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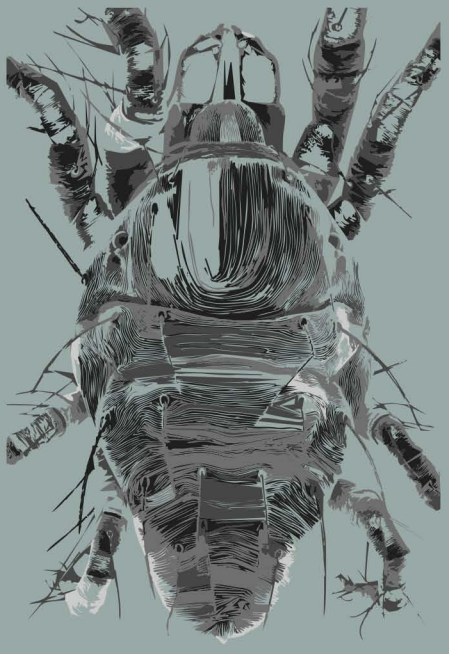

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RESEARCH ARTICLE

# The study of exposure times and dose-escalation of tick saliva on mouse embryonic stem cell proliferation

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**ASBTRACT:** The saliva of ticks contains numerous bioactive molecules with anti-hemostatic and immunomodulatory properties. Due to their abilities of self-renewal and pluripotency, stem cells hold considerable promise in the regenerative medicine and biomedical fields. The present study examines the viability and proliferation of mouse embryonic stem cells (mESCs) following the addition of tick salivary gland extracts obtained from three tick species (*Dermacentor marginatus*, *Rhipicephalus bursa* and *Hyalomma marginatum*) to the mESC medium in different quantities (0.2, 2, 20, 40, 80, and 160 µg/ml). On days 2, 5 and 7 of the treatment, the vitality and proliferation of the cells were determined with CellTiter-Glo and morphological tests. The results showed that the culture supplemented with *D. marginatus* salivary gland extract at a concentration of 80 µg/ml positively affected the proliferation rate of mESC. It was further shown that all concentrations of the salivary gland extracts derived from *H. marginatum* and *R. bursa* had a negative effect on the proliferation rate of mESC when compared to the controls.

**Keywords:** Mouse, stem cell, tick saliva, proliferation.
**Zoobank:** http://zoobank.org/0F8E7E30-A14D-4E5A-90D9-4335F2636532

## INTRODUCTION

Ticks (Acarina: Ixodida) represented by around 900 species identified to date throughout the world are obligate hematophagous ectoparasites. Unlike many other blood-sucking arthropods, they remain attached to the host skin for several hours to days and need to overcome some difficulties in this feeding process, such as creating a feeding pool in the host skin around their mouth parts, suppressing the blood clotting process, and combating the host immunity (Sonenshine and Roe, 2014) mainly by modulating the innate and acquired immune responses (Kotál et al., 2015; Wikel, 2018). Consequently, saliva of ticks, particularly of ixodid (Ixodidae) species, was reported to be arguably the most complex saliva of any animal (Nuttall, 2019). Up to the present, more than 500 different proteins and peptides have been described in tick saliva (Esteves et al., 2017, Perner et al., 2018). In addition to proteins and peptides, many non-peptidic molecules have been determined, such as nucleoside, adenosine, microRNAs, arachidonic acid derivative, prostaglandins (PGE2, F2α, I2, D2 and A2/B2), and endocannabinoids (Nuttall, 2019). However, molecular composition of saliva can vary considerably depending on the tick species, their biological stages, sex and feeding periods (Kaufman, 2007; Francischetti et al., 2009), and it is known that the molecule diversity and their amounts are particularly elevated in the late/accelerated stage of feeding in females (Leboulle et al., 2002; Mudenda et al., 2014; Karim and Ribeiro, 2015). Some molecules in the saliva of dozens of tick species have been identified to have quite different and effective bioactivity. However, there is no data about the majority of the tick saliva molecules (Mudenda et al., 2014; Ribeiro et al., 2017; Nuttall, 2019). There are a few comprehensive and recent reviews on the composition and role of tick saliva. Tick salivary molecules have a promising therapeutic potential for treatment of some human diseases associated with hemostatic and immunological disorders (Štibrániová et al., 2019; Nuttal 2019; Aounallah et al., 2020). Transcriptome and proteome studies of tick salivary glands (SGs) discovered an enormous protein diversity and unique proteins belonging to novel protein families with unknown functions (Štibrániová et al., 2019). Furthermore, it has been proposed that many substances found in tick saliva can be worth examining for different purposes due to their special properties, such as cytotoxic, apoptotic, anti-angiogenetic, immunomodulatory, anti-inflammatory, antiplatelet, and anticoagulant activities (Kazimírová and Štibrániová 2013; Sousa et al., 2015).

Stem cells are undifferentiated cells with the ability of self-renewal and can transform into multiple types of functional and specialized cells under appropriate conditions in the body (*in vivo*) or in the laboratory (*in vitro*). These self-renewing cells are derived from the inner cell mass (ICM) of mouse blastocysts (Evans and Koufman, 1981; Martin, 1981). Due to their unique properties, embryonic stem cells (ESCs) have potential for use in a wide range of areas, such as embryology, tissue regeneration, tissue-organ engineering, production of transgenic animals, etc. (Smith and Chepko, 2001; Iijima et al., 2010).

Although considerable progress has been achieved in stem cell studies since the discovery of ESCs, ESCs culture procedures are still complex and expensive. As such, there is still considerable interest in identifying the culture conditions that enable ECSs to renew themselves preventing their differentiation and promoting their proliferation (Silva-Cote and Cardier, 2011). It is essential that stem cells, which hold great promise in the regenerative medicine and biomedical fields due to their abilities of self-renewal and pluripotency, be prevented from differentiation, thus preserving their abilities, and it is well known that ESC quality depends on the culture conditions in current practice (Smith and Chepko, 2001; Iijima et al., 2010). In this regard, a great need has been expressed to create culture conditions that mimic a stem cell microenvironment in which the cells can preserve their character and potential (Tamm et al., 2013).

The present study examines the effects of six different concentrations (0.2 µg/ml, 2 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml) of salivary gland extracts (SGE) from three tick species (*D. marginatus*, *R. bursa* and *H. marginatum*) on mouse embryonic stem cells (mESCs) through their addition to a medium containing two inhibitors (PD03259010 and CHIR99021) without serum (Eiselleova et al., 2008). Counts were made and assessed at three different time points (days 2, 5 and 7), at which the experiments were terminated. Viable cell numbers were determined by a CellTiter-Glo® (CTC) test. Morphological characteristics of the mESCs lines obtained at the end of the experiments were examined and analyzed through alkaline phosphatase staining, being a marker of pluripotent cells. This study is intended to improve the efficiency and optimization of traditional stem cell cultivation conditions, and to this end, aims to answer the question of whether tick SGE has an accelerating effect on the proliferation of mESCs..

## MATERIALS AND METHODS

### Collection of Salivary Glands from Ticks

The main material of the study was adult female ticks of the *H. marginatum, R. bursa* and *D. marginatus* species. These ticks originated from Namık Kemal University, Department of Biology, were specific pathogens free and fed on adult New Zealand rabbits (*Oryctolagus cuniculus*) for continuation. The process of feeding the colonies on rabbits was carried out in the Namık Kemal University Application and Research Center for Experimental Animals, after permission for the study was granted by the Namık Kemal University the Local Ethics Committee for Animal Experiments (07.04.2016 - 9).

For the feeding of the adult ticks, a white cotton fabric bag was placed over the ears of the rabbits, and six unfed adult female and eight male ticks were placed on each ear. The ticks were monitored daily on a regular basis and females observed to have entered the rapid – feeding phase (the last phase of feeding), and mostly engorged female ticks (Sousa et al., 2015) were removed from the ears via forceps with a utmost care so as not to damage the tick, i.e., not to put pressure on its body and not to rupture the mouth parts. The collected female ticks were washed with distilled water and placed in a petri dish containing sterile phosphate buffered saline (PBS) (+4°C) and their salivary glands were removed with a microsurgery set under a stereo microscope (Patton et al., 2012). The removed salivary glands were separated meticulously from other tissue residues in a separate petri dish in new sterile PBS. The salivary glands were transferred into tubes and washed three times with sterile PBS, and then stored at -80°C until extraction.

### Preparation of Tick Salivary Gland Extracts

The tick salivary glands, were taken from the freezer (-80ºC), disrupted and homogenized with stainless steel beads at 50 Hz. at +4°C for 5 minutes by tissue lyser (until no tissue debris is visible) (Tissue Lyser LT Qiagen). The obtained suspension was transferred into new centrifuge tubes and centrifuged at 15,000 rpm for 5 minutes. Afterwards, the supernatant was removed (salivary gland extract - SGE) and stored at -80ºC until needed in the next step (Tietz and Anderson, 1986; Hudson and Hay, 1989).

To prepare the stored tick SGEs for use in the *in vitro* treatment of the cell culture, they were sterilized by being passed through a 0.22-µm-filter. A "Thermo Fisher Scientific-Pierce™ BCA (bicinchoninic acid) Protein Assay" (23225) kit was used to measure the protein concentration and the SGE with determined protein concentrations were divided into stocks with equal concentrations.

### Preparation of mESCs

This study tested at six different concentrations (0.2, 2, 20, 40, 80, 160 µg/ml) of SGEs from the different tick species (*H. marginatum*, *R. bursa*, *D. marginatus*) in a mouse stem cell (mESC) medium at three different timepoints (early, middle, late stages). As the mESC medium; KnockOut™ DMEM (Gibco, 10829018) containing 15% KnockOut Serum Replacement (KOSR, Gibco, 10828-028), 1% non-essential amino acids (NEAA Gibco, 11140-050), 1% penicillin streptomycin (Pen/Strep, Gibco, 15070063), 1% L -Glutamine (Gibco, 25030081), 0.1 mM 2-Mercaptoethanol (Gibco, 21985-023), 1000 IU/ml LIF (Prospec Protein Specialists, CYT-645), 1 µM PD0325901 (Tocris, 4192) and 3 µM CHIR99021 (Tocris, 4423) was used (Tamm et al., 2013). The SGEs were then added to the stem cell media at the abovementioned concentrations.

### Application of Tick Salivary Gland Extracts to mESCs

The experiments were designed to run in 96-well culture plates in a petri dish at three timepoints (day 2, day 5 and day 7), with three technical replicates for each group. Before the procedure, the petri dishes were coated with 1% gelatin for 15–20 minutes, after the gelatin was aspirated, the mitomycin-inactivated feeder cell layer (MEF) was seeded, with equal numbers (~$10^4$ cells) per well. The MEFs were attached to the gelatin, by being incubated overnight in the D10 medium (DMEM basal medium (Gibco, 41966029), 10% FBS (fetal bovine serum Gibco, 16000044) and a 1% Pen/Strep medium at 37°C and 5% CO2 for one day. After overnight incubation, equal num-

bers of mESCs, were isolated from E13.5 CB6F1 (C57BL/6J X Balb/c) embryos (Taskin et al. 2019) at the Koç University Animal Research Facility / MEFs. D10 was replaced by the ESC medium incubated for one day to allow the mESCs to attach to the base of the culture plates. On the following day (day 0 of the experiment), all of the ESC media in the petri dishes were withdrawn and replaced with a freshly prepared ESC medium containing SGEs at different concentrations. After two days, CellTiter-Glo (CTG) test was performed for early stage experimental groups (day 2). Afterwards, cell viability rate was measured by the CTG test for other experimental groups (day 5 and day 7) (Fig. 3). Every two days following the first day of the experiment, 50% of the ESC 2i (PD0325901 and CHIR99021) medium containing SGE was replaced with fresh medium (Czechanski et al., 2014).

### Cell Viability Assay

To examine the effects of the tick SGE on the proliferation of mESCs, the cell viability of each group was analyzed with a CTG test. After the study, results for three different tick species were obtained. CTG test was applied to determine the negative or positive effects of the SGEs on the proliferation of mESCs. The number of viable cells was determined using a "CellTiter-Glo® (CTG) Luminescent Cell Viability Assay" (G7570-Promega) kit, which is an approach that is based on the measurement of the amount of adenosine triphosphate (ATP) in viable cells. During the procedure, 96-well black microplates were used, and 500 MEFs were seeded into each well. Using the petri dishes with the completed experimental process, the Cell Titer-Glo solution was added to the ESC medium at a ratio of 1:10 in accordance with the kit protocol. The culture plates were placed into the reader (Synergy H1, Bio-Tek) and proliferation of the cells in the wells were examined at 567 nm. Relative fold change of cell viability was normalized to untreated negative control (Tamm et al., 2013).

### Morphology of mESC Lines

Mouse stem cell colonies were first selected based on their morphology, such as compact rounded cells and colonies with distinct edges. Pluripotency of the mESC lines were assessed by alkaline phosphatase staining.

### Alkaline Phosphatase (ALP) Staining

ALP staining was performed to demonstrate whether the pluripotent properties of the mESCs exposed to SGEs at different time points had been maintained. After removing the medium, the cells were washed three times with PBS (PBS-Thermo Fisher, 10010-023), after which, 4% paraformaldehyde was added to the wells which were then incubated for 5 min at room temperature in a shaking incubator. The cells were fixed with paraformaldehyde and washed twice with PBS. The staining solution was then added, following the protocol (Sigma Cat. 86R-1KT), and the cells were then incubated at room temperature for 60 minutes (Czechanski et al., 2014), washed again with PBS and examined under a microscope.

### Statistical Analysis

A two-way ANOVA (mixed model) was used for the statistical assessment of the differences between the media containing different tick SGEs and concentrations. The Geisser–Greenhouse correction and Dunnett´s test on multiple comparison were used. Differences at <0.05 (p-value) were considered statistically significant. The experiments were carried out in two biologically independent experiments with three technical replicates for each experiment.

## RESULTS

The study made use of the salivary glands of female ticks that were in the last feeding phase, and that had entered the rapid-feeding phase, although the feeding process did not continue in the same way, even in ticks that were fed on the same day. It took 8–12 days for the ticks to reach the desired feeding phase. The total number and weight of the ticks used were as follows: 28 *D. marginatus* females with an average weight of 0.3167 g (min 0.2001 g–max 0.5558 g), 24 *R. bursa* females with an average weight of 0.3355 g (min 0.2242 g–max 0.5645 g) and 26 *H. marginatum* females with an average weight of 0.3712 g (min 0.2557 g–max 0.6897 g). The number of salivary glands obtained from these ticks were deemed sufficient for the study.

The effects of SGE of three tick species (*H. marginatum*, *R. bursa and D. marginatus*) have been compared on mESC proliferation. For *H. marginatum*, all the media, with six different concentrations of SGE had a negative effect on the proliferation of mESCs in every stage of the culture (day 2, day 5 and day 7) (Fig. 1). In the cell cultures with the *D. marginatus* and *H. marginatum*, the maximum positive effect was observed in the middle stages (day 5) of the cultures with high concentrations of tick SGE (40 µg/ml, 80 µg/ml, 160 µg/ml) (Figs 1 - 3).

