplam *Demodex* sayısının, *Demodex* rastlanan katılımcı sayısına bölünmesi ile hesaplandı.

Verilerin istatistiksel değerlendirmesi SPSS 23.0 (Sosyal Bilimler için İstatistik Programı) (Chicago, IL, USA) programı kullanılarak yapıldı. Değişkenlerin normal dağılıma uygunluğu Kolmogorov-Smirnov testi kullanılarak incelendi. Gruplar arası karşılaştırmalar yapılırken nonparametrik testlerden Mann-Whitney U ve Kruskall Wallis testi kullanıldı. Kategorik verilerin değerlendirilmesinde ise Ki kare testi kullanıldı. *Demodex* prevalansı ve yoğunluk oranları %95 güven aralıkları ile maksimum olabilirlik tahmini yöntemi kullanılarak hesaplandı. ''P'' değerinin 0.05'den küçük olması durumunda istatistiksel olarak anlamlı kabul edildi.

BULGULAR VE TARTIŞMA

Zihinsel engellilik terimi genel olarak zekâ geriliğini (mental retardasyon) tanımlamak için kullanılır. Mental retardasyon, standardize edilmiş testler vasıtasıyla zekâ katsayısı (Intelligence Quotient, IQ) bulunarak ölçülmektedir. Ortalama puanı 100 olan IQ seviyesinin 70'in altında olması zekâ geriliği olarak kabul edilir. Amerikan Psikiyatri Birliği dört zekâ geriliği düzeyi belirlemiştir: hafif derecede zekâ geriliği (IQ 50-70 arası), orta derecede zekâ geriliği (IQ 35-55 arası), ağır zekâ geriliği (IQ 20-40 arası) ve derin zekâ geriliği (IQ 20-25'in altında) (Kömerik vd., 2012). Çalışmaya Erzincan il merkezinde faaliyet gösteren 7 ayrı rehabilitasyon merkezinde bakım ve destek hizmeti almakta olan 217 zihinsel engelli katılımcı dahil edildi (135 kadın, 82 erkek, ortalama yaş 25,69 ± 12,82). Katılımcıların rehabilitasyon merkezindeki dosya bilgilerine göre 80'inin hafif (%36,9), 96'sının orta (%44,2) ve 41'inin ağır düzeyde (%18,9) zihinsel engele sahip olduğu ve herhangi bir dermatolojik şikayeti bulunmadığı tespit edildi (Tablo 1).

Çalışmada zihinsel engellilerin %58'inde *D. folliculorum* (ortalama 8,34/cm^2), %25'inde *D. brevis* (ortalama 1,26/cm^2) olmak üzere toplam %61'inde *Demodex* akar (ortalama 9,59/cm^2) tespit edildi.

Tablo1. Katılımcıların yaş ve cinsiyetleri.

	Zihinsel Engel Düzeyi			
	Hafif (n: 80/217) (%36,9)	**Orta (n: 96/217) (%44,2)**	**Ağır (n: 41/217) (%18,9)**	**Toplam (n: 217) (%100)**
Yaş (yıl)				
Ortalama ± Standart sapma	19,51 ± 7,52	28,01 ± 14,42	32,29 ± 12,13	25,69 ± 12,82
Ortanca (en az – en çok)	17 (10-60)	25 (11-60)	29 (14-60)	22 (10-60)
Cinsiyet				
Kadın	51/80 (%64)	60/96 (%63)	24/41 (%59)	135/217 (%62)
Erkek	29/80 (%36)	36/96 (%37)	17/41 (%41)	82/217 (%38)

Tablo 2. Katılımcılarda *Demodex* akar prevalansı ve yoğunluğu.

	Zihinsel Engel Düzeyi				
	Hafif (n: 80/217) (%36,9)	**Orta (n: 96/217) (%44,2)**	**Ağır (n: 41/217) (%18,9)**	**Toplam (n: 217) (%100)**	**p**
***Demodex* Prevalansı**					
D. folliculorum	38/80 (%48) (GA: %36-59)	59/96 (%61) (GA: %52-71)	28/41 (%68) (GA: %53-83)	125/217 (%58) (GA: %51-64)	0,054 [b]
D. brevis	14/80 (%18) (GA: %9-26)	34/96 (%35) (GA: %26-45)	7/41 (%17) (GA: %5-29)	55/217 (%25) (GA: %20-34)	0,010 [b]
Demodex spp.	42/80 (%53) (GA: %41-64)	63/96 (%66) (GA: %56-75)	28/41 (%68) (GA: %53-83)	133/217 (%61) (GA: %55-68)	0,122 [b]
Ortalama *Demodex* Yoğunluğu [a]					
D. folliculorum	6,29 (GA: 0,45-12,12)	10,30 (GA: 4,92-15,68)	7,00 (GA: 2,33-11,77)	8,34 (GA: 5,09-11,58)	0,008 [c]
D. brevis	0,95 (GA: 0,33-1,57)	1,65 (GA: 0,83-2,47)	0,82 (GA: 0,02-1,63)	1,26 (GA: 0,80-1,72)	0,030 [c]
Demodex spp.	7,24 (GA: 0,91-13,56)	11,95 (GA: 5,86-18,04)	7,82 (GA: 2,35-13,30)	9,59 (GA: 5,96-13,23)	0,002 [c]

GA: %95 güven aralığı.

[a] *Demodex*/cm^2 yoğunluğunun hesaplanmasında sadece *Demodex* pozitif katılımcılar hesaba katılmıştır.

[b] Ki-kare testi

[c] Kruskal-Wallis testi

Katılımcılar *Demodex* prevalansı bakımından değerlendirildiğinde *D. folliculorum*'un *D. brevis*'e göre yaklaşık 2,3 kat daha yaygın olduğu belirlendi. Benzer şekilde cm^2'deki ortalama akar sayısı bakımından değerlendirildiğinde *D. folliculorum*'un, *D. brevis*'ten yaklaşık 7 kat daha yoğun olduğu tespit edildi. Diğer taraftan zihinsel engel şiddeti arttıkça *Demodex* akar prevalansının arttığı belirlenirken, *Demodex* yoğunluğunun en fazla orta düzey zihinsel engele sahip katılımcılarda olduğu tespit edildi. Zihinsel engelli grupları *Demodex* yoğunluğu bakımından karşılaştırıldığında aradaki farklar istatistiki bakımdan anlamlı bulundu (Tablo 2).

Günümüze kadar gerek Türkiye'de gerekse diğer ülkelerde dermatolojik ve oftalmolojik gibi, hasta gruplarında ve sağlıklı bireylerde *Demodex* akar prevalansını belirlemeye yönelik çok sayıda epidemiyolojik çalışma yapılmıştır. Ancak zihinsel engelli bireylerde bu konuda günümüze kadar yapılmış herhangi bir çalışma bulunmamaktadır. Ülkemizde yapılan çalışmalara bakıldığında; Gaziantep'te rozaseli 38 hastanın %26,3'ünde ortalama 6,68/cm^2, 38 kontrolün %13,1'inde ortalama 2,86/cm^2 tane *D. folliculorum* saptandığı belirtilmiştir (Erbağcı ve Özgöztaşı, 1998). Malatya'da böbrek yetmezliği olan 67 hastanın %40,2'sinde ortalama 6,12/cm^2, 67 kontrolün %29,8'inde 0.31/cm^2 tane *D. folliculorum* bildirilmiştir (Karıncaoğlu vd., 2005). Aydın'da üniversitede öğrenim gören 102 öğrencinin %34,8'inde ortalama 1,41/cm^2 tane *D. folliculorum* bildirilmiştir (Okyay vd., 2006). Diyarbakır'da 87 hemodiyaliz hastasının %19,54'ünde ortalama 5,11/cm^2, 87 kontrolün %10,34'ünde ortalama 2,55/cm^2 tane *D. folliculorum* tespit edilmiştir (Düzgün ve Aytekin, 2007). Sivas'ta kronik böbrek yetmezliği bulunan 47 hasta ve aktif spor yapan 38 sağlıklı bireyin sırası ile %25,53'ü ve %18,42'sinde *D. folliculorum* tespit edilmiştir (Özçelik vd., 2007). Afyonkarahisar'da romatoit artritli 41 hasta ve 27 kontrolde *D. folliculorum* yaygınlığı sırası ile %12 ve %8 olarak bildirilmiştir (Çiftci vd., 2007).

Tablo 3. Katılımcıların yaş ve cinsiyetleri ile *Demodex* prevalansı ve yoğunluğu arasındaki ilişki.