For *R. bursa*, all of the media, with six different concentrations of SGE had negative effects on the proliferation of mESCs in every examined time point of the treatment (day 2, day 5 and day 7) (Fig. 2). In the cell cultures with the SGEs of *H. marginatum* and *R. bursa* separately, the maximum positive effect was observed in the early stages (day 2) of the cultures with low concentrations of the extract (0.2 µg/ml, 2 µg/ml, 20 µg/ml) (Figs 1 - 2).

For *D. marginatus*, all the media, with six different concentrations of SGE, had a negative effect on the proliferation of mESCs in the early (day 2) and late (day 7) stages of the culture. Again, the numbers of mESCs in the middle stages (day 5) of the cultures with extracts at a concentration of 40 µg/ml and 160µg/ml were close to those of the controls. On the other hand, while a positive effect was observed on mESC proliferation in the middle stage (day 5) of the culture medium with an extract at a concentration of 80 µg/ml, the medium no longer had a positive effect in the late stage (day 7) (Fig. 3).

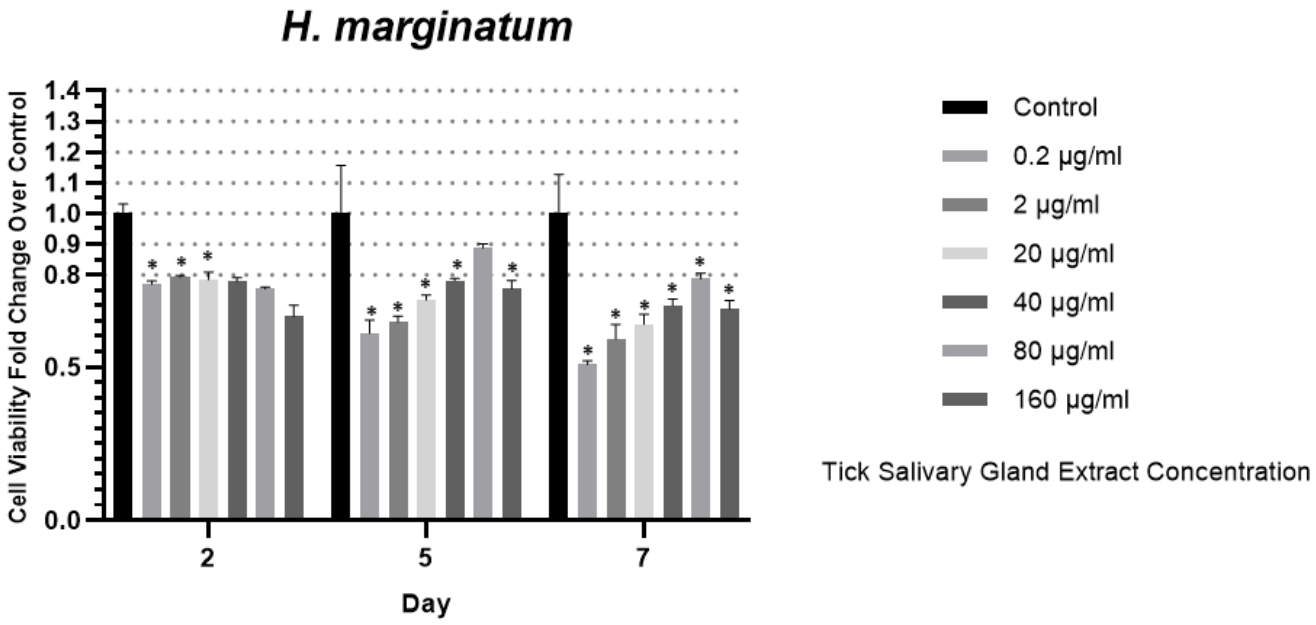


**Figure 1.** Effect of *H. marginatum* SGE (0-160 µg/ml) on mouse embryonic stem cell proliferation and viability. Values represent relative fold change of cell viability normalized to untreated negative control. The Geisser–Greenhouse correction and Dunnett´s test on multiple comparison were used. The results are mean ± standard deviation (SD) from a representative experiment carried out in triplicate and were seeded in equal amount in 3 different 96-well cultured plates.

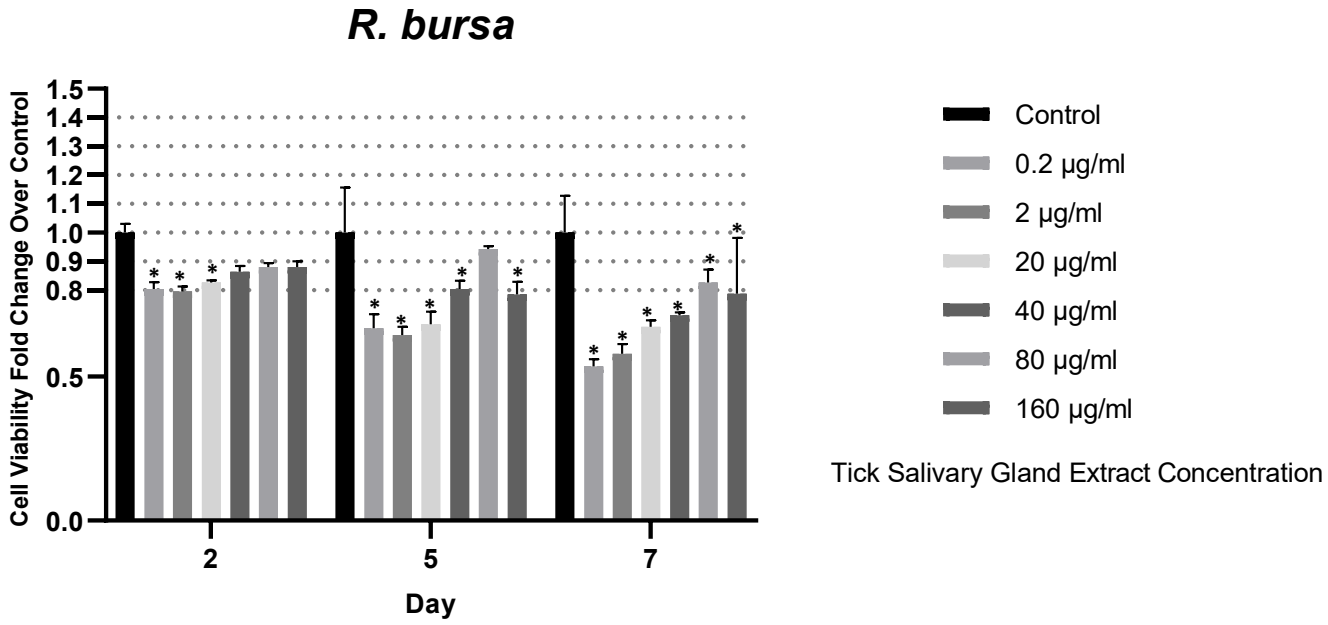


**Figure 2.** Effect of *R. bursa* SGE (0-160 µg/ml) on mouse embryonic stem cell proliferation and viability. Values represent relative fold change of cell viability normalized to untreated negative control. The Geisser–Greenhouse correction and Dunnett´s test on multiple comparison were used. The results are mean ± standard deviation (SD) from a representative experiment carried out in triplicate and were seeded in equal amount in 3 different 96-well cultured plates.

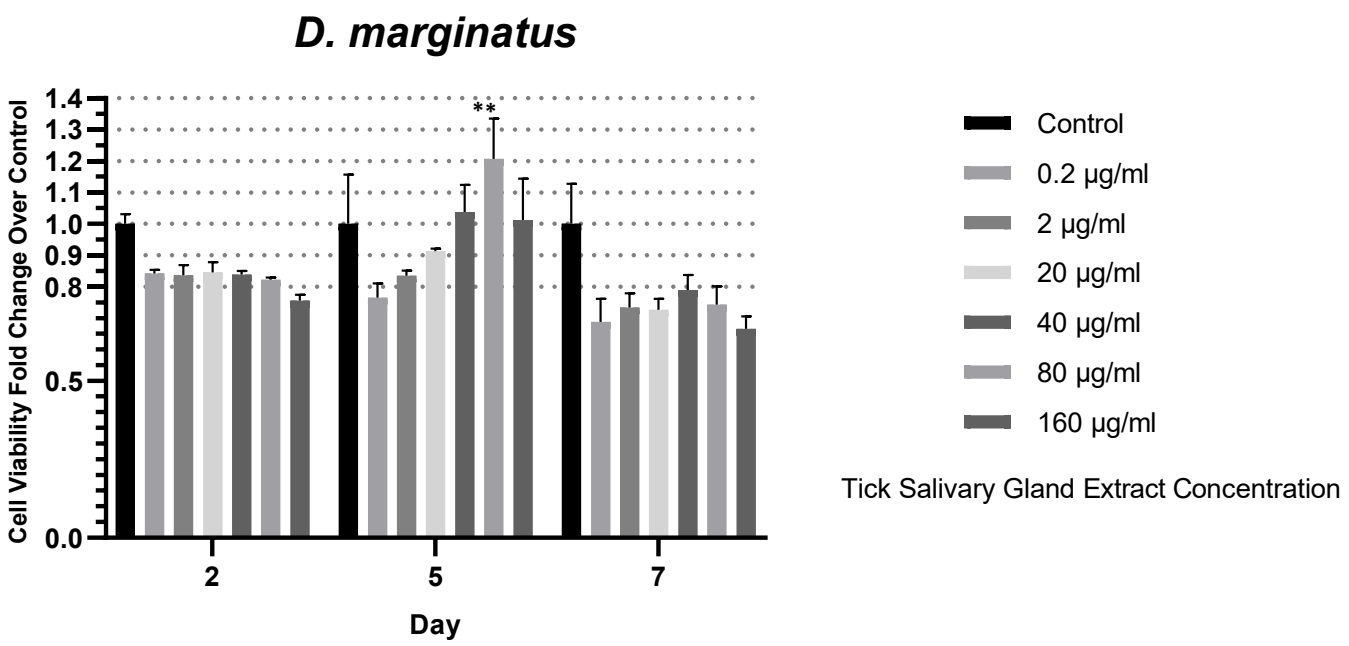


**Figure 3.** Effect of *D. marginatus* SGE (0-160 µg/ml) on mouse embryonic stem cell proliferation and viability. Values represent relative fold change of cell viability normalized to untreated negative control. The Geisser–Greenhouse correction and Dunnett´s test on multiple comparison were used. All experiments have the P value <0.05, on three different time laps. The results are mean ± standard deviation (SD) from a representative experiment carried out in triplicate and were seeded in equal amount in 3 different 96-well cultured plates.

One common remarkable feature noted with all media supplemented by three different tick SGE was that those containing low concentrations of SGE positively the proliferation more positively in the early stages. As the concentrations were increased, the proliferation was higher in the middle stage, however all the values were lower than the control. In these processes, the *D. marginatus* SGE differed from the others, since the highest proliferation was observed above the control values. This feature was absent for *H. marginatum* and *R. bursa* SGE.

## DISCUSSION

In this study, we investigated the effect of six concentrations of SGE from three tick species on mESC proliferation in the course of three different days. It was demonstrated that specific SGE concentrations SGE of the species increased proliferation of mESC in middle stages of the culture.

On the other hand, previous studies on tick saliva or SGE reported negative effects on, e.g., endothelial cells. *Ornithodoros brasiliensis* salivary gland homogenates interfered with endothelial cell proliferation, which is a key phenomenon in wound healing. SGE of *O. brasiliensis* significantly delayed skin wound healing (Reck et al., 2013). This is also supported by previous work showing that saliva of the *Ixodes scapularis* (≤ 1:500 dilutions) or salivary gland (0.1–0.5 pairs / assay) dose-dependently inhibited microvascular endothelial cell (MVEC) proliferation (Francischetti et al., 2005). Inhibition was also detected with the saliva of the cattle tick *Boophilus microplus* but not with the salivary gland of *Anopheles gambiae* (Francischetti et al., 2005). Our results support inhibition effects of tick SGE on mESCs proliferation, except one species. In the future, it will be of interest to investigate the mechanisms behind the positive effects.

Tick salivary glands produce and secrete into the feeding site in the host skin an impressive amount and variety of bioactive molecules modulating hemostatic, inflammatory and immune responses as well as wound healing. The composition of tick saliva is highly complex and changes through the feeding process (Francischetti et al., 2009; Kotal et al., 2015; Blisnick et al., 2017; Šimo et al., 2017; Nuttall, 2019; Wikel, 2018). Studies have reported that tick SGE increases the proliferation on B and T lymphocytes and marked degranulation of mast cells and basophils (Gill, 1986; Ushio et al., 1993). The immunomodulatory effect of tick saliva on the production of proinflammatory cytokines released by dendritic cells (DC) stimulated by lipopolysaccharide (LPS) is associated with the inhibition of the activation of extracellular signal-regulated kinases (ERK1/2) and mitogen-activated protein (MAP) kinases (Ferreira and Silva, 1998; Oliveira et al., 2010; Lieskovská and Kopecký, 2012). It is known that the inhibition of the ERK1/2 and MAP kinase pathways also positively affects the pluripotency of embryonic stem cells (Ohtsuka et al., 2015).

In recent years several new ES cell culture protocols have been published; however, most laboratories still use the traditional method that involves culturing the ESCs in mitotically inactivated MEFs with media supplemented

with FBS and LIF (Tamm et al., 2013). In the present study, we added tick salivary gland extracts to the 2i medium containing KOSR in a standard culture medium and investigated its effects on ESCs.

It is difficult to establish the causality of the mechanism behind the negative effect or the dose-related positive effect specific to *D. marginatus* SGE observed in this study. It is known that several molecules found in tick saliva interact with many types of receptors which activate different signal transduction pathways involved in different cellular processes such as proliferation, differentiation etc. (Arolas et al., 2005; Brake et al., 2010; Akagi et al., 2012; Carneiro-Lobo et al., 2012; Cao et al., 2013). It is believed that the positive effect specific to *D. marginatus* is attributable to the positive impact of the inhibition of the ERK1/2 and MAP kinase pathways on the pluripotency of the mESCs due to the various proteins in the extract (Oliveira et al., 2010). When the extract concentration was doubled (160 µg/ml), the positive effect on proliferation disappeared, which possibly because the proteins that have a positive effect at low doses negatively affect proliferation in high doses; or some other proteins in SGE that have a negative effect may become more dominant after the dose is increased, although both explanation may be the cause.

In conclusion, it is necessary to diversify the SGE concentrations of *D. marginatus* in the range of 40–160 µg/ml. Although maximum proliferation was observed at the 80 µg/ml concentration in the present study, another concentration may exceed the value we found. Other chemicals or conditions should be tested with the potential to be substituted by the *D. marginatus* extract in mESC culturing methods. Further studies should also include proteomic analyses to reveal which protein or proteins of the tick SGE affect proliferation. In addition, it is necessary to investigate the pathways affecting the proliferation of mESCs, and the expression levels of the important genes that belong to these pathways.

## Authors' contributions

**Ahmet Kocabay:** Methodology, investigation, visualization, data curation, formal analysis (supporting), writing - original draft. **Ayyub Ebrahimi:** Visualization, data curation, formal analysis (supporting), writing - original draft. **Ali Cihan Taşkın:** Project administration, supervision, formal analysis, writing - review & editing. **Sırrı Kar:** Conceptualization, project administration, supervision, formal analysis, writing - review & editing.

## Statement of ethics approval

This study approved by Namık Kemal University the Local Ethics Committee for Animal Experiments (approval number: 07.04.2016-9).

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## Conflict of Interest

The authors declared that there is no conflict of interest.

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RESEARCH ARTICLE

# Species complexes of leaf-inhabiting mites on *Prunus laurocerasus* L. (Rosaceae) trees in Ordu, Turkey

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**ABSTRACT:** *Prunus laurocerasus* L. (Rosaceae) is a summer fruit and evergreen species belonging to the family Rosaceae. It is also known as cherry laurel, common laurel, and sometimes English laurel. This plant grows naturally in the North-Eastern region of Turkey. The aim of the study was to investigate the mite species on *P. laurocerasus* trees in eleven municipalities (Altınordu, Akkuş, Çaybaşı, Fatsa, Gülyalı, İkizce, Kabadüz, Kumru, Perşembe, Ulubey and Ünye) of the Ordu province (Eastern Black Sea Region, Turkey) in 2015 and 2016. Leaf samples were taken weekly from late April until early October in each year. At each sampling date, leaves were taken from different parts of the tree canopy, i.e. lower, middle and upper. The number of sampled trees was determined according to the total number of the trees in each orchard. Approximately 20 leaves per *P. laurocerasus* tree were taken. A total of eighteen mite species belonging to three orders, six families and twelve genera were identified including Phytoseiidae (7), Tydeidae (5), Tetranychidae (2), Stigmaeidae (2), Iolinidae (1) and Winterschmidtiidae (1) during the study. According to the results, *P. laurocerasus* trees have an especially rich fauna of beneficial mites probably due to lack of pesticide usage.

**Keywords:** Cherry laurel, fauna, diversity, pest mites, predatory mites.
**Zoobank:** http://zoobank.org/7273CC64-63E1-491E-871E-91410A111F66

## INTRODUCTION

*Prunus laurocerasus* L. is an evergreen shrub or small to medium-sized tree, growing to 5 to 15 meters tall, with ovoid dark purple to blackish fruit 8-20 mm in diameter belonging to the family Rosaceae (Kolaylı et al., 2003; Bracewell, 2005; Sulusoglu and Cavusoglu, 2010). It is also known as cherry laurel, common laurel, and sometimes English laurel. This plant grows naturally in the region bordering the Black Sea in southwestern Asia and southeastern Europe. It is widely distributed in the North-Eastern region of Turkey. Moreover, it is declared that this tree imported to Europe from Turkey in the 16th century.

This plant is exhibited its diuretics and antidiabetic effects, also used to treatment of digestive system problems, stomach ulcers, hemorrhoids, eczemas, bronchitis (Milan, 1984; Baytop, 1989; Kolaylı et al., 2003). *P. laurocerasus* is also known to have insecticidal activity on arthropods (Rattan and Sharma, 2011; Akyazı et al., 2015). The fruit which has unique taste is consumed directly both fresh and dried and used as raw material for the preparation of food product such as jam, pulp, marmalade, and drinks (Ayaz, 1997; Kolaylı et al., 2003). It is widely consumed in the eastern Black Sea region (Kolaylı et al., 2003). It can also be used to create a tall sized hedge or screen and has become popular garden plants (Sulusoglu and Cavusoglu, 2010). Cherry laurel has endless benefits, and it is clear that the plant seems promising for the future. In this case, until now, it is a big gap of the lack of an investigation about pests, damage to cherry laurel, and their related natural enemies in Turkey. Moreover, few studies have focused on this subject abroad. Among them, it is reported by Navajas et al. (1996) and Witters et al. (2007) that *P. laurocerasus* is among one of the host plants of citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae). Hale (2007) and Denmark et. al. (2018) shared just a photo of symptoms of *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) on *P. laurocerasus* leaves.

To fill these literature gaps, the overall goal of this work was to determine mite species on *P. laurocerasus* trees in eleven municipalities (Altınordu, Akkuş, Çaybaşı, Fatsa, Gülyalı, İkizce, Kabadüz, Kumru, Perşembe, Ulubey and Ünye) of the Ordu province (Eastern Black Sea Region, Turkey) during 2015-2016.

## MATERIALS AND METHODS

The surveys were carried out during 2015 and 2016 on *P. laurocerasus* trees in Ordu provinces. Leaf samples were collected from eleven municipalities including Altınordu, Akkuş, Çaybaşı, Fatsa, Gülyalı, İkizce, Kabadüz, Kumru, Perşembe, Ulubey and Ünye of Ordu province in the Black Sea region (Fig. 1). Geographical coordinates were recorded using a GPS mobile device.

### Sampling of *Prunus laurocerasus* trees

Samplings were carried out weekly between April and October in 2015 and 2016. On each sampling date, leaves were taken from different parts of the tree canopy, i.e. lower, middle and upper. The number of sampled trees per site was determined according to the total number of the trees in each orchard (Table 1). Approximately 20 leaves per tree were collected. The samples were put into paper bags placed inside plastic bags, labeled, and transferred to the laboratory.

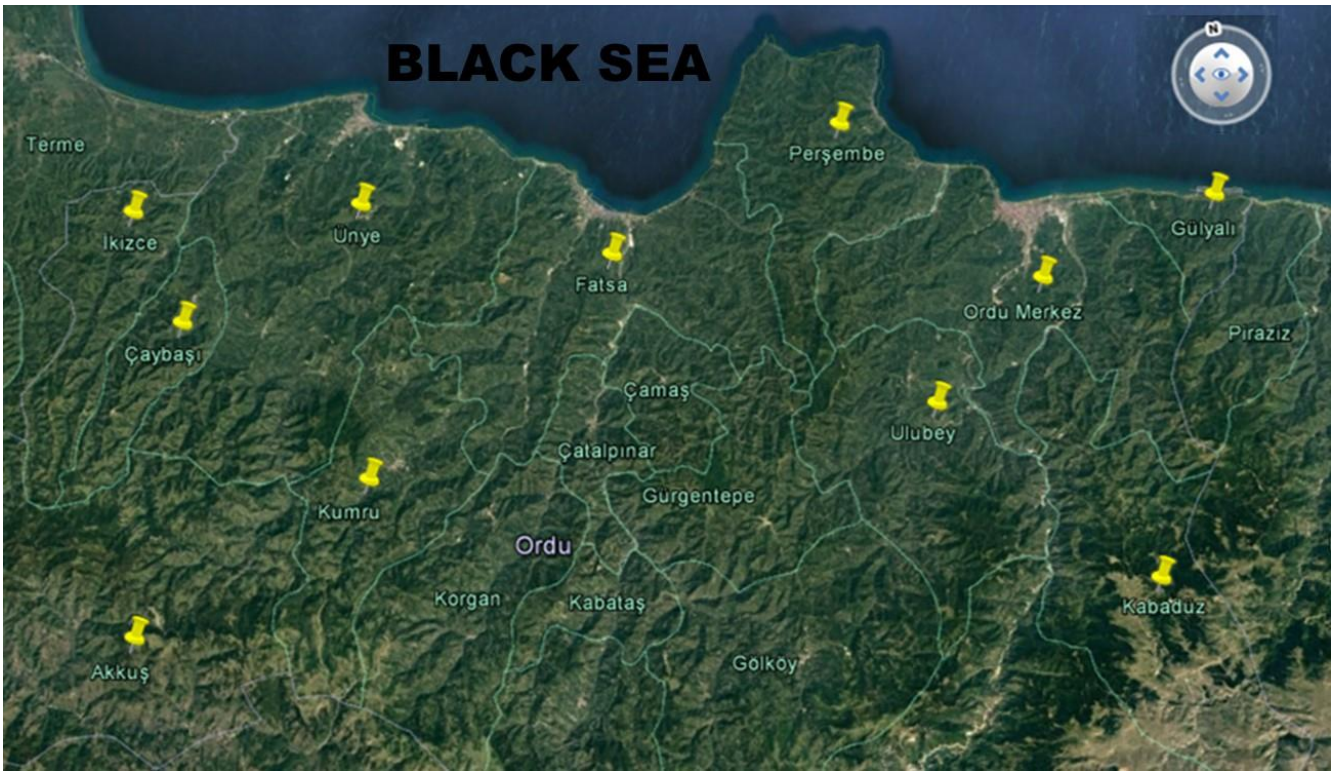


**Figure 1.** Eleven sampled areas in Ordu, Turkey (Black Sea Region) during the 2015 and 2016.

**Table 1**. The number of sampled *Prunus laurocerasus* trees during the 2015 and 2016 growing seasons in Ordu, Turkey (Black Sea Region), according to the total number of trees in each sampling area (Madanlar, 1991).

| Total number of trees in each sampling area | The number of sampled trees |
|---|---|
| 0- 50 | All trees |
| 51-200 | 50 trees |
| 201-400 | 60 trees |
| More than 400 | 20% of the total number of trees |

### Extraction and preparation of mite specimens

The mites were collected with a 0 or 00 paint brush under a stereomicroscope (Leica S8 APO) directly from the leaves. Specimens were preserved in vials containing 70% ethanol. All mites were cleared in lactophenol. Each mite was mounted in Hoyer's medium on microscope slides and dried for 5-7 days in an oven at 50°C according to the method of Krantz and Walter (2009).

### Identification of mite specimens

The mites were identified to species level using the relevant identification keys such as Pritchard and Baker (1955), Meyer (1987), Zhang et al. (2002), Zhang (2003), Seeman and Beard (2011), Ueckermann and Çobanoğlu (2012), Auger et al. (2013) for the family Tetranychidae, Gonzalez-Rodriguez (1965), Fan and Zhang (2005), Saccaggi and Ueckermann (2018) for Stigmaeidae, Baker (1968, 1970), Castagnoli (1984), Andre (2011), Ueckermann (2013), Ripka et al. (2013), Akyazı et al. (2017), Ueckermann et al. (2019) for Iolinidae and Tydeidae, Muma and Denmark (1970), Çobanoğlu (1989a,b,c, 1993a,b,c,d), Faraji et al. (2007, 2011), Tixier et al. (2009), Döker et al. (2014a,b, 2016, 2020) for Phytoseiidae, Fain (1972), Moser and Bogenschütz, (1984), Fain and Rack (1987) and Krantz and Walter (2009) for Winterschmidtiidae. Mite species were identified under a light microscope (Leica DM 2500, Heerbrugg, Switzerland) equipped with phase contrast.

Confirmation of species identification and some of identifications were made in School of Biological Sciences/Zoology, North-West University by Prof. Dr. Edward Albert Ueckermann. The mite specimens were deposited in the Mite Collection at the Ordu University, Agricultural Faculty, Plant Protection Department, Ordu, Turkey.

## RESULTS AND DISCUSSION

During the study, a total of 344 mite specimens in various development stages were examined: 341 adults (308 females, 33 males), 3 nymphs. A total of 18 mite species belonging to 3 orders, 6 families and 12 genera were identified as follows: seven species of Phytoseiidae, five Tydeidae, two Tetranychidae, two Stigmaeidae, one for each of Iolinidae and Winterschmidtiidae (Table 2).

Family **Phytoseiidae** Berlese

***Transeius wainsteini*** (Gomelauri)

Material examined: 6♀♀ (Altınordu, 29.06.2016), 4♀♀ (Altınordu, 21.07.2015), 2♀♀ (Altınordu, 31.07.2015), 6♀♀, 1♂ (Perşembe, 12.08.2015), 10♀♀ (Ulubey, 20.09.2016), 2♀♀ (Ulubey, 01.10.2015), 14♀♀, 1♂ (İkizce, 11.08.2016), 3♀♀, 1♂ (Fatsa, 11.08.2015), 2♀♀, 1♂ (Fatsa, 14.06.2016), 7♀♀, 1♂ (Ünye, 03.08.2015), 1♀ (Ünye,

22.06.2016), 1♀ (Gülyalı, 19.08.2015), 2♀♀ (Kabadüz, 09.08.2016), 4♀♀, 1♂ (Çaybaşı, 11.08.2016)

Comments: *Transeius wainsteini* has been recorded in Denmark, Georgia, Germany, Russia, Iran, Slovakia and Turkey (Demite et al., 2017). In Turkey, it was found on *Rosa canina* L. (Rosales) in Giresun (Faraji et al., 2011), persimmon trees (Akyazı et al., 2016, 2017), vegetables (Soysal and Akyazı, 2018), stone (Altunç and Akyazı, 2019) and pome (Akyol, 2019) fruit trees in Ordu. It was collected among the *Panonychus ulmi* (Koch) (Trombidiformes: Tetranychidae) and *Aceria* sp. (Trombidiformes: Eriophyidae) population on wild apple trees in Iran by Rahmani et al. (2010). On the other hand, it was found in association with *T. urticae* in hazelnut orchards and sunflower by the same researchers. Moreover, Tajmiri et al. (2014) notified that this predator has probably survived using alternative food (such as pollen) at the lack of prey times.