	***Demodex* Prevalansı**	**p**	**Ortalama *Demodex* yoğunluğu**	**p**
Yaş (yıl)				
10 – 20	55/104 (%53) (GA: %43-63)		4,93 (GA: 3,03-6,82)	
21 – 40	58/85 (%68) (GA: %58-78)	0.049 [b]	13,16 (GA: 5,47-20,84)	0,046 [c]
41 - 60	20/28 (%71) (GA: %54-89)		12,10 (GA: 3,94-20,26)	
Toplam	133/217 (%61) (GA: %55-68)		9,59 (GA: 5,96-13,23)	
Cinsiyet				
Kadın	86/135 (%64) (GA: %55-72)	0.349 [b]	9,57 (GA: 5,68-13,46)	0,642 [d]
Erkek	47/82 (%57) (GA: %46-68)		9,64 (GA: 1,99-17,29)	
Toplam	133/217 (%61) (GA: %55-68)		9,59 (GA: 5,96-13,23)	

GA: %95 güven aralığı.

[a] *Demodex*/cm^2 yoğunluğunun hesaplanmasında sadece *Demodex* pozitif katılımcılar hesaba katılmıştır.

[b] Ki-kare testi

[c] Kruskal-Wallis testi

[d] Mann-Whitney U testi

Afyonkarahisar'da yapılan başka bir çalışmada fototerapi alan 45 hastanın %28,9'unda ortalama 3,22/cm^2, 43 kontrolün %7'sinde 0,97/cm^2 *D. folliculorum* bildirilmiştir (Kulaç vd., 2008). Yine Afyonkarahisar'da tıp fakültesinde öğrenim gören 100 öğrencinin %11'inde *D. folliculorum* saptanmıştır (Miman vd., 2008). Ordu'da yapılan bir çalışmada devlet hastanesindeki laboratuvar çalışanları, mutfak personeli, temizlik işçileri ve hemşirelerden alınan 95 örnekten %74,7'sinin *Demodex* spp. bakımından pozitif olduğu belirtilmiştir (Fırat vd., 2010). Elazığ'da 258 üniversite öğrencisinin %10,07'sinde *Demodex* spp. saptanmıştır (Kaplan vd., 2012). Ordu'da yapılan başka bir çalışmada 300 üniversite öğrencisinin %37'sinde *Demodex* spp.'ye rastlanılmıştır (Karaman vd., 2014). Hatay'da 63 depresyon hastası ve 63 kontrolün sırası ile %23,8'i ve %9,5'inde *Demodex* spp. tespit edilmiştir (Kokaçya vd., 2014). Hatay'da yapılan başka bir çalışmada polikistik over sendromlu 30 hasta ve 30 kontrolde *D. folliculorum* pozitifliği sırası ile %30 ve %6,7 olarak bildirilmiştir (Benk Silfeler vd., 2015). Giresun'da sağlık bilimleri fakültesinde öğrenim gören 270 öğrenciden %29,7'sinde *D. folliculorum*, %19,5'inde *D. brevis* olmak üzere %47,4'ünde *Demodex* spp. saptanmıştır (Özdemir vd., 2015). Erzincan'da üniversitede öğrenim gören 385 öğrencinin %80,3'ünde ortalama 6,6/cm^2 *D. folliculorum*, %3,6'sında ortalama 1,3/cm^2 *D. brevis*, %50,1'inde ortalama 7,1/cm^2 *Demodex* spp. bildirilmiştir (Zeytun vd., 2017). Erzincan'da yapılan başka bir çalışmada *kronik obstrüktif akciğer hastalığı* (KOAH)olan 101 hastada %82,2 *D. folliculorum* (ortalama 21,78/cm^2), %40,6 *D. brevis* (ortalama 4,70/cm^2) olmak üzere %87,1 *Demodex* spp. (ortalama 22,74/cm^2) pozitifliği rapor edilmiştir (Zeytun ve Ölmez, 2017). Diğer ülkelerde yapılan çalışmalara bakıldığında ise; Yunanistan'da rozaseli 92 hastanın %90,2'sinde ortalama 2,03/cm^2, 92 kontrolün %11,9'unda ortalama 0,16/cm^2 *D. folliculorum* saptandığı belirtilmiştir (Georgala vd., 2001). Meksika'da herhangi bir sağlık problemi olmayan 315 bireyin %16,2'sinde *D. folliculorum*, %11,1'inde *D. brevis* olmak üzere %27,3'ünde *Demodex* spp. pozitifliği bildirilmiştir (Hana vd., 2004). Meksika'da başka bir çalışmada rozaseli 30 hastanın %80'inde ortalama 1,90/cm^2, 30 kontrolün %30'unda ortalama 0,71/cm^2 *D. folliculorum* tespit edilmiştir (Rios-Yuil ve Mercadillo-Perez, 2013). Slovenya'da perioral dermatitli 82 hastanın %62,2'sinde ortalama 3,23/cm^2, 70 kontrolün %31,4'inde ortalama 0,66/cm^2 tane *D. folliculorum* bildirilmiştir (Dolenc Voljc vd., 2005). Mısır, Kahire'de dermatozlu 40 hastanın %82'sinde ortalama 11,82/cm^2, 40 kontrolün %47,5'inde ortalama 1,77/cm^2 *D. folliculorum* bildirilmiştir (el-Bassiouni vd., 2005). Çin'de 756 üniversite öğrencisinin %60,3'ünde *D. folliculorum*, %30,7'sinde *D. brevis* olmak üzere %67,6'sında *Demodex* spp. saptanmıştır (Zhao vd., 2011). Malezya'da tıp fakültesinde öğrenim gören 390 öğrencinin %17,2'sinde *Demodex* spp. saptandığı bildirilmiştir (Isa vd., 2011). Fransa'da rozaseli 50 hastanın %96'sında ortalama 4,9/cm^2, 47 kontrolün %74'ünde ortalama 0,84/cm^2 *D. folliculorum* bildirilmiştir (Casas vd., 2012). İran'da rozaseli 34 hastanın %47,1'inde ortalama 8,78/cm^2, 34 kontrolün %20,6'sında ortalama 4,11/cm^2 tane *Demodex* spp. saptandığı bildirilmiştir (Talghini vd., 2015).

Yukarıda özetlenen çalışmalar dikkate alındığında *D. folliculorum*, *D. brevis* ve *Demodex* spp. prevalansı ve cm^2 deki ortalama akar yoğunluğu değişim aralığının sırasıyla %7 - 96 (ortalama 0,16-21,78/cm^2), %3,6 - 40,6 (ortalama 1,3-4,70/cm^2) ve %9,5 - 87,1 (ortalama 4,11-22,74/cm^2) ol-

duğu görülmektedir. Çalışmamamızda *D. folliculorum*, *D. brevis* ve *Demodex* spp. prevalansı ve cm^2 deki ortalama akar yoğunluğu bakımından elde edilen bulgular yapılan diğer çalışmaların verileri ile uyumluluk göstermektedir. Bununla birlikte çalışmamızda ve diğer çalışmalarda *D. folliculorum*'un, *D. brevis*'ten daha yaygın ve yoğun olduğu tespit edilmiştir. Bunun sebebi *D. brevis*'in cilt yüzeyinden daha derinlerde (kıl foliküllerinin altındaki sebase bezlerde) yaşaması, *D. folliculorum*'un ise kıl foliküllerinin dışa bakan kısmında (cilt yüzeyine daha yakın bölgede) yaşaması ve dolayısıyla daha kolay izole edilebilir olmasından kaynaklanıyor olabilir (Tilki vd., 2017; Zeytun 2017; Zeytun ve Ölmez, 2017; Zeytun ve Yazıcı, 2019; Zeytun ve Karakurt, 2019).

Katılımcıların yaş ve cinsiyet özellikleri ile *Demodex* prevalansı ve yoğunluğu arasındaki ilişki Tablo 3'te ayrıntılı olarak verilmiştir. Çalışmada *Demodex* yoğunluğunun erkek ve kadın katılımcılarda hemen hemen aynı olduğu, ancak *Demodex* prevalansının kadınlarda erkeklerden daha fazla olduğu tespit edildi. Diğer taraftan yaş artışı ile birlikte *Demodex* prevalansının arttığı belirlenirken, *Demodex* yoğunluğunun da benzer şekilde arttığı ancak en fazla yoğunluğa 21-60 yaşlardaki katılımcıların sahip olduğu belirlendi. Yaş grupları *Demodex* prevalansı ve yoğunluğu bakımından karşılaştırıldığında aradaki farklar istatistiki bakımdan anlamlı bulundu. Yapılan diğer çalışmalarda *Demodex* prevalansının; erkeklerde daha fazla (Roihu ve Kariniemi, 1998; Okyay vd., 2006; Yazar vd., 2008; Isa vd., 2011; Kaplan vd., 2012; Karaman vd., 2014; Durmaz vd., 2015; Zeytun vd., 2017; Tilki vd., 2017; Zeytun ve Ölmez., 2017; Sarı vd. 2019; Zeytun ve Yazıcı., 2022), kadınlarda daha fazla (Fırat vd., 2010; Sönmez vd., 2013; Özdemir vd., 2015; Kokaçya vd., 2014; Zeytun, 2017) ya da erkek ve kadınlarda eşit olduğu bildirilmektedir (Hana, 2004; Zhao vd., 2011b). Bununla birlikte pek çok çalışmada *Demodex* prevalansı ve yoğunluğunun yaş artışı ile birlikte arttığı rapor edilmiştir (Aycan vd., 2007; Zhao vd. 2011; Zeytun vd., 2017; Tilki vd., 2017; Zeytun, 2017; Zeytun ve Ölmez., 2017; Sarı vd., 2019; Zeytun ve Karakurt., 2019; Zeytun ve Yazıcı., 2019).

Sonuç olarak gerek sağlıklı bireylerde gerek birçok hasta grubunda olduğu gibi zihinsel engelli bireylerde de *D. folliculorum* ve *D. brevis*'in yaygın ve yoğun olduğu tespit edildi. Çalışmamızda elde edilen bulguların dermatolojik şikayetleri olan zihinsel engelli bireylerin klinik değerlendirme sürecinde göz önünde bulundurulmasının faydalı olabileceği düşünüldü.

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RESEARCH ARTICLE

First record of the genus *Rhinothrombium* (Trombidiformes: Tanaupodidae) from Türkiye: *Rhinothrombium nemoricola* (Berlese)

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ABSTRACT: The genus *Rhinothrombium* (Tanaupodidae) is represented in the world by three species: *R. nemoricola* (Berlese, 1886), *R. inopinum* Hull, 1918 and *R. wuxiensis* Zhang, 1993. In this study, morphological characters, micrographs of various organs and distribution of postlarval forms of *R. nemoricola* are given. This is the first record of the genus *Rhinothrombium* from Türkiye.

Keywords: Acari, biodiversity, distribution, first record, Parasitengonina.
Zoobank: https://zoobank.org/A3C86D4A-DF8D-4ABA-AB8C-22FE5FE20696

INTRODUCTION

Tanaupodidae Thor, 1935 comprise nine genera: two fossil (*Atanaupodus* Judson and Mąkol, 2009, *Propolyssenia* Mąkol, Konikiewicz and Klug, 2018) and seven extant (*Eothrombium* Berlese, 1910, *Lassenia* Newell, 1957, *Neotanaupodus* Garman, 1925, *Polydiscia* Methlagl, 1928, *Rhinothrombium* Berlese, 1910, *Tanaupodus* Haller, 1882, and *Tignyia* Oudemans, 1937) (Mąkol and Featherstone, 2021). Members of the family are mainly distributed in the Palaearctic region, where they are found in decomposing organic matter and soil. The fauna of Türkiye represents only two known tanaupodid species; *Lassenia hemsinensis* Noei, Saboori and Çobanoğlu, 2018 and *Eothrombium siculum* Berlese, 1910 (Sevsay, 2017; Noei et al., 2018; Karakurt and Sevsay, 2020). The genus *Rhinothrombium* has the fewest species in the family and includes three species: *R. nemoricola* (Berlese, 1886), *R. inopinum* Hull, 1918, and *R. wuxiensis* Zhang, 1993 (Mąkol and Wohltmann, 2012). *Rhinothrombium* can be easily identified by the following characteristics: Scutum with naso, and idiosoma cuticle striate (Zhang, 1993). We here introduce a new record to contribute to the knowledge to mite diversity of Türkiye.

MATERIALS AND METHODS

Mite specimens were collected from Erzincan and Tunceli provinces of Eastern Türkiye. The specimens were extracted by Berlese-Tullgren funnels. The examined materials were preserved in 70% ethanol and cleared in 9% KOH. Specimens for microscope studies were fixed on slides in Hoyer's medium (Walter and Krantz, 2009). The morphological terminology follows those of Mąkol (2007) and Saboori et al. (2009). For measurements and micrographs an Olympus BX63 and Leica 3000 microscope were used. Micrometers (µm) is used for all measurements. The slides are deposited in the Acarology Laboratory of Erzincan Binali Yıldırım University, Erzincan, Türkiye (EBYU).

RESULTS

Family: Tanaupodidae Thor, 1835

Genus: *Rhinothrombium* Berlese, 1910

***Rhinothrombium nemoricola* (Berlese, 1886)**

Larva. Unknown.

Adult (n=2): Colouration reddish to brown. Idiosoma slightly elongate, length 1553-1650 and width 989-1075.

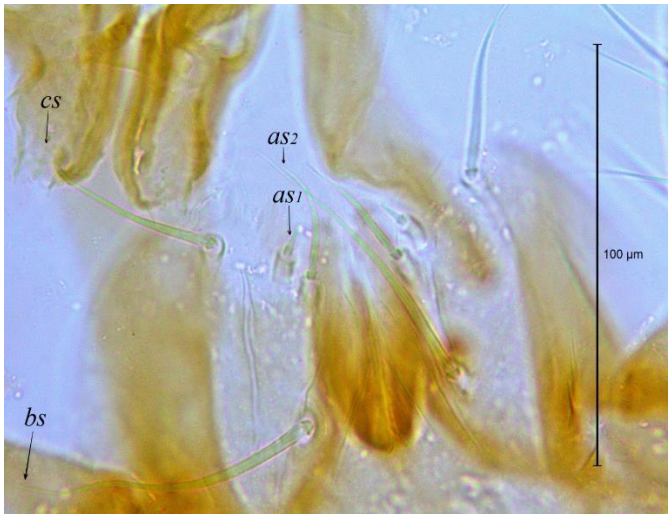

Figure 1. *Rhinothrombium nemoricola* (adult). Ventral view of gnathosoma.

One pair of nude galealae (*cs*, 47-49), two pairs of nude anterior hypostomalae (as_1, 10-12 and as_2, 30-34), one pair of nude subcapitular setae (*bs*, 58-72), *bs* longer than *cs* (Fig. 1). Cheliceral blade serrated along the inner edge. Palp tarsus slightly narrowing towards the end, rounded distally and extending behind the termination of palp tibial claw, covered with numerous solenidia at the top (Fig. 2A).