**Table 2.** Mite species collected from *Prunus laurocerasus* trees during the 2015 and 2016 growing season in the eleven different sampling areas (Altınordu, Akkuş, Çaybaşı, Fatsa, Gülyalı, İkizce, Kabadüz, Kumru, Perşembe, Ulubey and Ünye) in Ordu, Turkey (Black Sea Region).

| **Order** | **Family/Species** | **Number of mite specimens** | | | |
|---|---|---|---|---|---|
| | | **♀** | **♂** | **N** | **TOTAL** |
| Mesostigmata | Phytoseiidae | **163** | **8** | **-** | **171** |
| | *Transeius wainsteini* (Gomelauri) | 64 | 6 | | 70 |
| | *Amblyseius herbicolus* Chant | 54 | - | - | 54 |
| | *Amblyseius andersoni* (Chant) | 36 | 2 | - | 38 |
| | *Amblyseius bryophilus* Karg | 3 | - | | 3 |
| | *Euseius stipulatus* (Athias-Henriot) | 3 | - | - | 3 |
| | *Euseius gallicus* Kreiter and Tixier | 1 | - | - | 1 |
| | *Paraseiulus triporus* (Chant and Yoshida-Shaul) | 2 | - | - | 2 |
| Trombidiformes | Tetranychidae | **34** | **5** | **3** | **42** |
| | *Panonychus citri* (McGregor) | 28 | 4 | 2 | 34 |
| | *Tetranychus urticae* Koch | 6 | 1 | 1 | 8 |
| | Stigmaeidae | **2** | - | - | **2** |
| | *Agistemus collyerae* Gonzalez | 1 | - | - | 1 |
| | *Zetzellia mali* (Ewing) | 1 | - | - | 1 |
| | Tydeidae | **88** | **20** | - | **108** |
| | *Tydeus californicus* (Banks) | 45 | 9 | - | 54 |
| | *Tydeus goetzi* Schruft | 42 | 9 | - | 51 |
| | *Tydeus calabrus* (Castagnoli) | - | 1 | - | 1 |
| | *Tydeus plumosus* Karg | 1 | - | - | 1 |
| | *Brachytydeus paraobliqua* Panou & Emmanuel | - | 1 | - | 1 |
| | Iolinidae | **4** | - | - | **4** |
| | *Pronematus ubiquitus* (McGregor) | 4 | - | - | 4 |
| Sarcoptiformes | Winterschmidtiidae | **17** | - | - | **17** |
| | *Calvolia* sp. Oudemans | 17 | - | - | 17 |
| **TOTAL** | | **308** | **33** | **3** | **344** |

N: Nymph

***Amblyseius herbicolus*** Chant

Material examined: 7♀♀ (Ulubey, 01.10.2015), 2♀♀ (Ulubey, 20.09.2016), 2♀♀ (Ünye, 03.08.2015), 17♀♀ (Perşembe, 12.08.2015), 8♀♀ (Gülyalı, 19.08.2015), 9♀♀ (Altınordu, 21.07.2015), 9♀♀ (Altınordu, 31.07.2015).

Comments: *Amblyseius herbicolus* has very wide distribution in the world (Demite et al., 2017). In Turkey, this predatory species was firstly reported on persimmon trees in Ordu by Akyazı et al. (2016). Altunç and Akyazı (2019), Akyol (2019) and Döker et al. (2020) also found it on stone, pome and citrus fruit trees, respectively. This mite was classified as Subtype III-c-Generalist predators living in confined space on dicotyledonous plants by McMurtry et al. (2013).

***Amblyseius andersoni*** (Chant)

Material examined: 2♀♀ (Altınordu, 29.06.2016), 13♀♀ (Altınordu, 21.07.2015), 1♀, 1♂ (Perşembe, 12.08.2015), 8♀♀ (Ünye, 03.08.2015), 2♀♀ (Ünye, 22.06.2016), 1♀ (İkizce, 11.08.2016), 4♀♀ (Fatsa, 11.08.2015), 2♀♀ (Ulubey, 20.09.2016), 1♀ (Ulubey, 01.10.2015), 1♀ (Gülyalı, 19.08.2015), 1♀, 1♂ (Kumru, 29.07.2016).

Comments: *Amblyseius andersoni* is a very common predatory mite species which shows distribution in more than thirty countries (Demite et al., 2017). In Turkey, it was reported on different plants by many researchers (Çobanoğlu, 1992; Akyazı and Ecevit, 2003, 2005; Çobanoğlu, 2004; İnal, 2005; Yanar and Ecevit, 2005; Bayram and Çobanoğlu, 2007; Kumral and Kovancı, 2007; Özşişli and Çobanoğlu, 2011; Yeşilayer and Çobanoğlu, 2011; Faraji et al., 2011; Özsayın, 2012; Satar et al., 2013; Kasap et al., 2013; Gençer-Gökçe, 2015; Kumral and Çobanoğlu, 2015a,b; Çobanoğlu and Kumral, 2016; Çobanoğlu and Güldalı, 2017; Akyazı et al, 2017; Soysal and Akyazı, 2018; Altunç and Akyazı, 2019; Çobanoğlu et al., 2020; Döker et al., 2020; Ersin et al., 2020). It was classified as Type III Lifestyle-generalist predators by McMurtry et al. (2013).

***Amblyseius bryophilus*** Karg

Material examined: 1♀ (Altınordu, 21.07.2015), 1♀ (Altınordu, 31.07.2015), 1♀ (Kabadüz, 09.08.2016).

Comments: *Amblyseius bryophilus* was firstly recorded from Germany by Karg (1970). It is also known in France, Hungary, Poland, Serbia, and Turkey (Demite et al., 2017). In Turkey, *A. bryophilus* was recorded for the first time from Rize province by Döker et al. (2014a). This species was also found on pome fruit trees in Ordu (Akyol, 2019) and *Phaseolus vulgaris* (Fabaceae) in Rize (Döker et al., 2020). *Amblyseius* spp. were classified as generalist predators-Type III lifestyle (Croft et al., 2004).

***Euseius stipulatus*** (Athias-Henriot)

Material examined: 2♀♀ (Gülyalı, 19.08.2015), 1♀ (Altınordu, 29.06.2016).

Comments: *Euseius stipulatus* is a common predatory mite worldwide (Demite et al., 2017). It was recorded on citrus (McMurtry, 1977; Çobanoğlu, 1989b), cucumber (Çobanoğlu, 1989a), pome fruits (Akyol, 2019), walnut leaves (Çakır et al., 2020) and olive trees (Ersin et al., 2020) in Turkey. This species was classified as Type IV Lifestyle-Pollen feeding generalist predators by McMurtry et al. (2013).

***Euseius gallicus*** Kreiter and Tixier

Material examined: 1♀ (Ünye, 22.06.2016).

Comments: *Euseius gallicus* was reported for the first time on *Tilia platyphyllos* Scopoli (Tiliaceae), *Prunus cerasus* L. (Rosaceae), *Aesculus hippocastanum* L. (Sapindaceae) and *Viburnum tinus* L. (Adoxaceae) in France (Tixier et al., 2009). Later, it was determined in Belgium, France, Germany, Italy, Netherlands, Slovenia, Tunisia and Turkey (Demite et al., 2017). In Turkey, Döker et al. (2014b) recorded this predatory mite on *Ipomoea* sp. (Convolvulaceae). It was also found on vegetables in Ordu (Soysal and Akyazı, 2018) and walnut leaves in Samsun province (Çakır et al., 2020). This mite is a Type IV- pollen feeding generalist predator (Kreiter et al., 2020).

***Paraseiulus triporus*** (Chant and Yoshida-Shaul)

Material examined: 1♀ (Gülyalı, 19.08.2015), 1♀ (Altınordu, 21.07.2015).

Comments: *Paraseiulus triporus* was reported from almost thirty countries (Demite et al., 2017). In Turkey, this predatory species was firstly reported on quince, apple and cranberry by Çobanoğlu (2004). So far, many researchers found it on the different plants from different regions of Turkey (Kasap and Çobanoğlu, 2007; Yeşilayer and Çobanoğlu, 2011; Özsisli and Çobanoğlu, 2011; Özsayın, 2012; Erdoğan, 2013; Kasap et al., 2013; Satar et al., 2013; Gençer-Gökçe, 2015; Akyazı et al., 2017; İnak and Çobanoğlu, 2018; Akyol, 2019; Altunç and Akyazı, 2019; Keskin, 2019). McMurtry et al. (2013) classified *Paraseiulus* spp. as Subtype I-c- specialized predators of tydeids.

Family **Tetranychidae** Donnadieu

***Panonychus citri*** (McGregor)

Material examined: 5♀♀ (Altınordu, 21.07.2015), 1♀ (Altınordu, 31.07.2015), 6♀♀, 1 nymph (Perşembe, 12.08.2015), 4♀♀, 3♂♂ (Fatsa, 14.06.2016), 1♀ (Ünye, 03.08.2015), 4♀♀ (Ünye, 22.06.2016), 7♀♀, 1♂, 1 nymph (Gülyalı, 19.08.2015).

Comments: *Panonychus citri* is a major pest of citrus and occasionally attacks grapes, ornamental flowers and evergreen shrubs grown in greenhouses and nurseries (Zhang, 2003). It was reported from all regions of the world (Migeon et al., 2011). *P. citri* was collected from different plants by Düzgüneş (1952), İnal (2005), Satar et al. (2013), Altunç and Akyazı (2019) in Turkey as well.

***Tetranychus urticae* Koch** (Green form)

Material examined: 2♀♀, 1♂, 1 nymph (Gülyalı, 19.08.2015), 1♀ (Ünye, 22.06.2016), 2♀♀ (Fatsa, 14.06.2016), 1♀ (Kumru, 29.07.2016).

Comments: *Tetranychus urticae* is a highly polyphagous and cosmopolitan species (Zhang, 2003; Migeon et al., 2011). It was reported by many researchers in Turkey as well (Ulusoy et al., 1999; Özman and Çobanoğlu, 2001; İncekulak and Ecevit, 2002; Yanar and Ecevit, 2005; İnal, 2005; Çetin et al., 2006; Ertop, 2006; Kumral and Kovancı, 2007; Kasap et al., 2008; Elma and Alaoğlu, 2008; Güven, 2008; Özsayın, 2012; Satar et al., 2013; Erdoğan, 2013; Çobanoğlu and Kumral, 2014; Gençer-Gökçe, 2015; Kumral and Çobanoğlu, 2015a,b; Kutlu, 2016; Akyazı et al., 2017; Çobanoğlu and Güldalı, 2017; Soysal and Akyazı, 2018; Akyol; 2019; Altunç and Akyazı, 2019; Keskin, 2019; Cilbircioğlu and Çobanoğlu, 2020; Çobanoğlu et al., 2020; Erdoğan and Çobanoğlu, 2020). On the other hand, we collected few individuals of *T. urticae* from each location. So, we think that these findings may be originated from accidental infestations. The sampled trees were close to hazelnut orchards. The hazelnut trees and also floor vegetation plants under *P. laurocerasus* trees can harbor *T. urticae* and may become a source of infestation for *P. laurocerasus* trees. This assumption might be addressed in future studies.

Family **Stigmaeidae** Oudemans

***Agistemus collyerae*** Gonzalez

Material examined: 1♀ (Perşembe, 12.08.2015).

Comments: The genera *Agistemus* Summers and *Zetzellia* Oudemans are the second most important predator group after Phytoseiidae (Gerson et al., 2003). Gonzalez-Rodriguez (1963) firstly reported *A. collyerae* in New Zealand. It was also found Australia, Iran, Italy, Portugal, Turkey (Fan et al., 2016) and South Africa (Saccaggi and Ueckermann, 2018). Saccaggi and Ueckermann (2018) declared that it was detected on products imported from USA, Chile, Yemen, Spain and France to South Africa. In Turkey, the predatory mite was found on *Quercus robur* L. (Fagaceae) and *Cupressocyparis leylandii* (A.B. Jacks. & Dallim.) (Cupressaceae) in İstanbul (Yeşilayer and Çobanoğlu, 2013) and *Malus domestica* Borkh. (Rosales) in Çanakkale (Kasap et al., 2013). It is known to feed on *Tetranychus lambi* Pritchard & Baker (Trombidiformes: Tetranychidae) and *Aculus fockeui* (Nalepa & Trouessart) (Trombidiformes: Eriophyidae) (Saccaggi and Ueckermann, 2018).

***Zetzellia mali*** (Ewing)

Material examined: 1♀ (Fatsa, 11.08.2015).

Comments: *Zetzellia mali* is widely distributed in the Holarctic region worldwide (Gonzalez-Rodriguez, 1965; Dönel and Doğan, 2013). It was found for the first time in Turkey by Düzgüneş (1963). Later, this species was collected from many provinces by many researchers (Akyazı and Ecevit, 2003; İnal, 2005; Kasap and Çobanoğlu, 2007; Elma and Alaoğlu, 2008; Kasap et al., 2008, 2013; Özsayın, 2012; Satar et al., 2013; Çobanoğlu and Kumral, 2014; Kasap et al., 2014; Gençer-Gökçe, 2015; Kumral and Çobanoğlu, 2015a,b; Akyazı et al., 2016, 2017; Soysal and Akyazı, 2018; Akyol, 2019; Altunç and Akyazı, 2019; Keskin 2019; Çobanoğlu et al., 2020; Erdoğan and Çobanoğlu, 2020). According to Croft (1994), it preys on eggs and immature stages of European red mite and active stages of apple rust mite. Khanjani and Ueckermann (2002) also declared that *Z. mali* tends to feed on eriophyid mite than adult tetranychid mites. It is known that *Z. mali* may feed on other predator mite eggs as well (Kain and Nyrop, 1995). Croft (1994) also noted that it can prey on eggs of the predatory phytoseiid mites *Typhlodromus occidentalis* (Nesbitt) and *Typhlodromus pyri* Scheuten (Mesostigmata: Phytoseiidae) and other *Z. mali.*

Family **Tydeidae** Kramer

***Tydeus californicus*** (Banks)

Material examined: 6♀♀, 1♂ (Altınordu, 29.06.2016), 10♀♀, 1♂ (Ünye, 03.08.2015), 14♀♀, 5♂♂ (Ünye, 22.06.2016), 1♀ (Fatsa, 14.06.2016), 1♀ (Çaybaşı, 11.08.2016), 2♀♀ (İkizce, 11.08.2016), 2♀♀ (Ulubey, 01.10.2015), 3♀♀ (Gülyalı, 19.08.2015), 4♀♀, 2♂♂ (Kumru, 29.07.2016), 2♀♀ (Akkuş, 04.06.2016).

Comments: *Tydeus californicus* is a cosmopolitan species (Tempfli et al., 2015). It has been reported on various hosts in Turkey by many researchers (Çobanoğlu, 1992; Çobanoğlu and Kazmierski, 1999; Özman and Çobanoğlu, 2001; İncekulak and Ecevit, 2002; Akyazı and Ecevit, 2003; Yanar and Ecevit, 2005; Kasap and Çobanoğlu, 2007; Kumral and Kovancı, 2007; Güven, 2008; Kasap et al., 2008; Özsisli and Çobanoğlu, 2011; Yeşilayer and Çobanoğlu, 2011; Özsayın, 2012; Erdoğan and Yanar, 2015; Kasap et al., 2013; Satar et al., 2013; Kasap et al., 2014; Gençer-Gökçe, 2015; Akyazı et al., 2017; Soysal and Akyazı, 2018; Akyol, 2019; Altunç and Akyazı, 2019; Çobanoğlu et al., 2020). Tempfli et al. (2015) notified that this species can play an important role in the management of rust mites.

***Tydeus goetzi*** Schruft

Material examined: 12♀♀, 4♂♂ (Altınordu, 31.07.2015), 4♀♀, 2♂♂ (Altınordu, 21.07.2015), 4♀♀ (Altınordu, 29.06.2016), 7♀♀, 2♂♂ (Perşembe, 12.08.2015), 14♀♀ (Gülyalı, 19.08.2015), 1♀ (Ünye, 03.08.2015), 1♂ (Kabadüz, 09.08.2016).

Comments: *Tydeus goetzi* has shown limited distribution in world. It was reported in Germany (Schruft, 1972), France (Andre, 2011), and Turkey (Akyazı et al., 2017; Soysal and Akyazı, 2018; Altunç and Akyazı, 2019; Akyol, 2019). According to Schruft (1972), *T. goetzi* feeds on *Colomerus vitis* (Pagenstecher) and *Calepitrimerus vitis* (Nalepa) (Trombidiformes: Eriophyidae).