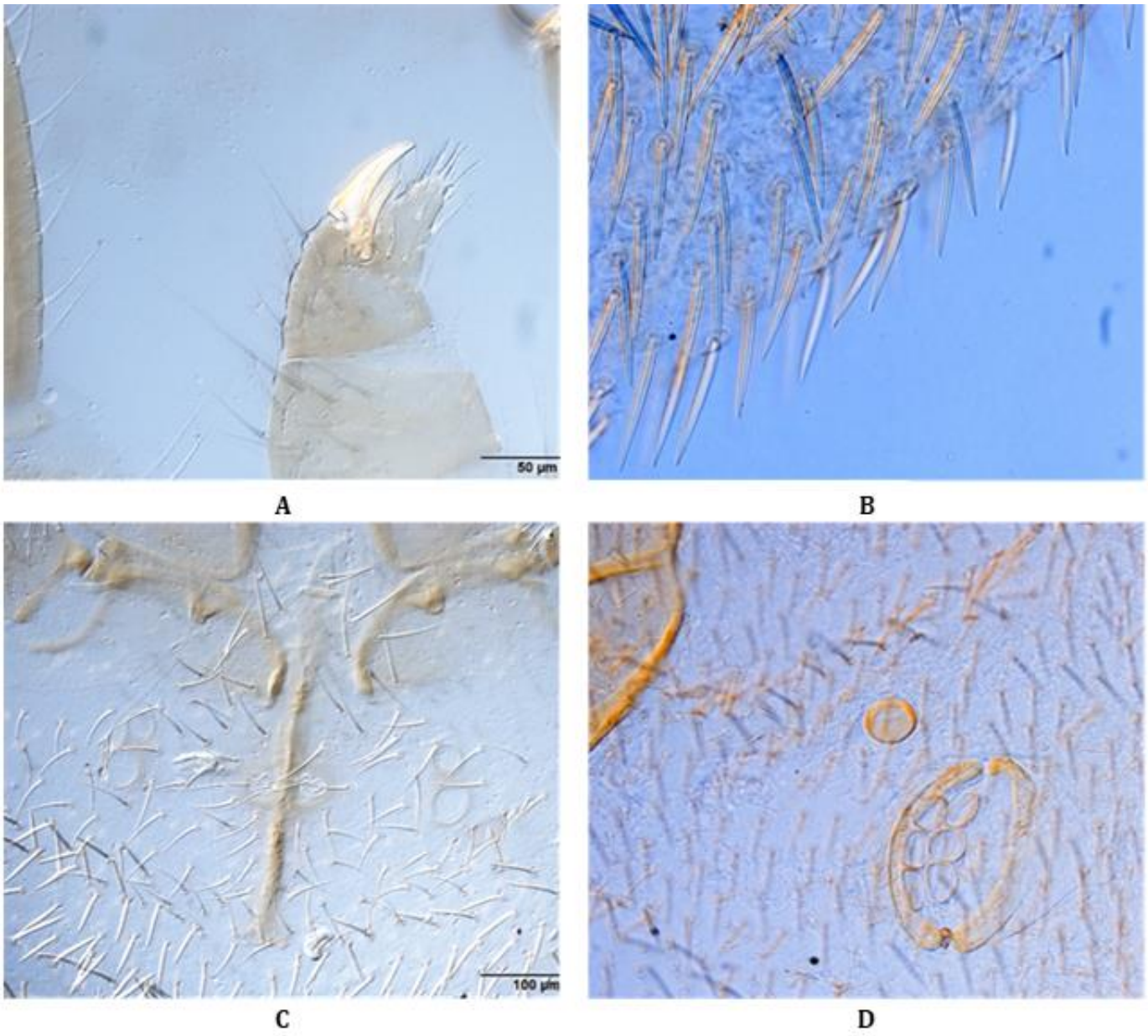

Figure 2. *Rhinothrombium nemoricola* (adult). A) Palp tarsus, B) Posterior dorsal idiosomal setae, C) Crista metopica, D) An unpaired sclerite in front of the genital opening.

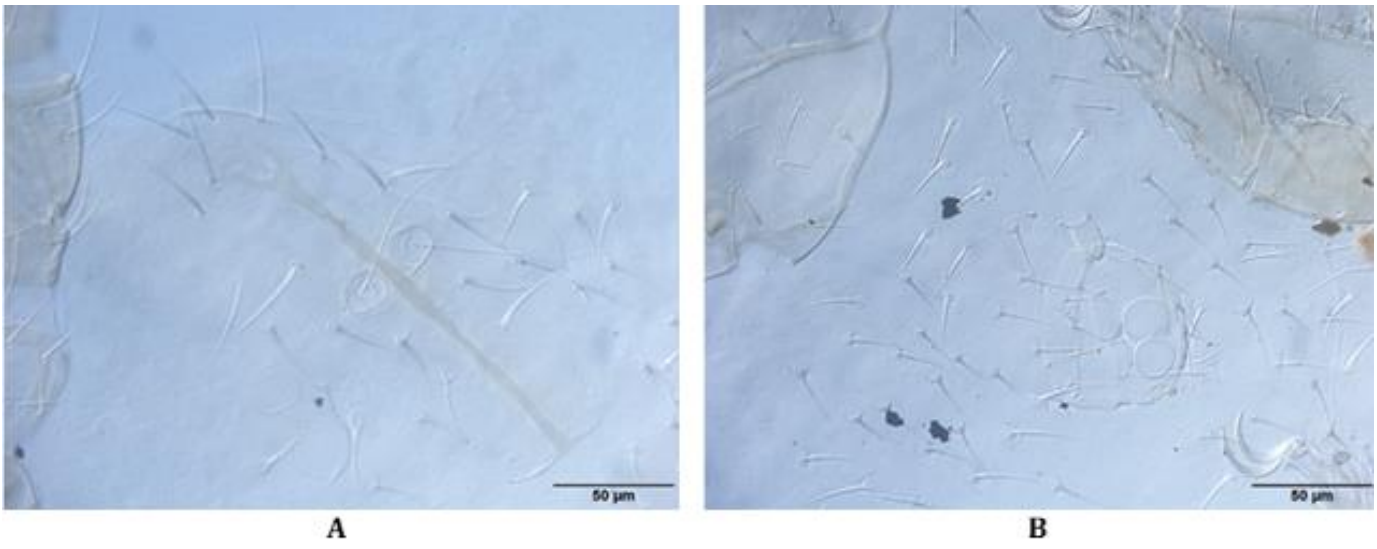

Figure 3. *Rhinothrombium nemoricola* (deutonymph). A) Crista metopica, B) The genital opening.

Table 1. Leg measurements.

	Adult-1	Adult-2	Deutonymph
Leg I			
Ta_I (L/W)	310/150	284/147	147/72
Ti_I	225	214	102
Ge_I	183	163	86
Tf_I	156	145	72
Bf_I	164	157	47
Tr_I	96	68	35
Cx_I	179	175	100
Leg II			
Ta_II	215	192	92
Ti_II	134	127	58
Ge_II	115	98	47
Tf_II	95	88	45
Bf_II	96	94	48
Tr_II	71	63	35
Cx_II	198	181	87
Leg III			
Ta_III	208	198	90
Ti_III	163	133	59
Ge_III	124	100	43
Tf_III	103	88	37
Bf_III	99	86	40
Tr_III	102	89	47
Cx_III	172	164	89
Leg IV			
Ta_IV	272	247	119
Ti_ IV	250	236	100
Ge_ IV	173	181	68
Tf_ IV	139	153	44
Bf_ IV	113	110	48
Tr_ IV	154	136	49
Cx_ IV	217	229	98
IP	4424	4199	1935

Dorsal opistosomal setae simple (49-66), uniform, slightly thickened stem, narrowing towards tip, spine-like, without setulose, inserted on prominent sclerites (Fig. 2B). Crista metopica strongly sclerotized. The border of the anterior region of the crista metopica not clear. Scutum with projecting naso (45-52) with two setae. Posterior region prominent (113-117) shorter than anterior region (132-135). Two pairs of sessile eyes laterally to crista metopica on ocular sclerites. Anterior lenses (20-26) shorter than posterior lenses (30-35). Middle part of crista widened at the level of sensillary area. Sensillae smooth (125-137) (Fig. 2C). Genital opening at level of coxae IV with three pairs of acetabulae, surrounded by paired sclerites of similar shape. Anterior to genital opening an unpaired round sclerite (Fig. 2D).

Legs stout, shorter than idiosoma. Tarsus I oval in shape. All tarsi terminated with double claws. The length of the leg segments are given in Table 1.

Deutonymph (n=1): Body smaller than adult. Idiosoma length 902 and width 595. Other characters as in adults. Gnathosoma. One pair of nude galealae (*cs*, 23), two pairs of nude anterior hypostomalae (as_1, 8 and as_2, 23), one pair of nude subcapitular setae (*bs*, 38). Palps not as robust as in adults. Dorsal opisthosomal setae similar to those in adults but shorter (35-42) (Fig. 3A). Two pairs of genital acetabula present (Fig. 3 B).

Specimens examined

Esenyurt Village, Üzümlü district, Erzincan, Türkiye 39°38′28″N 40°03′19″E, 2007 m a.s.l., 11 October 2021, one adult and one deutonymph from mossy soil (Leg. A. Torunlar). Mutu district, Tunceli, Türkiye, 39°32'41''N 39°54'40''E, 1511 m a.s.l., 29 July 2018, one adult from moss on stone (Leg. E. Buğa). The adults were kept in the glass vials, but no larvae were observed.

DISCUSSION

Two genera of the family Tanaupodidae are known from Türkiye: The genus *Eothrombium*; *E. siculum* Berlese 1910 and the genus *Lassenia*; *L. novoseljensis* Haitlinger and Šundić from Aydın and *L. hemsinensis* Noei et al. from Rize (Noei et al., 2018; Oner et al., 2021). In our country, the first record of the genus *Rhinothrombium* is given. All species of

Rhinothrombium are rather rare, their biology remains almost unknown. *Rhinothrombium* is exclusively known from mountainous areas. The specimens obtained in this study are grassy, moss, litter plain areas. Species of *Tanaupodus* inhabit lowlands and seem confined to hygric biotopes (Wohltmann et al., 2007). Members of the family were known only from Europe (Berlese 1887) until found in Iran (Yazdanpanah et al., 2013). Three species of the genus *Rhinothrombium* are known to date:

Rhinothrombium inopinum Hull, 1918 [Postlarvae]

Distribution: Great Britain (Mąkol and Wohltmann, 2012).

Rhinothrombium nemoricola (Berlese, 1886) [Postlarvae]

Distribution; Austria, France, Germany, Great Britain, Hungary, Italy, Norway, Poland, Romania, Spain (Mąkol and Wohltmann, 2012). It is newly recorded species for fauna of Türkiye.

Rhinothrombium wuxiensis Zhang, 1993 [Postlarvae]

Distribution: China (Mąkol and Wohltmann, 2012).

Karakurt (2016) gave some of specimens as *Rhinothrombium nemoricola* in his PhD thesis and later this species was published as *Eothrombium siculum* due to the structure of the anterior part of the crista (Karakurt, 2016; Karakurt and Sevsay, 2020). The members of *Eothrombium* separate from other postlarval of *Rhinothrombium* by the shape of the anterior part of crista. While end of anterior part of crista is bifurcate in *Eothrombium*, it is linear in *Rhinothrombium*. Also, naso is absent in *Eothrombium* while *Rhinothrombium* has naso.

The knowledge of the systematics and biology of the group tanaupodids is still fragmentary, and is caused mainly by the rarity of occurrence of these mites, and the consequent limited material that has been published about them (Mąkol and Featherstone, 2021). The number of individuals in two sexes is very low. As specimens number of both deutonymph and adult stages increases, the differences between them will be more understandable.

Authors' contributions

Alper Torunlar and **Evren Buğa:** Methodology, investigation, visualization, writing - review & editing. **Sevgi Sevsay:** Supervision, project administration, resources, investigation, methodology, writing - review & editing. This study is a part of the first author's MSc thesis.

Statement of ethics approval

Not applicable.

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Conflict of interest

All authors declare that there is no any potential conflict of interest.

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RESEARCH ARTICLE

Aculus taihangensis (Acari: Prostigmata: Eriophyidae), a potential biological control agent identified from the highly invasive pest plant, tree of heaven, in Türkiye

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ABSTRACT: Invasions by alien plant species are rapidly increasing in both their extent and intensity, leading to the widespread degradation of terrestrial and aquatic ecosystems across the world. One of the most widely dispersed, invasive, alien plant species in Europe, North America and many other countries, including Türkiye, is the tree of heaven, *Ailanthus altissima*. Numerous potential biological control agents, including eriophyoid mites, have been reported from this plant within its native range. A widespread collection of leaf specimens from the tree of heaven in Türkiye yielded only the eriophyoid, *Aculus taihangensis* (=*A. mosoniensis*), a new record for Türkiye. No obvious damage was observed on any of the leaf specimens. It appears highly unlikely that *A. taihangensis* is currently playing a meaningful role in the biological control of the tree of heaven in Türkiye.

Keywords: *Ailanthus altissima*, *Aculus mosoniensis*, eriophyid mite, new record, invasive species.
Zoobank: https://zoobank.org/297499EC-D59A-4DA3-BCBB-F488A5CB1562

INTRODUCTION

Invasions by alien plant species are rapidly growing in their extent and severity across the world, leading to an alarming level of degradation of both terrestrial and aquatic ecosystems. One of the most widely distributed, invasive, alien plant species in Europe and North America, and in many other countries, is the tree of heaven [*Ailanthus altissima* (Mill.) Swingle, 1916] (Sapindales: Simaroubaceae). It is native to northern and central China, Korea and Vietnam and is considered one of the worst invasive plants in Europe (Ding et al., 2006; Nava, 2014; Sladonja et al., 2015; EPPO, 2023). The tree of heaven is cultivated as an ornamental plant in many towns across Türkiye and has become naturalized (Cullen, 1967; Uludağ, 2015; Ulus et al., 2021). That means that this plant has been invading the landscape of Türkiye for more than 55 years.

Kashefi et al. (2022) did a short review of the history of the spread of the tree of heaven, its pest status in various jurisdictions, and potential biological control agents. They attributed the invasiveness of the tree of heaven to five characteristics, namely, tolerance of extreme environmental conditions, production of numerous allelopathic substances, high production and viability of seeds, clonal proliferation with copious sprouting after cutting, and limited herbivory by insects. Apart from its negative ecological impacts, the plant has also been reported to harm human health; the pollen of the tree causes sensitization, allergic rhinitis and asthma (Mousavi et al., 2017; Samei et al., 2020; Werchan et al., 2023).

In a literature review of natural enemies of the tree of heaven in China, Ding et al. (2006) listed 46 phytophagous arthropods, 16 fungi and one potyvirus, some apparently causing substantial damage, with the arthropods including three eriophyid mite species, *Aculops ailanthi* Lin, Jin & Kuang, 1997, *Aculops taihangensis* Hong & Xue, 2005 and *Aculus altissimae* Xue & Hong, 2005. *Aculops ailanthi* has since been reported from the tree of heaven in the United States of America (Gardner, 2008; Skvarla et al., 2021).

Later, *Aculus mosoniensis* (Ripka, 2014) was described from the tree of heaven in Hungary (Ripka and Ersek, 2014; de Lillo et al., 2017). However, the suggested synonymy between *Aculops taihangensis* and *Aculus mosoniensis* (de Lillo et al., 2017) was supported by the recent work of de Lillo et al. (2022) who stated that, based on new morphological and molecular data, *A. mosoniensis* has to be considered a junior synonym of *Aculus taihangensis* (Hong & Xue, 2005) new combination, with *Aculops taihangensis* reassigned to the genus *Aculus*. de Lillo et al. (2022) also described the deutogyne of *Aculus taihangensis*. In Europe, only *A. taihangensis* (=*A. mosoniensis*) has been reported from the tree of heaven; it has now been reported from at least 13 European countries (Ripka and Ersek, 2014; de Lillo et al., 2017, 2022; Marini et al., 2021; Kashefi et al., 2022).

Aculus taihangensis can form dense populations on the under surface of the leaflets of the young compound leaves of the tree of heaven, causing the leaf edges to curl upwards and turn yellowish. Drying of the upper parts of the stem can occur on heavily infested plants, and young plants can become water stressed and lose leaves prematurely (de Lillo et al., 2017). Host range tests on *A. taihangensis* indicated that it is a safe biological control agent that could help to control this highly invasive tree (Marini et al., 2021).

MATERIALS AND METHODS

Leaf samples were collected during the vegetation period of tree of heaven in many parts of Türkiye in 2022 and 2023 (Table 1, Fig. 1). Apart from some trees in parks, most of the plants were on disturbed sites, especially along roadsides in both urban and rural areas. Almost all were self-seeded plants that were often growing in small clumps or thickets. Four or five young compound leaves were randomly collected at each site. They were wrapped in paper, labelled, and then kept in cold storage until they were processed. Both surfaces of the leaflets were observed under a stereomicroscope (Olympus® SZ 61) at 45x magnification. The collected eriophyoid mites were stored in 70% ethyl-alcohol until they were identified. For that purpose, they were mounted in Hoyer's medium without the use of fibers, and then kept on a heating plate for two hours at 80 °C. The permanent slides were examined under an Olympus® BX51 phase-contrast microscope for identification which was made following Ripka and Ersek (2014) and de Lillo et al. (2010, 2017, 2022). All morphological measurements were done according to de Lillo et al. (2017, 2022). Measurements are given in micrometers (µm). Images were taken with a digital camera (Toupcam E31SPM20000KPA). The examined specimens are in the mite collection of the Acarology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Türkiye.

Figure 1. Map of Türkiye showing the provinces from which leaf samples were collected from the tree of heaven in 2022 and 2023 (* indicates the site in Çanakkale Province at which the eriophyid mite, *Aculus taihangensis*, was collected).

Table 1. Collection dates and locations for leaf sampling from the tree of heaven in Türkiye in 2022 and 2023.