***Tydeus calabrus*** (Castagnoli)

Material examined: 1♂ (Ünye, 03.08.2015).

Comments: *Tydeus calabrus* is a rarely- seen species of genus *Tydeus* Koch. Little is known of its distribution. Çobanoğlu and Kazmierski (1999) recorded this species from Turkey. Sadeghi et al. (2012) also mentioned it as a new record for Iran.

***Tydeus plumosus*** Karg

Material examined: 1♀ (Gülyalı, 19.08.2015).

Comments: *Tydeus plumosus* was recorded in apple orchards of Serbia (Stojnic et al., 2002) and on wheat in Diyarbakır (Sur) in Turkey (Ueckermann et al., 2019).

***Brachytydeus paraobliqua*** Panou and Emmanuel

Material examined: 1♂ (İkizce, 11.08.2016).

Comments: *Brachytydeus paraobliqua* was found in Greece (Panou and Emmanouel, 1996), Hungary (Ripka et al., 2002; Tempfli et al., 2015) and Turkey. In Turkey, it was firstly reported in Samsun by Özman-Sullivan et al. (2005). Later, it was found on *Diospyros kaki* Thunb. and *Diospyros lotus* L. (Ebenaceae) by Akyazı et al. (2017) and stone fruit trees by Altunç and Akyazı (2019) in Ordu.

Family **Iolinidae** Pritchard

***Pronematus ubiquitus*** (McGregor)

Material examined: 3♀♀ (Fatsa, 14.06.2016), 1♀ (Ünye, 22.06.2016).

Comments: This predator species is widely distributed in USA, Egypt and Africa (Baker, 1968). In Turkey, it was reported by Çobanoğlu (1992); Göven et al. (2009); Can and Çobanoğlu (2010); Kumral and Çobanoğlu (2015b); Akyol (2019); Ueckermann et al. (2019). According to Abou-Awad et al. (1999), *P. ubiquitus* can develop from larva to adult stage when feeds on individuals of the fig bud mite, *Eriophyes ficus* Cotte and the fig leaf mite, *Rhyncaphytoptus ficifoliae* Keifer (Trombidiformes: Eriophyidae).

Family **Winterschmidtiidae** Oudemans

***Calvolia* sp.**

Material examined: 3♀♀ (Fatsa, 11.08.2015), 5♀♀ (Altınordu, 31.07.2015), 1♀ (Perşembe, 12.08.2015), 6♀♀ (Kabadüz, 09.08.2016), 2♀♀ (Altınordu, 29.06.2016).

Comments: Different species within the genus *Calvolia* have been reported from Germany (Moser and Bogenschütz, 1984), Bangladesh (Gupta and Sanyal, 2004), Poland, America and Ukrain (Krantz and Walter, 2009). In Turkey, *Calvolia* sp. was collected from vegetable (Soysal, 2016) and stone fruit (Altunç and Akyazı, 2019) leaves in Ordu. *Calvolia* spp. are deemed fungivorous by Krantz and Walter (2009).

In spite of seventeen females in the material, the species could not be identified to species level because of their damaged body parts, poor preparation of slides, lack of literature. This is an issue for future research to explore.

## Authors' contributions

**Rana Akyazı:** Planning the research, project administration, investigation, resources, survey and laboratory studies, assemble data, writing the manuscript, review and editing. **Mete Soysal:** Investigation, survey and laboratory studies, assemble data. **Yunus Emre Altunç:** Investigation, survey and laboratory studies, assemble data.

## Statement of ethics approval

Not applicable.


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## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.


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RESEARCH ARTICLE

# A contribution to avian ectoparasite fauna of Turkey: the reports of feather mites and tick on the Great tit (*Parus major* L.)

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**ASBTRACT:** Birds have a symbiotic relationship with many ectoparasite groups such as chewing lice (Mallophaga: Amblycera, Ischnocera), fleas (Siphonaptera), keds (Diptera: Hippoboscidae), mites (Acari: Sarcoptiformes, Trombidiformes) and ticks (Acari: Ixodidae, Argasidae). In fact, this relationship is mainly based on parasitism, but feather mites are mostly classified as commensal ectosymbionts, compared to other parasitic insects and arachnids. This study reports tick and feather mite species detected on a dead specimen of the Great Tit (*Parus major* L.) that was brought to the Department of Parasitology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey. As a result of the parasitological examination, the tick samples were identified as *Ixodes frontalis* (Panzer) and feather mites were identified as *Analges mucronatus* (Buchholz) and *Proctophyllodes stylifer* (Buchholz). With this study, *A. mucronatus* and *P. stylifer* have been reported for the first time from Turkey, and the feather mite fauna of Turkey has reached 42 identified species.

**Keywords:** *Analges mucronatus*, *Ixodes frontalis*, *Proctophyllodes stylifer*.
**Zoobank:** http://zoobank.org/6EEF0F27-2B00-47BB-90B6-C0343C01CC6C

## INTRODUCTION

Feather mites (Acari: Astigmata) are obligatory parasitic or commensal ectosymbionts permanently living on birds. These mites are most commonly found in the plumage of their hosts, especially on the wing and tail feathers (Gaud and Atyeo, 1996; Dabert and Mironov, 1999; Proctor, 2003). They are currently classified under two superfamilies (Analgoidea, Pterolichoidea) in the Astigmata (Krantz and Walter, 2009), although experts of 20th century recognized one more superfamily, Freyanoidea (Gaud and Atyeo, 1978, 1996; Mironov, 2019). More than 2500 species have been known to date, and it is estimated that there could be over 16000 feather mite species in the world (Peterson, 1975; Gaud and Atyeo, 1996; Dabert and Mironov, 1999).

Feather mites have developed various specialized morphological adaptations, depending on hosts and attachment regions. Usually, these mites are classified into four morpho-ecological groups according to their general adaptations and locations on hosts: wing and tail feather mites, contour feather mites, quill mites and skin mites (Mironov, 1987, 1999; Dabert and Mironov, 1999).

The relationship between feather mites and host birds is thought to be a phenomenon between commensalism and parasitism. Some authors consider these organisms to be commensal creatures that feed on oil and feather debris secreted from the uropygial gland (Peterson, 1975; OConnor, 1982; Gaud and Atyeo, 1996). However, in the study conducted by Blanco et al. (2001) showed that the nutrient contents of the intestines of *Pterodectes rutilus* (Robin) (Proctophyllodidae) and *Scutulanyssus nuntiaeveris* (Berlese) (Pteronyssidae) included algae, fungi and pollen apparently also taken from the feather surfaces. In addition, as in other symbiotic relationships, feather mites, especially representatives of the family Epidermoptidae, may exert a possible pressure on the immune system due to stress and direct damage of the skin surface (Esch et al., 1975).

Contrary to the feather mite studies in the world, the studied are insufficient from Turkey (Bakırcı and Güleğen, 2005; Aksin, 2007, 2010, 2011; Gürler et al., 2013; Per and Aktaş, 2018; Eren et al., 2021). In these studies, 40 idendified, 2 unidentified feather mites were reported from birds (Table 1). Feather mites are mostly ignored in the field of Veterinary Parasitology from Turkey, as they do not usually cause general condition disorders or infections in domestic and wild birds, unlike other parasitic mites.

Ticks (Acari: Ixodida) have been studied more in avian ectoparasites because of their long distance transportation by birds and their ability to overcome geographical barriers, and also they are vectors for many viral, bacterial and parasitic agents of medical and veterinary importance (Hasle, 2013; Brinkerhoff et al., 2019). As in the world, many studies have been carried out about tick infestation on birds from Turkey (Table 2). In these studies; *Amblyomma*, *Argas*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Ornithodoros* and *Rhipicephalus* infestations were detected (Kurtınar, 1954; Merdivenci, 1970; Oğuz et al., 2015; Keskin and Erciyas-Yavuz, 2016, 2019; Keskin et al., 2017).

The great tit (*Parus major* L.) is a passerine bird species distributed in the western Palearctic region in areas suitable for its habitat such as deciduous or mixed woodlands, gardens, parks, shrublands and sometimes coniferous forests (Beaman and Madge, 2010).

**Table 1.** List of feather mite species detected so far in Turkey.

| Family | Species | References |
|---|---|---|
| **Alloptidae** | *Alloptoides aythinae* | Gürler et al. (2013) |
| **Analgidae** | *Analges mucronatus**, *A. passerinus*, *A. spiniger*, *A. turdinus*, *Diplaegidia columbae*, *Megninia ginglymura*, *Strelkovi-acarus quadratus* | Aksin (2011); Gürler et al. (2013); Per and Aktaş (2018); *present study |
| **Avenzoariidae** | *Avenzoaria totani* | Gürler et al. (2013) |
| **Dermoglyphidae** | *Dermoglyphus* sp. | Aksin (2010) |
| **Falculiferidae** | *Falculifer rostratus* | Bakırcı and Güleğen (2005) |
| **Freyanidae** | *Freyana anatina*, *Freyana nyrocae* | Aksin (2007); Gürler et al. (2013) |
| **Proctophyllodidae** | *Dolichodectes edwardsi*, *Joubertophyllodes modularis*, *Monojoubertia microphylla*, *Proctophyllodes cetti*, *P. clavatus*, *P. doleophyes*, *P. lusciniae*, *P. mesocaulus*, *P. rube-culinus*, *P. scolopacinus*, *P. stylifer**, *P. sylviae*, *P. troncatus*, *Proctophyllodes* sp. | Gürler et al. (2013); Per and Aktaş (2018); *present study |
| **Pterolichidae** | *Grallolichus minutus*, *Pelargodacna heteromorpha, Periexo-caulus anacanthus*, *Pseudolichus solutocurtus*, *Xoloptes claudicans* | Aksin, 2011; Gürler et al. (2013); Eren et al. (2021) |
| **Pteronyssidae** | *Pteronyssoides striatus*, *Scutulanyssus hirundicola* | Gürler et al. (2013) |
| **Ptiloxenidae** | *Ptiloxenus major* | Aksin (2010) |
| **Psoroptoididae** | *Temnalges mesalgoides* | Gürler et al. (2013) |
| **Trouessartiidae** | *Trouessartia bifurcata, T. inexpectata*, *T. jedliczkai*, *T. kratochvili*, *T. microcaudata*, *T. reguli*, *T. rubecula*, *T. trouessarti* | Gürler et al. (2013); Per and Aktaş (2018) |

**Table 2.** List of tick species detected so far on birds from Turkey

| | Tick species | References |
|---|---|---|
| **Ixodidae** | *Amblyomma* sp. | Keskin and Erciyas-Yavuz (2016) |
| | *Dermacentor marginatus* | Keskin and Erciyas-Yavuz (2016) |
| | *Haemaphysalis concinna*, *H. punctata*, *H. parva*, *H. sulcata, Haemaphysalis* sp. | Merdivenci (1970); Leblebicioglu et al. (2014); Keskin and Erciyas-Yavuz (2016, 2019); Dik and Kandir (2021) |
| | *Hyalomma marginatum*, *Hyalomma* sp. | Merdivenci (1970); Leblebicioglu et al. (2014); Keskin and Erciyas-Yavuz, (2016, 2019); Dik and Kandir (2021) |
| | *Ixodes arboricola*, *I. eldaricus*, *I. festai*, *I. frontalis**, *I. hexagonus*, *I. redikorzevi*, *I. ricinus, Ixodes* sp. | Merdivenci (1970); Leblebicioglu et al. (2014); Keskin et al. (2014); Keskin and Erciyas-Yavuz (2016, 2019; Dik and Kandir (2021); *present study |
| | *Rhipicephalus bursa, R. turanicus* | Leblebicioglu et al. (2014); Oğuz et al. (2015) |
| **Argasidae** | *Argas persicus*, *A. reflexus* | Kurtınar (1954); Parrish (1961); Merdivenci (1970) |
| | *Ornithodoros coniceps* | Merdivenci (1970) |

Turkey has a rich ornithofauna due to geographically different habitats and being on many migration routes, but ectoparasite studies in birds are unfortunately insufficient. We believe that this study will contribute to the literature on feather mites and tick infestations on birds from Turkey.

## MATERIALS AND METHODS

The specimen of the Great Tit, *Parus major* L. (Passeriformes: Paridae), found dead at the tram stop in the university campus (41°22'01.3"N 36°11'35.6"E), was brought to the Department of Parasitology Laboratory,

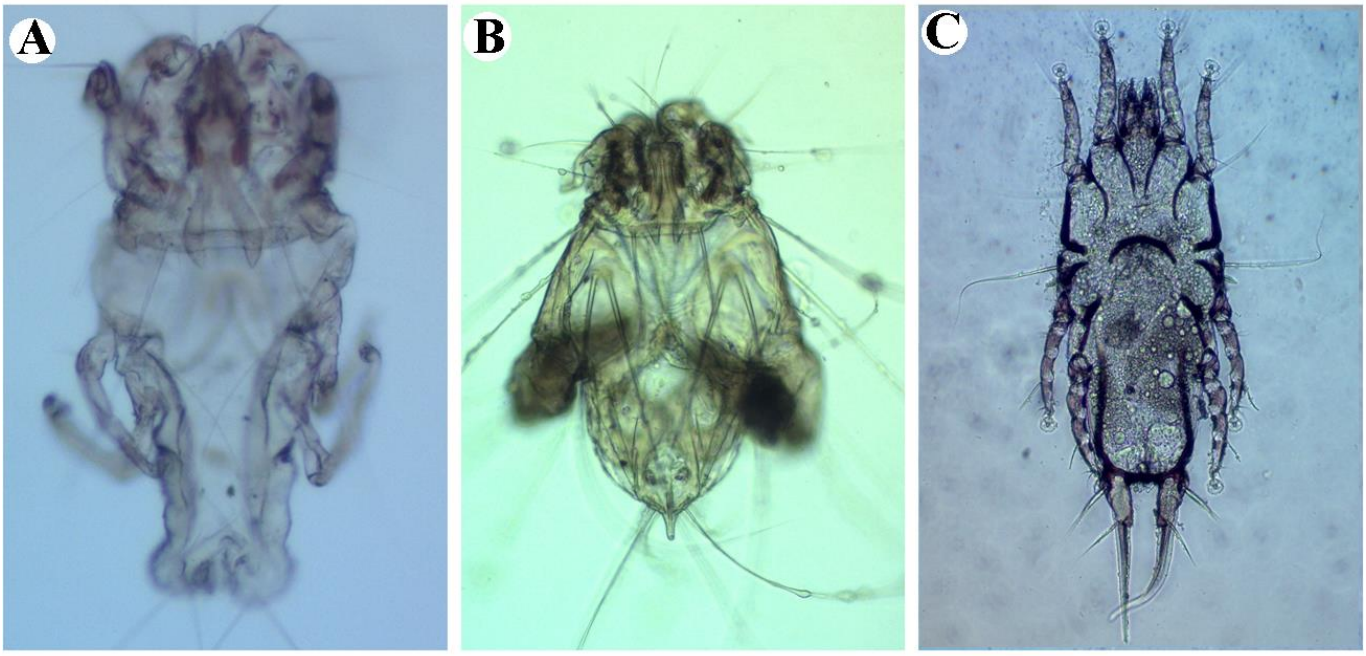


**Figure 1. A.** *Analges mucronatus* (female), **B.** *Analges mucronatus* (male), **C.** *Proctophyllodes stylifer* (female).