Collection Date	Location (Town/Province)	Collection Date	Location (Town/Province)
09.08.2022	Hekimhan/Malatya	12.06.2023	City center/Kırşehir
11.08.2022	City center/Bingöl	12.06.2023	Ürgüp/Nevşehir
13.08.2022	City center/Şırnak	12.06.2023	Uçhisar/Nevşehir
15.08.2022	City center/Şanlıurfa	12.06.2023	Gülpınar/Gülağaç/Aksaray
09.09.2022	Koparan/Çorum	13.06.2023	University campus/Niğde
09.09.2022	Delice/Kırıkkale	13.06.2023	Acıkuyu/Ereğli/Konya
10.09.2022	Tepebaşı/Eskişehir	13.06.2023	Pozantı/Adana
11.09.2022	City center/Balıkesir	13.06.2023	Karacalar/Osmaniye
12.09.2022	Burhaniye/Balıkesir	14.06.2023	Altınçağ/Dörtyol/Hatay
13.09.2022	Adatepe/Ayvacık/Çanakkale	14.06.2023	Ceyhan/Adana
14.09.2022	Kilitbahir/Çanakkale	15.06.2023	Darısekisi/Toroslar/Mersin
14.09.2022	Uzunköprü/Edirne	17.06.2023	Çaybaşı/Muratpaşa/Antalya
15.09.2022	Ataşehir/İstanbul	19.06.2023	Palm Center/Köyceğiz/Muğla
16.09.2022	Sapanca/Sakarya	19.06.2023	Döğüşbelen/Köyceğiz/Muğla
17.09.2022	City center/Bolu	20.06.2023	Selçuk/İzmir
17.09.2022	Ilgaz/Çankırı	20.06.2023	University campus/Aydın
05.10.2022	Cihanbeyli/Konya	21.06.2023	Baklacı/Alaşehir/Manisa
05.10.2022	Tavşancalı/Konya	21.06.2023	Güre/Uşak
09.10.2022	City center/Isparta	21.06.2023	Aliağa/Gediz/Kütahya
09.10.2022	Kargı/Bucak/Burdur	22.06.2023	Adapazarı/Sakarya
09.10.2022	Avşar/Afyonkarahisar	22.06.2023	Darıdere/Bozüyük/Bilecik
10.10.2022	Polatlı/Ankara	22.06.2023	Döngelli/Akçakoca/Düzce
10.10.2022	Elmadağ/Kırıkkale	23.06.2023	Safranbolu/Karabük
10.05.2023	İlkadım/Samsun	23.06.2023	City center/Zonguldak
13.05.2023	Atakum/Samsun	24.06.2023	Asağıçaylı/İnebolu/Kastamonu
11.06.2023	Yerköy/Yozgat	30.06.2023	Yılgın/Tirebolu/Giresun

Table 2. Morphological measurements of *Aculus taihangensis* females.

Characters	Protogyne (n=5)	Deutogyne (n=3)	Protogyne (n=7) (de Lillo et al., 2017)	Deutogyne (n=18) (de Lillo et al., 2022)
Body (including gnathosoma)	242-290	216-222	237-275	194-292
Body thickness	64-67	45-58	54-70	42-60
Body width	56-60	42-58	55-57	42-56
Gnathosoma	21-22	21-22	-	21-25
Palp	21-23	22	22-27	-
Chelicerae	19-20	19-20	17-18	16-19
Shield length (including anterior lobe)	42-45	35-36	40-45	35-40
Shield width	46-48	34-46	47-49	35-45
Anterior lobe	4-6	-	4-7	-
Setae *sc*	68-77	42-43	68-81	36-43
Spacing *sc*	27-30	22-24	27-29	22-26
Leg I	42-44	40-45	40-45	41-46
Tibial setae *l´*	5-7	5-7	5-7	4-7
Tarsal setae *ft´*	20-21	21-23	19-21	21-23
Tarsal setae *ft´´*	24-26	25-28	23-27	24-29
Empodium	6-7	6-7	6	6-7
Empodium rays	5	5	5	5
Solenidion	8-9	10	8-9	9-10
Leg II	38-40	38-41	37-41	36-42
Tarsal setae *ft´*	6-7	6-7	6-7	5-8
Tarsal setae *ft´´*	21-25	25-29	22-27	24-32
Empodium	6-7	6-7	6	6-7
Empodium rays	5	5	5	5
Solenidion	8-9	10	8-9	9-10
Setae *1b*	12-13	10-13	12-15	10-14
Setae *1a*	26-32	34-47	27-38	32-45
Setae *2a*	37-45	52-59	38-42	51-63
Dorsal semiannuli	44-46	66-75	45-50	66-76
Ventral semiannuli	71-79	77-80	73-85	67-80
Semiannuli between coxae and genitalia	7-8	5-6	7	5-6
Setae *c2*	26-27	22-29	22-28	17-26
Location *c2*	9-10	8-9	9-10	8-10
Setae *d*	72-88	68-82	73-85	52-83
Location *d*	24-25	22-25	24-27	23-27
Setae *e*	27-32	22-27	32-37	22-27
Location *e*	41-49	43-48	49-52	42-48
Setae *f*	38-39	45-47	36-39	36-47
Location *f*	67-74 5 annuli to the rear	71-73 5 annuli to the rear	68-77 5 annuli to the rear	63-75 5 annuli to the rear
Genitalia width	21-22	22-24	21-22	21-25
Genitalia length	10-12	10-12	10-12	10-14
Coverflap ridges	10	10	10	8-10
Setae *3a*	22-26	28-29	19-29	20-28
Spacing *3a*	21-22	17-18	21-22	14-17
Setae *h2*	102-124	107-142	92-134	62-150
Setae *h1*	4-5	4-5	4	4-5

RESULTS

Eriophyid mites were found on leaf samples of the tree of heaven, *Ailanthus altissima*, collected at only one site, Adatepe (39°34'12.3"N 26°37'10.7"E) on 13.09.2022 in Çanakkale Province, in northwest Türkiye, of a total of 52 sites across 42 provinces of Türkiye, despite the large number of leaves checked (Table 1, Fig. 1). Fifty seven slides, that included protogynes, deutogynes and males, were prepared. The specimens were identified as *Aculus taihangensis* (Hong & Xue, 2005), which is a new record for Türkiye. Both the protogynes and deutogynes that were examined had very similar morphological characters and measurements to the specimens of de Lillo et al. (2017, 2022) (Figs 2-5, Table 2).

Protogyne (n=5). Body fusiform, prodorsal shield including rounded anterior lobe; shield pattern composed of a network of lines with a short median line and complete admedian lines; three transverse lines on the shield, first two transverse lines from the rear connect the median line with the admedian lines, forming four cells; the third transverse line joins the admedian lines, forming two median cells; third transverse line continues as arched submedian lines, forming five cells between the submedian lines and the anterior edge of the shield; five more pairs of cells between the rear margin of the shield and the admedian and submedian lines. Setae *sc* directed posteriorly, tubercles subcylindrical, on rear shield margin; solenidia distally tapered and empodium simple and 5-rayed on both legs; opisthosoma dorsally arched, dorsal semiannuli almost half the number of ventral semiannuli; dorsal opisthosoma with elliptical, elongated microtubercles, last 6-7 ventral semiannuli with elongated and linear microtubercles; genital coverflap with longitudinal striae (Fig. 2A, Table 2).

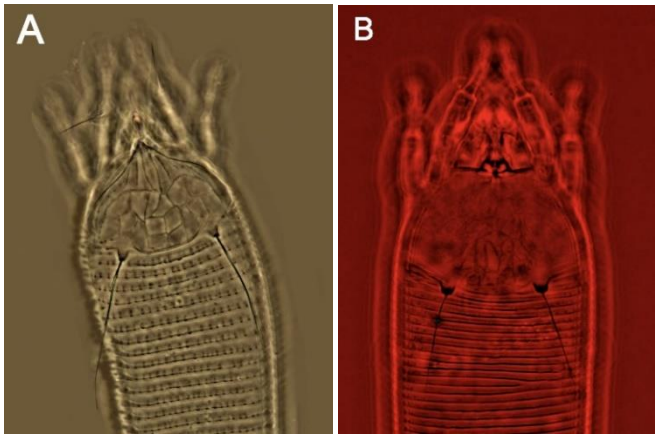

Figure 2. *Aculus taihangensis*. Prodorsal shield and part of dorsal opisthosoma: A. Protogyne, B. Deutogyne.

Deutogyne (n=3). Body vermiform, prodorsal shield including rounded anterior lobe; shield pattern indefinite, lines thinner than those of the protogyne; complete median and admedian lines connected by three transverse lines, forming cells; scapular setae *sc* directed posteriorly, tubercles *sc* subcylindrical, on rear shield margin; solenidia distally rounded, empodium simple and 5 rayed on both legs, same as protogyne; opisthosoma dorsally arched, number of the dorsal semiannuli and ventral semiannuli almost same; dorsal opisthosoma with not well defined microtubercles, last 5 dorsal and ventral semiannuli with elongated microtubercles; genital coverflap with longitudinal striae (Figs 2B, 3A-B, Table 2).

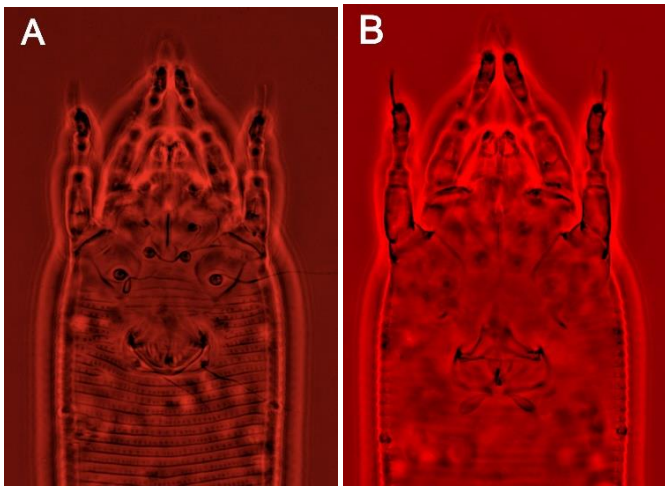

Figure 3. *Aculus taihangensis* – Deutogyne: A. Coxigenital region, B. Internal genitalia.

On the infested leaflets, there were quite dense aggregations of eggs, nymphs and adults of *A. taihangensis* along the midribs on the lower surfaces of the leaflets but no leaf curling, yellowing or other damage was observed (Fig. 5).

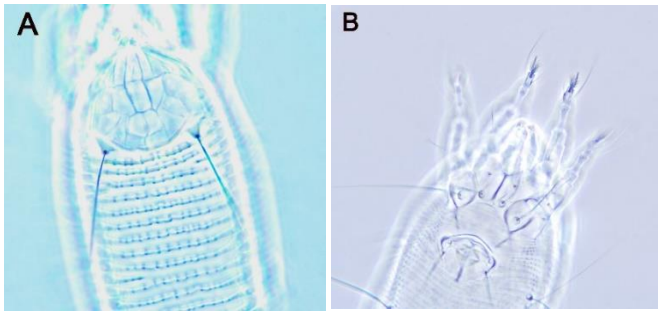

Figure 4. *Aculus taihangensis* – Male: A. Prodorsal shield and part of dorsal opisthosoma, B. Coxigenital region.

Figure 5. Dense aggregation of *Aculus taihangensis* along the midrib of a leaflet of the tree of heaven.

DISCUSSION

The tree of heaven, *Ailanthus altissima*, is an invasive plant in Türkiye where it has colonized forest edges, agricultural areas, historical sites and railway corridors, and its planting continues, without consideration of its invasiveness. Therefore, it will become a much bigger problem in the future, considering climate change scenarios for Türkiye (Uludağ, 2015). That point was reinforced by Ulus et al. (2021) who stated that there is no regional or national strategy for the management and control of *A. altissima* in Türkiye, even though it is accepted as an invasive plant species. The current study, which was focused on the collection of potential eriophyoid biological control agents of the tree of heaven, further confirmed its invasiveness in that large numbers of individual plants and clumps were observed in disturbed areas in 42 provinces across Türkiye in 2022 and 2023.

In Europe, *A. taihangensis* (=*A. mosoniensis*), was first reported from the tree of heaven in Hungary (Ripka and Ersek, 2014), and later in another 12 countries, namely Albania, Austria, Bulgaria, Croatia, Greece, Italy, Macedonia, Montenegro, Romania, Serbia, Slovenia and France (Cristofaro et al., 2018; Marini et al., 2021; de Lillo et al., 2022). The current study reports *A. taihangensis* from Türkiye for the first time.

With the presence of the tree of heaven confirmed in 27 countries in Europe (EPPO, 2023), and the presence of *A. taihangensis* (=*A. mosoniensis*) confirmed in 13 European countries, including two of its neighbouring countries, Bulgaria and Greece (Marini et al., 2021; de Lillo et al., 2022), it was not surprising to find the eriophyid, *A. taihangensis,* in Türkiye. However, despite the collection of a substantial number of leaf samples from *A. altissima* at numerous sites across Türkiye, *A. taihangensis* was detected at only one site, Adatepe, in Çanakkale Province.

It is possible that *A. taihangensis* may have been present in small numbers in some areas but inactive during the hot, dry summer period. However, there was no evidence of leaf curl or other evidence of damage on the collected leaves. A detailed study on the population dynamics of *A. taihangensis* during the entire vegetation period, and considering its protogyne and deutogyne forms, would elicit useful information on its biology and ecology.

Conclusions

The eriophyid mite, *Aculus taihangensis* (=*A. mosoniensis*), was detected on the leaves of the tree of heaven, *Ailanthus altissima*, at only one site, despite a widespread collection of samples across Türkiye. Also, no evidence of leaf damage was observed. It therefore seems highly unlikely that *A. taihangensis* is presently playing a meaningful role in the biological control of the tree of heaven in Türkiye.

Authors' contributions

Sebahat K. Ozman-Sullivan: conceptualization (equal), Methodology (equal), investigation (lead), data curation (lead), writing-original draft (equal), writing-review & editing (equal), project administration (equal). **Gregory T. Sullivan:** Conceptualization (equal), writing-original draft (equal), writing-review & editing (equal), investigation (equal). **Philipp E. Chetverikov:** Conceptualization (equal), methodology (equal), writing-review & editing (equal), project administration (equal). **Esma Kaplan:** Methodology (supporting), investigation (equal), data curation (supporting).

Statement of ethics approval

Not applicable.

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Conflict of interest

The authors have no conflicts of interest to declare in relation to the subject matter of this research.

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RESEARCH NOTE

Amblyomma scutatum Neumann, 1899 (Ixodida: Ixodidae) parasitizing *Ctenosaura similis* (Gray, 1831) (Squamata: Iguanidae) in Costa Rica

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ASBTRACT: A total of 38 ticks were collected on black spiny-tailed iguana, *Ctenosaura similis*, in a secondary dry forest from northern lowlands of Guanacaste, Costa Rica. At the time of finding, the animal has not mobility of the hind legs and tail. Ticks were identified morphological and molecularly as *Amblyomma scutatum*. PCR amplification tests to *Rickettsia*, Anaplasmataceae and *Borrelia* were negative. Possible caused of the lack of mobility is discussed.

Keywords: *Ctenosaura similis, Amblyomma scutatum*, lowland dry forest, Costa Rica.
Zoobank: https://zoobank.org/C5A85DFD-4743-4244-BF7D-CCA9CD5FDEE7

Amblyomma scutatum Neumann, 1899 is a tick species that maintains a distribution through dry areas in northern Mexico, Guatemala, Honduras, El Salvador, Nicaragua, and Costa Rica (Guglielmone et al., 2021). All stages of this species are parasites of Iguanidae (*Iguana* and *Ctenosaura*) (Guglielmone et al., 2021). Even so, there is one report of a female parasitizing a Cane toad, *Rhinella marina* L. 1758 (Romero et al., 2021). The identification of this species is difficult, since there are morphological differences found in ticks from different countries, mainly in the shape of the scutum, the ornamentation patterns, the presence and form of spurs in the coxae, and the dentition of the hypostoma (Guglielmone et al., 2021). Therefore, molecular tools are being used for more accurate taxonomic identification. Romero et al. (2021) reported *Candidatus* Rickettsia colombianensi in *A. scutatum* in El Salvador. However, limited information about distribution and host data presents a challenge to this tick records.

In Costa Rica, *A. scutatum* has been previously reported on the black spiny-tailed iguana *Ctenosaura similis* (Gray, 1831) and *Ctenosaura* sp. under the name garrobos in 10 sites of the Puntarenas and Guanacaste provinces (Álvarez-Calderón et al., 2005; U.S. National Tick Collection (USNTC) records). The aim of this paper is to present new information about *A. scutatum* in Costa Rica, based on morphological and molecular identification.

Host locality and observation: In June 2021 a *C. similis* female was observed without mobility in its pelvic limbs and flaccid tail in Daria (10.231453 N, -85.546432 W), Santa Cruz, Guanacaste province, Costa Rica (Fig. 1). This region is in a secondary lowland dry forest at 75 meters above sea level. During the observation, the iguana only moved using its front limbs, with no movement at the pelvic limbs and tail. Despite the efforts, the animal could not be trapped for veterinary examination. Ninety days later the animal was found dead with severe injuries due to the bites and scratches caused by other animals; in addition, many ticks were observed on its body (Fig. 2). The ticks were collected, placed in 70% alcohol, and transported to the Parasitology Laboratory of the Veterinary School, Universidad Nacional de Costa Rica (VS-UNA-CR).