Faculty of Veterinary Medicine, Ondokuz Mayıs University (Samsun, Turkey) for parasitological examination. In the process of parasitological examination, samples of mites and ticks were collected by using point tip tweezers and placed in vials with 70% ethanol. Then, feather mite specimens were cleaned and softened for 24 hours with lactophenol and mounted in slides with Hoyer's medium (Evans, 1992). The tick sample was cleared for 48 hours in lactophenol, and mounted in slides with Canada Balsam. Subsequently, the samples were identified under a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) using the taxonomic works on corresponding mite taxa with diagnostic keys (Atyeo and Braasch, 1966; Mironov, 1985; Pfäffle et al., 2017). The photographs of the ectoparasites were taken with a camera (Mshot Mdx4-t, Guangzhou, China) integrated with the microscope (Figs 1, 2). The collected mite and tick of samples are preserved in 70% ethanol in the parasitology laboratory (Karatepe and Karatepe, 2015).

## RESULTS

As a result of microscopic examination, the following ectoparasites have been identified: the hard tick *Ixodes frontalis* (Panzer, 1795) (1 larva) and feather mites *Analges mucronatus* (Buchholz, 1969) (1 male and 1 female) and *Proctophyllodes stylifer* (Buchholz, 1869) (1 female) were identified.

*Analges mucronatus* (male and female). The size of idiosoma (length × width) in male is 290 × 195 µm, and in female is 340 × 160 µm. Among species the genus *Analges* (Analgidae: Analginae) this species can be unmistakably identified based on a unique structure of terminal lamella in males, which is modified in a finger-like process with the tip curved ventrally (Fig. 1B).

*Proctophyllodes stylifer* (female). The size of idiosoma: length 360 µm, width 164 µm. Among species of the genus *Proctophyllodes* (Proctophyllodidae: Proctophyllodinae) associated with European passerines, females of this species can be readily identified in having strongly contrasting sclerotization of the hysteronotal shield, which is almost colourless in most part but with wide dark-brown bands along lateral margins (Fig. 1C).

*Ixodes frontalis* (larva). Numbers of setae on the scutal, alloscutal and ventral of body are as follows, on both sides: scutal (Sc), 5; central dorsal (Cd), 6; marginal dorsal (Md), 8; supplementary (S), 5; sternal (St), 3; preanal (Pa), 3; premarginal (Pm), 5; marginal ventral (Mv), 5. The scutum is longer than its width, and the idiosomal setae are also quite long. The hypostome is pointed and the dental formula of hypostome are arranged as 3/3 (Fig. 2B); base of the hypostome with two lateral protrusions. Numbers of setae and spurs on coxae I-III are 2-1-1, respectively (Fig. 2A).

## DISCUSSION

Few studies have been conducted on feather mites in Turkey, and so far 40 identified, two unidentified species of feather mites have been identified in these studies (Bakırcı and Güleğen, 2005; Aksin, 2007, 2010, 2011; Gürler et al., 2013; Per and Aktaş, 2018; Eren et al., 2021). *Analges mucronatus* (Buchholz, 1869). The genus *Analges* (Analgidae: Analginae) is the most species-rich in this family with 64 species described so far (Mironov, 2019). Mites of this genus are characterized by the weakly sclerotized body and a number of macrosetae found on both the dorsal and ventral body surfaces, as for most analgides, and also spine-like ventral extensions in the tibiae and tarsi of the first two pairs of legs (Gaud and Atyeo, 1996). Although, *Analges* species are predominantly distributed on members of the order Passeriformes also known as songbirds. A total of five species have been recorded from birds of other orders, each from a single host from Apodiformes, Columbiformes, Cuculiformes, Piciformes and Psittaciformes; all these host associations are considered questionable and probably were results of

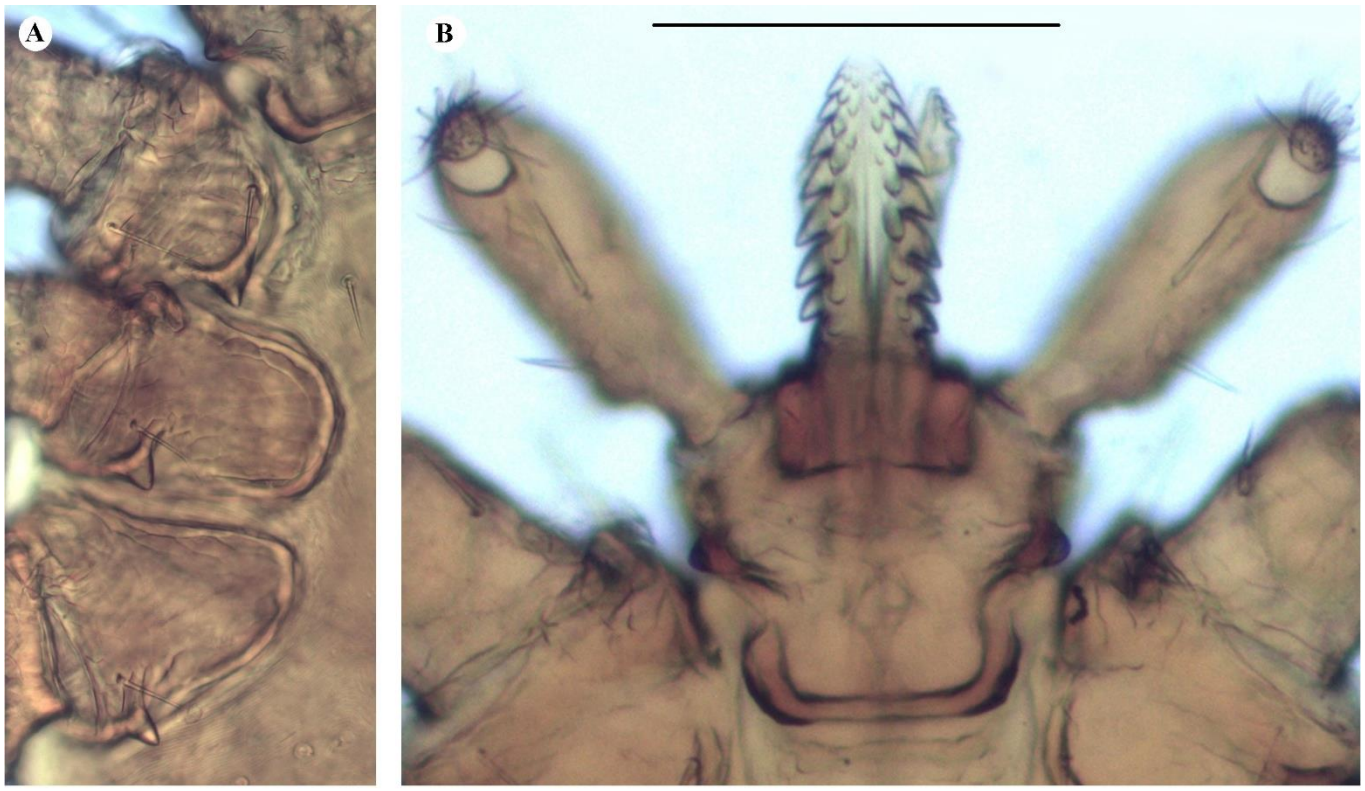


**Figure 2.** *Ixodes frontalis* (larva), **A.** Coxae; **B.** Ventral of capitulum. Scale bar: 150 µm.

contamination (Mironov, 2019). The genus *Analges* is one of most complicate genera of feather mites, because its taxonomy and species identification are based almost exclusively on heteromorphic males (Dabert et al., 2018; Mironov, 2019). *Analges mucronatus* was first described from the Blue Tit (*Cyanistes caeruleus* L.) in Germaly (Buchholz, 1869); further it was reported from this host and from *Parus major* across Western Palearctic (Mironov, 1985, 1996). In Turkey, three *Analges* species were previously known (Table 1). *Analges mucronatus* is reported for the first time in this country.

*Proctophyllodes stylifer* (Buchholz, 1969). The genus *Proctophyllodes* (Proctophyllodidae: Proctophyllodinae) is the richest among all feather mites with over 150 presently known species classified into 12 species groups (Atyeo and Braasch, 1966; Mironov, 2012; Klimov et al., 2017). In this genus, taxonomy and identification of species are mainly based on male individuals (with the terminal lamellae and genital area as the most important diagnostic characters). Although most members of the genus inhabit birds of the order Passeriformes, several *Proctophyllodes* species are specific to particular hosts from the orders Apodiformes, Charadriiformes, and Piciformes. *Proctophyllodes stylifer* has been reported so far from various titmice species in Western Palearctic: the Blue Tit (*C. caeruleus* L.), Great Tit (*P. major*), Marsh Tit (*Poecile palustris* L.) and Willow Tit (*Poecile montanus* Conrad von Baldenstein) (Atyeo and Braasch, 1966; Behnke et al., 1985; Mironov 1996; 2012). In Turkey, nine *Proctophyllodes* species have been previously reported (Table 1). With the present study, *P. stylifer* is reported from this country for the first time.

Ticks, which are obligate blood-sucking arthropods, transmit many bacterial, viral and protozoal agents while feeding on hosts. In addition, birds with a good body condition score, sometimes known as tick-related syndrome, cause acute depressive disorder or death characterized by mild or moderate haemorrhage and oedema in the head and neck area where the tick is attached (Monks et al., 2006). *Ixodes frontalis*, which has a three-host life cycle, is a species that needs birds at every life stage (larva, nymph and adult). It can be easily distinguished from other larva of *Ixodes* species by looking at its morphological features, especially with dorsal and ventral chaetotaxy and two lateral protrusions in the base of the hypostome. It is known that some hard ticks have been identified using larval chaetotaxy (Clifford and Anastos, 1960). It often causes infestation in members of the order Passeriformes, but it can also cause infestations in members of the Anseriformes, Falconiformes, Galliformes, Gruiformes, and Strigiformes (Pfäffle et al., 2017). Although known specifically as a bird parasite, it has sometimes been reported in mammals such as badgers and martens (Hillyard, 1996) and also humans (Keskin et al., 2017). In the studies conducted so far in Turkey, *I. frontalis* has been reported from 23 different bird species: Blackbird (*Turdus merula* L.), Black-headed Warbler (*Sylvia atricapilla* L.), Black Redstart (*Phoenicurus ochruros* Gmelin), Bluethroat (*Luscinia svecica* L.), Cetti's Warbler (*Cettia cetti* Temminck), Common Chiffchaff (*Phylloscopus collybita* Vieillot), Dunnock (*Prunella modularis* L.), Eurasian Penduline Tit (*Remiz pendulinus* L.), Eurasian Reed Warbler (*Acrocephalus scirpaceus* Hermann), Eurasian woodcock (*Scolopax rusticola* L.), Eurasian wren (*Troglodytes troglodytes* L.), Goldcrest (*Regulus regulus* L.), Great Reed Warbler (*A. arundinaceus* L.), Great Tit (*P. major* L.), Finch (*Fringilla coelebs* L.), House Sparrow (*Passer domesticus*

L.), Robin (*Erithacus rubecula* L.), Red-breasted Flycatcher (*Ficedula parva* Bechstein), Sardinian Warbler (*S. melanocephala* G.), Savi's Warbler (*Locustella luscinioides* Savi), Song Thrush (*Turdus philomelos* Brehm), Thrush Nightingale (*Luscinia luscinia* L.), and Willow Warbler (*Phylloscopus trochilus* L.) (Keskin and Erciyas-Yavuz, 2016, 2019).

Although Turkey has a rich ornithofauna, which increases day by day and approaching 500 species, parasitological studies on birds are poorly conducted. Including this study so far, about 50 hosts, which are almost 10% of the ornithofauna of Turkey, have been studied and 42 identified, two unidentified species of mites have been identified (Table 1). In addition, a total of 18 species of infestation have been reported in tick-focused studies (Table 2). In the near future, multidisciplinary studies including ornithology, veterinary parasitology and acarology will contribute to the diversity and distribution of Turkey's feather mite and tick fauna, as well as other parasitic arthropods such as chewing lice, fleas, keds and parasitic mites.

## Authors' contributions

**Gökhan Eren:** Conceptualization, methodology, resources, visualization, writing - original draft, writing - review & editing. **Mustafa Açıcı:** Conceptualization, methodology, resources, supervision, visualization, writing - original draft, writing - review & editing.

## Statement of ethics approval

Ethics approval is not required for the study material consists of parasite specimens collected from a bird found as dead at the tram stop.


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## Conflict of interest

The authors declared that there is no conflict of interest.


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RESEARCH ARTICLE

# Contribution to the water mite (Acari, Hydrachnidia) fauna of Turkey

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**ASBTRACT:** This study provides new records of water mites from Erzincan, Gümüşhane and Tunceli provinces (Turkey). Thirteen species have been registered as new for the hydrachnid water mite fauna of the study area. *Parathyas colligera* (K. Viets, 1923) is a new record for the fauna of Turkey. So far, no water mite records have been given from the provinces of Tunceli and Gümüşhane. Including the new data, the total number of taxa recorded from Erzincan province tallies 81 species in 17 families.

**Keywords:** *Parathyas,* moist moss, new record, Turkey.
**Zoobank:** http://zoobank.org/E71298D2-82DA-46C1-AC9E-DC876D174D0B

## INTRODUCTION

Turkey is very rich in terms of freshwater mite diversity and to date, 336 species in 62 genera and 25 families of water mites have been reported from Turkey (Erman et al., 2010, 2019; Esen, 2021). Although there have been many studies on this group in the last two decades, new records and new species are given from Turkey and the water mite fauna is still far from complete. Therefore, faunistic studies on water mites should continue for a while.

The family Hydryphantidae is third species-rich family with 39 species (in 12 genera) after families Arrenuridae (58 species) and Hygrobatidae (56 species). Up to now, only one species has been described belonging to genus *Parathyas*. *P. palustris* (K.Viets, 1923) recorded previously from Erzurum Province (Özkan and Erman, 1999).

The aim of this paper is to contribute to the knowledge of the diversity of Turkish hydrachnid water mites by studying the newly collected materials from Erzincan, Gümüşhane and Tunceli.