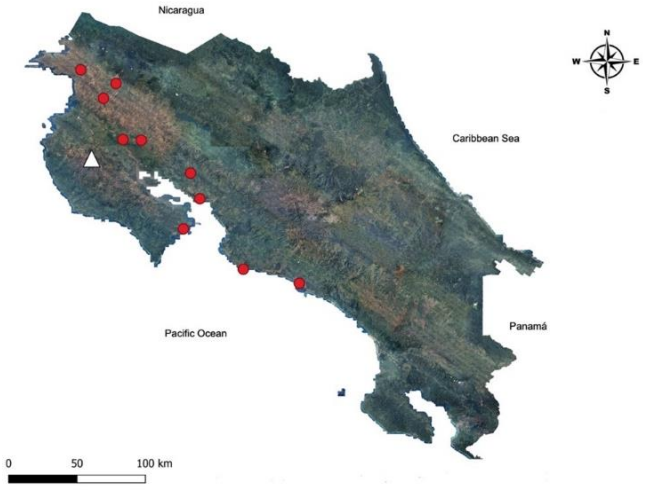

Figure 1. Distribution of *Amblyomma scutatum* in Costa Rica. Past collection sites in descending order: Santa Rosa, Hacienda la Norma, Liberia, Parque Nacional Palo Verde, Bebedero, Monteverde, Puntarenas, Curú, Jacó, Quepos (red circles), and current collection site: Diriá (white triangle).

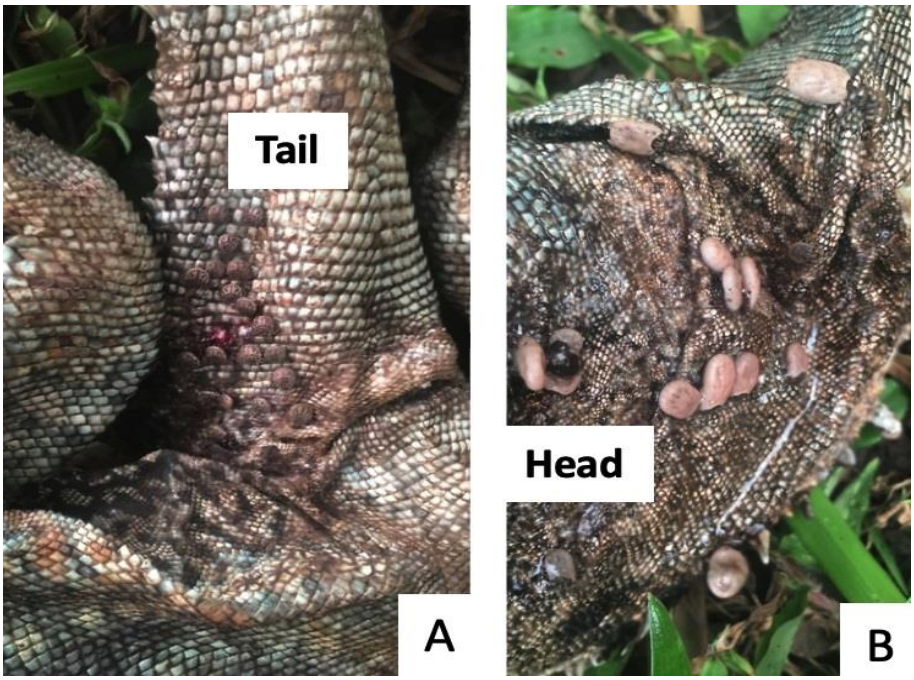

Figure 2. *Ctenosaura similis* parasitized by *Amblyomma scutatum*. **A.** Group of ticks at the ventral base of the tail, **B.** Partially and fully engorged females on the left side of the neck.

Morphological and molecular identification: The ticks was carried out using the taxonomic key of Guzmán-Cornejo et al. (2011), using a Nikon SMZ 800 stereo microscope and photographed with a 12 MP camera. DNA was extracted from three male and one female ticks using the Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer`s instructions, and molecular identification was performed through PCR amplification of a ~460 base pair (bp) fragment of the tick mitochondrial 16S rRNA gene using CCGGTCTGAACTCAGATCAAGT and GCTCAATGATTTTTTAAATTGCTGTGG sequences proposed by Norris et al. (1996). In addition, DNA from ticks were subjected to PCR assays targeting bacteria of the genera *Rickettsia*, *Borrelia* and the family Anaplasmataceae (Table 1). Amplifications were visualized in 1% agarose gels stained with GelRed™ Nucleic Acid Gel Stain (Biotium, 5 µg/ml). Positive samples were sent to Macrogen (Seoul, Korea) for sequencing. The sequences were edited and aligned using the Biological Sequence Alignment Editor (BioEdit version 7.2.5) (Hall 1999) and compared with sequences of the NCBI (National Center for Biotechnology Information) database using the BLASTn algorithm.

A total of 38 ticks (13 males and 25 females, out of which 22 engorged) were morphologically identified as *A. scutatum*, with few different morphological characteristics as compared to those reported by of Guzman-Cornejo et al. (2011). Previously, several authors have highlighted the morphological differences reported throughout the distribution of *A. scutatum* (Guglielmone et al., 2021). In our specimens, we found slight differences in the shape of the ornamentation of the scutum and size of the spurs of the coxa I (Fig. 3). According to some authors, these differences may be due to variations in the population or their distribution (see Guglielmone et al., 2021); however this fact should be better evaluated. In this sense, there are few sequences to compare this species, and include only the 16s rRNA gene, thus it is necessary to develop new analysis that allow a better morphological and molecular comparison that helps to define this species. In the present study the molecular analysis showed a 99.02% (403/407 bp) of similarity to *A. scutatum* from El Salvador (MW369633). The present partial sequence of 16S rRNA gene was deposited in GenkBank under the accession number GM OM691677, which correspond the first sequence of *A. scutatum* in Costa Rica. Voucher specimens of the ticks were deposited in VS-UNA-CR (PA-127-121). The DNA of *Rickettsia*, *Borrelia* or those of the Anaplasmataceae family could not be detected in our samples.

Although the lack of mobility of the *C. similis* female was not analyzed in this work, some hypotheses could be associated with lesions seen on the first day of observation of this animal, as such injury, pathology, climate, or a case of tick paralysis. This last assumption is supported by the literature, and in our case, the number of ticks found on the host. Tick paralysis is triggered by neurotoxins from the saliva of the ticks, particularly engorged females. Exposure to these neurotoxins can affect the host mobility and even cause the death of the hosts (Mans et al., 2004; Hanson et al., 2007). Because paralysis is associated with the presence of ticks on the host, the signs are reversible by removal of the ticks (Baeza, 1979; Hanson et al., 2007). This type of paralysis has been reported both in cold and warm-blooded animals, including humans (Dunn, 1918; Baeza, 1979; Mans, 2004; Hanson et al., 2007). Of the more than 70 species of ticks that have been associated with tick paralysis, *Amblyomma dissimile* Koch, 1844, *Amblyomma rotundatum* Koch, 1844, and *Robertsicus elaphensis* (Price 1959) (cit. as *Amblyomma elaphense*) have been implicated in paralysis in reptiles and amphibians in the Americas (Gothe and Neitz, 1991; Mans et al., 2004). Future studies are required to clarify the role of *A. scutatum* on *C. similis* in natural conditions.

Table 1. Details of the PCR protocols used for amplifying selected tick-borne agents.

Agent	Gene (PCR method)	Primers (Sequence 5'-3')	Fragment length (bp)	References
Anaplas-mataceae	16S rRNA (real time PCR)	**ECHSYBR-F** (AACACATGCAAGTCGAACGG) **ECHSYBR-R** (CCCCCGCAGGGATTATACA)	145-153	Li et al. (2001)
Borrelia spp.	Spacer region between the 5S and 23S rRNA (nested PCR)	**23SN1** (ACCATAGACTCTTATTACTTTGAC) **23SC1** (TAAGCTGACTAATACTAATTACCC) **23SN2** (ACCATAGACTCTTATTACTTTGACCA) **5SCB** (GAGAGTAGGTTATTGCCAGGG)	226	Rijpkema et al. (1995)
Rickettsia spp.	*gltA* (PCR)	**CS78** (GCAAGTATCGGTGAGGATGTAAT) **CS323** (GCTTCCTTAAAATTCAATAAATCAGGAT)	401	Labruna et al. (2004)

Figure 3. *Amblyomma scutatum* **A.** Dorsal view, female, **B.** Ventral view, female. **C.** Dorsal view, male, **D.** Ventral view, male.

Authors' contributions

Ana Jiménez-Rocha: Conceptualization, formal analysis, data curation (lead), writing (supporting); **Sergio Bermúdez Castillero:** Original draft, data curation (supporting), writing - review & editing (lead), visualization (lead). **Antony Solórzano-Morales:** Methodology (lead), formal analysis (supporting), software; **Ernesto Rojas**

Sánchez: Data curation (supporting), resources (supporting), visualization (supporting). **César Pérez:** Resources (supporting), investigation, methodology (supporting). **Gaby Dolz:** Project administration, resources (lead), supervision, validation. All authors participated in the writing of the last draft of the manuscript.

Statement of ethics approval

No ethical approval was necessary according to the Costa Rican laws.

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Conflict of interest

None.

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