## MATERIALS AND METHODS

Mites were collected from moss samples using Berlese-Tullgren funnels. Sampling studies were carried out after obtaining legal permissions from the General Directorate of Agricultural Research and Policies (50411936-604.02-E.2200901) and from the General Directorate of Nature Conservation and National Parks (72784983-488.04-44455 and 21264211-288.04-E.2187926), two units of TR Ministry of Agriculture and Forestry. Mite specimens were preserved in vials containing 70% ethanol. The specimens mounted in Hoyer's medium and dissected as described elsewhere (e.g., Gerecke et al., 2007). The specimens are deposited in the research collection of the Department of Biology, Bingöl University, Bingöl, Turkey.

The composition of the material is given as: (males/females/deutonymphs). All measurements are given in micrometers.

The following abbreviations are used: a.s.l. = above sea level, IV-L-6 = Fourth leg, sixth segment, Ac-3 = third acetabulum, dL = dorsal length, L = length, P-1 = palp, first segment, vL = ventral length, W = width.

## RESULTS

Family: Hydrovolziidae Thor, 1905

Genus: *Hydrovolzia* Thor, 1905

*Hydrovolzia cancellata* Walter, 1906

Material examined: Moist moss on the river bank, Pülümür Valley, Tunceli, 39°15'40"N, 39°52'25"E, 1443 m a.s.l., 13.10.2018 (2/1/0).

Records from Turkey: Afyon, Muş and Rize (Erman et al., 2010).

Distribution: Central and Eastern Mediterranean, Iran (Gerecke et al., 2007; Pešić and Saboori, 2007).

Family: Hydryphantidae Piersig, 1896

Genus: *Panisus* Koenike, 1896

*Panisus michaeli* Koenike, 1896

Material examined: Wet mosses, Harşit Valley, Gümüşhane, 40°36'26"N, 39°32'14"E, 2280 m a.s.l., 21.06.2014 (0/1/0).

Records from Turkey: Konya (Boyacı and Özkan, 1994), Antalya (Boyacı et al., 2012), Burdur (Gülle et al., 2017) and Isparta (Durucan and Boyacı, 2020).

Distribution: Europe, Turkey, Iran (Pešić and Saboori, 2007; Di Sabatino et al., 2010a; Erman et al., 2010).

*Panisus torrenticolus* Piersig, 1898

Material examined: Moist moss, Harşit Valley, 40°56'35"N, 38°51'13"E, 100 m a.s.l., 21.03.2014 (1/0/0); Moist and grasy soil on the river bank, Sansa, Erzincan, 39°33'30"N, 40°07'11"E, 1418 m a.s.l., 21.10.2020 (0/3/0); moist moss and grasy soil on the river bank, 39°34'16"N, 40°06'03"E, 1664 m a.s.l., 28.11.2020 (1/1/0).

Records from Turkey: Afyon, Erzurum (Erman et al., 2010), Antalya (Boyacı et al., 2012) and Isparta (Durucan and Boyacı, 2020).

Distribution: Europe (except Fennoscandia), Turkey, Iran (Pešić and Saboori, 2007; Di Sabatino et al., 2010a; Erman et al., 2010).

Genus: *Panisopsis* K. Viets, 1926

*Panisopsis thori* (Walter, 1907)

Material examined: Moist and grassy soil, Ahmetli, Erzincan, 39°52'49"N, 39°23'46"E, 2210 m a.s.l., 09.09.2014 (1/0/0).

Records from Turkey: Erzurum, Van (Erman et al., 2010) and Burdur (Gülle et al., 2017).

Distribution: Mediterranean, Alps, Central Europe, Turkey (Di Sabatino et al., 2010a; Erman et al., 2010).

*Panisopsis setipes* (K. Viets, 1911)

Material examined: Moist mosses on the river bank, Pülümür Valley, Tunceli, 39°15'40"N, 39°52'25"E, 1443 m a.s.l., 13.10.2018 (1/0/0).

Records from Turkey: Isparta (Aşçı and Boyacı, 2016; Durucan and Boyacı, 2020) and Burdur (Gülle et al., 2017).

Distribution: Western Palaearctic: Northern, Central and Southern Europe, Turkey (Di Sabatino et al., 2010; Erman et al., 2019).

Genus: *Tadjikothyas* Sokolow, 1948

*Tadjikothyas connexa schwoerbeli* Özkan, 1988

Material examined: Moist moss, Pülümür Valley, Tunceli, 39°33'28"N, 39°33'28"E, 1634 m a.s.l., 27.04.2019 (0/1/0); moist moss and grasy soil on ther river bank, Sansa, Erzincan, 39°34'16"N, 40°06'03"E, 28.11.2020 (3/7/0); 39°34'16"N, 40°07'11"E, 1334 m a.s.l., 17.08.2020 (1/1/0).

Records from Turkey: Kayseri, Muş (Erman et al., 2010) and Burdur (Boyacı et al. 2013).

Distribution: Turkey (Erman et al., 2010).

Genus: *Thyopsis* Piersig, 1899

*Thyopsis cancellata* (Protz, 1896)

Material examined: Wet moss on the river bank, Pülümür Valley, Tunceli, 39°24'28"N, 39°51'15"E, 1491 m a.s.l., 09.09.2014 (0/1/1); 39°41'59"N, 39°37'39"E, 30.01.2019 (0/2/0); Erzincan Province, Ekşisu Marshes, 39°43'15"N, 39°36'46"E, 1130 m a.s.l., 12.05.2015 (0/1/0).

Records from Turkey: Erzurum (Boyacı and Özkan, 2007).

Distribution: Western Palaearctic (Di Sabatino et al., 2010a; Erman et al., 2010).

Genus: *Trichothyas* K. Viets, 1926

*Trichothyas* (*Lundbladia*) *petrophila* (Michael, 1895)

Material examined: Moist moss, Harşit Valley, 40°56'35"N, 38°51'13"E, 100 m a.s.l., 21.03.2014 (1/0/0); wet moss, 40°36'N, 38°30'E, 2423 m a.s.l., 21.06.2014 (0/1/0); moist mosses on the river bank, Pülümür Valley, Tunceli, 39°15'40"N, 39°52'25"E, 1443 m a.s.l., 13.10.2018 (0/2/0).

Record from Turkey: Afyon Erzincan and Muş, (Erman et al., 2010; Esen et al., 2013).

Distribution: Southern and Western Europe, England, Balkan, Iran, Asia Minor (Pešić and Saboori, 2007; Di Sabatino et al., 2010; Erman et al., 2010).

Genus: *Parathyas* Lundblad, 1926

*Parathyas palustris* (Koenike, 1912)

Syn. *Thyas palustris* Koenike, 1912

*Thyas rivalis* Koenike, 1912

Material examined: Moist moss, Ahmetli, Erzincan, 39°57'25"N, 39°21'11"E, 2088 m a.s.l., 23.04.2014 (1/1/1).

Description: *Male* Idiosoma L/W 1005/743, integument papillate, frontale diameter 40, dorsal plates larger, pre- and postfrontale fused. Gnathosoma without short rostrum (Fig. 1E), vL 158. Palp segments dL and setation (in parenthesis) P-1 50 (1), P-2 84 (6), P-3 51 (2), P-4 140 (3-4) P-5 39 (2). Chelicera total L 226. Genital field L/W 220/141, genital flap L/W 173/70, posterior margins of genital flap bearing 8-9 setae, pregenital sclerite round and separate (Fig. 1F). Ac-3 located posterior Ac-2, their basal sclerites forming less elongated stalks. Excretory pore with anterior and posterior sclerites, anterior sclerite crescent shaped (Fig. 1G).

*Female* Idisoma L/W 1230/940, frontal eye plate large (diameter 55), pre- and postfrontale fused (Fig. 1A). Palp segments setation same as male (Fig. 1B), palp segments dL and setation (in parenthesis) P-1 53 (1), P-2 105 (6), P-3 62 (2), P-4 170 (3-4), P-5 40 (2). Chelicera total L 320. Genital field L/W 298/200, genital flap L/W 272/96, posterior margins of genital flap bearing 8-9 setae, pregenital sclerite fused with anterior genital sclerite (Fig. 1C). Excretory pore with similar anterior and posterior sclerite (Fig. 1D).

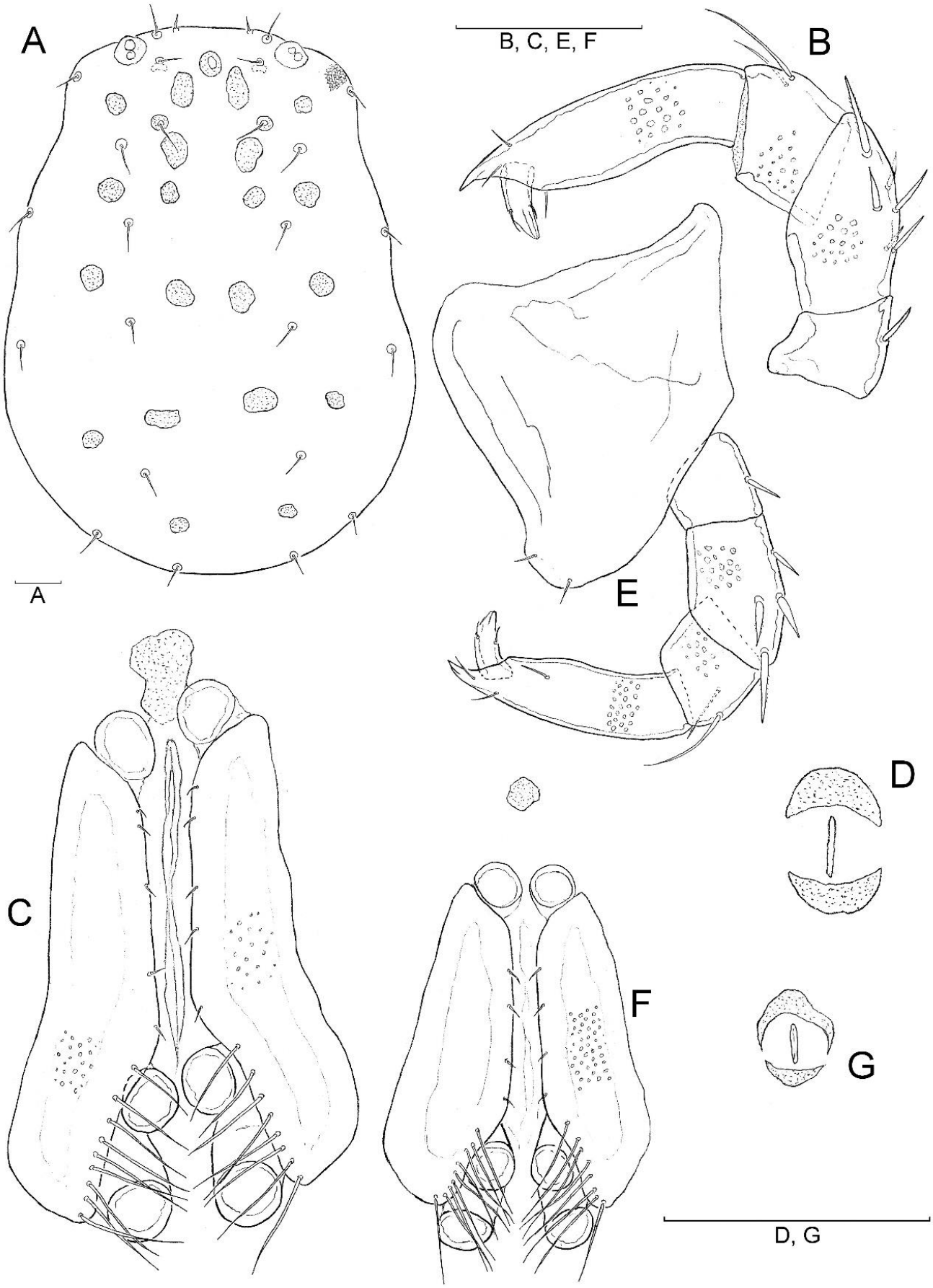


**Figure 1.** *Parathyas palustris*, female: **A**. Idiosoma, dorsal view, **B.** Palp, medial view, **C**. Genital field **D.** Excretory pore; Male: **E**. Gnathosoma, **F.** Genital field, **G.** Excretory pore (Scale bars = 100 μm).

Records from Turkey: Erzurum (as *Thyas rivalis*) (Özkan and Erman, 1999).

Distribution: Holarctic (in Europe not recorded from the Iberian Peninsula); North America, Turkey (Di Sabatino et al., 2010a; Erman et al., 2010).

*Parathyas colligera* (K. Viets, 1923)

Syn. *Thyas distinctus* Tuzovskij, 2007

Material examined: Wet moss, Ahmetli, Erzincan, 39°52'54"N, 39°20'31"E, 2048 m a.s.l., 08.05.2014 (1/2/0).

Description: *Male* Idiosoma L/W 1106/910, integument papillate, frontale diameter 50, dorsal plates smaller, pre- and postfrontale fused (Figs. 2A, 4A). Gnathosoma vL 220. Palp segments dL and setation (in parenthesis) P-1 50 (3), P-2 106 (6), P-3 60 (3), P-4 152 (3), P-5 32 (2). Chelicera total L 295. Genital field L/W 238/161, genital flap L/W 170/78, posterior margins of genital flap bearing 9-10 setae, pregenital sclerite round and separate (Fig. 2B). Ac-3 located posterior Ac-2, their basal sclerites forming more elongated stalks. Excretory pore with anterior and posterior sclerites, anterior sclerite crescent shaped, posterior sclerite small and roundish (Fig. 2C).

*Female* Idisoma L/W 1442/1160, frontal eye plate large (diameter 50), prefrontale and postfrontale not fused (Figs. 3A, 4B-C). Palp segments setation same as male (Figs 3B, C), palp segments dL and setation (in parenthesis) P-1 60 (3), P-2 112 (6), P-3 65 (2), P-4 163 (3-4), P-5 33 (2-3). Gnathosoma with short rostrum, vL 250, chelicera total L 351. Genital field L/W 297/208, genital flap L/W 240/100, posterior margins of genital flap bearing 8-9 setae, pregenital sclerite separate with anterior genital sclerite (Fig. 3E). Excretory pore with large anterior and small posterior sclerite (Fig. 3F).

New record for Turkey

Distribution: Germany, The Netherlands, Russia (Di Sabatino et al., 2010a).

Family: Anisitsiellidae Koenike, 1910

Genus: *Bandakia* Thor, 1913

*Bandakia concreta* Thor, 1913

Material examined: Moist moss, Ahmetli, Erzincan, 39°57'25"N, 39°21'11"E, 2088 m a.s.l., 23.04.2014 (0/1/0); wet moss on the river bank, Pülümür Valley, Tunceli, 39°24'28"N, 39°51'15"E, 1491 m a.s.l., 09.09.2014 (0/1/0).

Records from Turkey: Isparta (Boyacı and Özkan, 2004; Durucan and Boyacı, 2020) and Antalya (Boyacı et al., 2012).

Distribution: Europe, Turkey (Di Sabatino et al., 2010a; Erman et al., 2010).

Genus: *Nilotonia* Thor, 1905

*Nilotonia* (*Dartia*) *vietsi* Bader & Sepasgozarian, 1980

Material examined: Moist and grassy soil, Ahmetli, Erzincan, 39°52'49"N, 39°23'46"E, 2210 m a.s.l., 09.09.2014 (0/1/0); wet moss, Harşit Valley, Gümüşhane, 40°36'N, 38°30'E, 2423 m a.s.l., 21.06.2014 (0/3/0).

Records from Turkey: Erzurum, Muş (Erman et al. 2010) and Isparta (Durucan and Boyacı, 2020).

Distribution: Iran, Turkey (Pešić and Saboori, 2007; Erman et al., 2010).

*Nilotonia (Dartonia) rizeensis* Oezkan & Bader, 1988

Material examined: Wet mosses, Harşit Valley, Gümüşhane, 40°43'28"N, 39°02'40"E, 730 m a.s.l., 23.11.2013 (0/1/1).

Records from Turkey: Rize (Oezkan and Bader, 1988).

Distribution: Turkey (Erman et al., 2010; Esen et al., 2017).

Remark: This is the second record from Turkey except the type locality. To date, only the female of *Nilotonia (Dartonia) rizeensis* is known from Rize Province. In the collected specimens from Gümüşhane Province, a female and a deutonymph were found. The dorsal plates of deutonymph are similar with adults. There are two pairs of genital acetabulae in the genital field. Bearing a ventrodistally projection on pedipalp femur and three setae on the tip of the last segment of fourth leg are the most important characteristic features of adults. Although the general palp shape of deutonumph is similar to adults, there is no projection on P-2. Further, unlike adult individuals, there are two setae on the tip of the last segment of the fourth leg. Figure 5A-G show some morphological details of female and deutonymph from Gümüşhane Province.

## DISCUSSION

In this study totaly thirteen species belonging to families Hydrovolzidae, Hydryphantidae and Anisitsiellidae recorded from Erzincan, Gümüşhane and Tunceli provinces. All species are new for study area. *Parathyas colligera* (K. Viets, 1923) is newly recorded from Turkey. *P. palustris* (as *Thyas rivalis*) is previously recorded from Erzurum province by Özkan and Erman (1999). They stated that *T. rivalis* and *T. palustris* are very similar species, and whether they are synonymous or not can only be decided by examining more specimens of both species.

*Thyas* Koch, 1836*, Todothyas* Cook, 1974 and *Acerbitas* Özdikmen, 2006 are synonymized with *Parathyas* Lundblad, 1926 by Di Sabatino et al. (2010b). Previously, Tuzovskij (2007) proposed to synonymize *Parathyas* Lundblad, 1926 with *Thyas*, Koch. There are not significant differences between the type species of *Parathyas* and the character states diagnostic for *Thyas*. Thus, Di Sabatino et al. (2010b) refused the former subdivision of

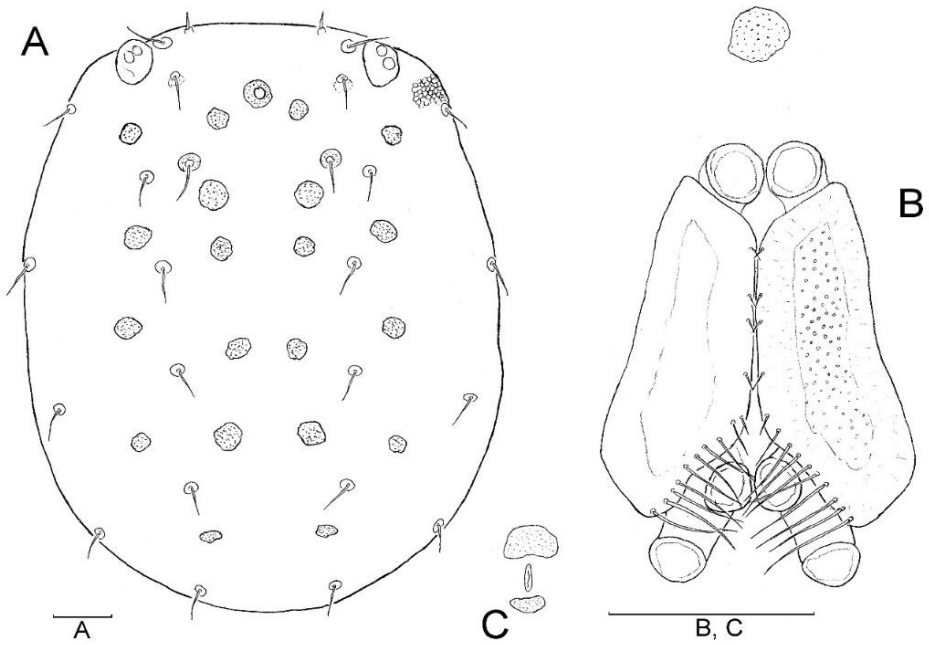


**Figure 2.** *Parathyas colligera*, male: **A.** Idiosoma, dorsal view, **B.** Genital field, **C.** Excretory pore (Scale bars = 100 μm).

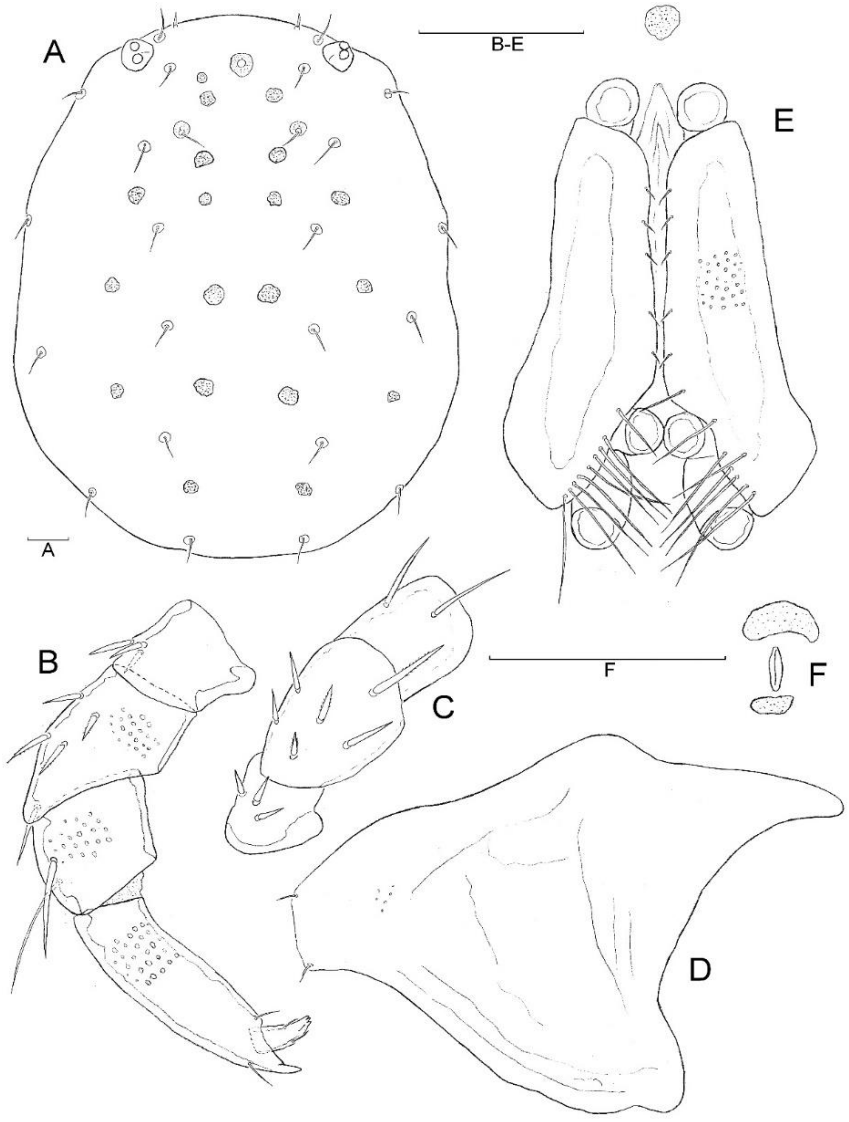


**Figure 3.** *Parathyas colligera*, female: **A.** Idiosoma dorsal view, **B.** Palp, lateral view, **C.** Palp, dordal view, **D.** Capitulum, lateral view, **E.** Genital field, **F.** Excretory pore (Scale bars = 100 μm).

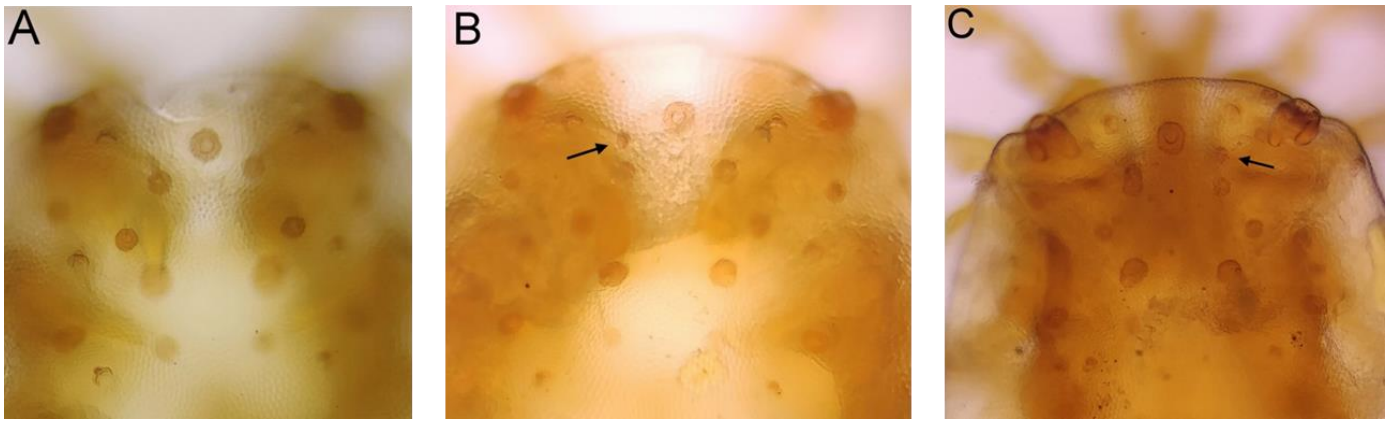


**Figure 4.** *Parathyas colligera*, **A.** Frontal area of male, **B-C.** Frontal area of female (arrows indicate prefrontale variability in females).

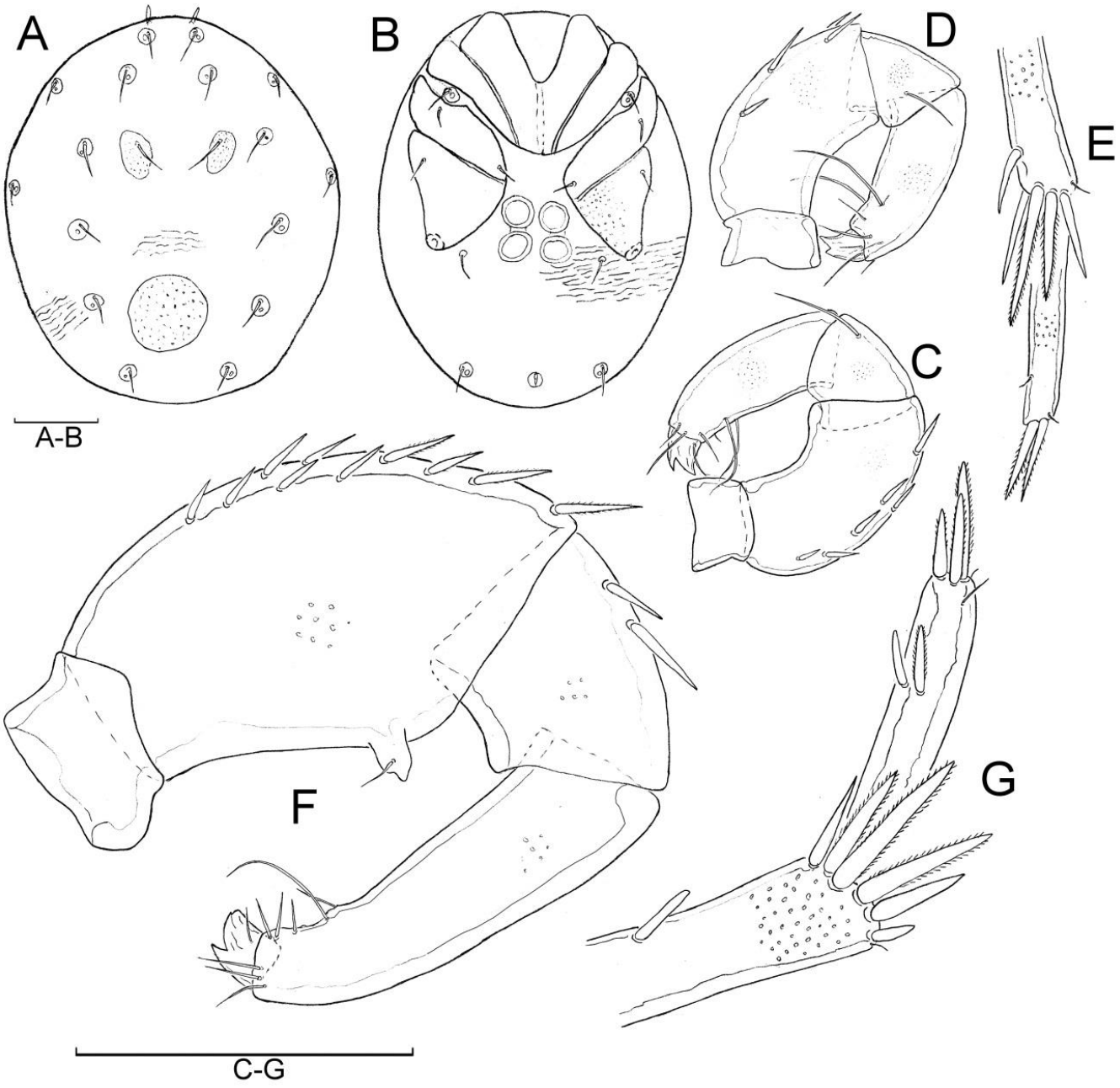


**Figure 5.** *Nilotonia* (*Dartonia*) *rizeensis*, deutonymph: **A.** Idiosoma, dorsal view, **B.** Idiosoma, ventral view, **C.** Palp medial view, **D.** Palp, lateral view, **E.** IV-L-6; Female: **F.** Palp, medial view, **G.** IV-L-6 (Scale bars = 100 µm).

the genus into subgenera and all species formerly placed in *Thyas* tranferred to *Parathyas*.

*Parathyas colligera* (K.Viets, 1923) is very similar to *P. palustris* Koenike, 1912 by the gnathosoma with longer rostrum and diameter of frontal eye platelet approximately equal to length of lateral eye capsule. *P. colligera* can be easily distinguished from *P. palustris* due to the P-1 with three setae, dorsal plates smaller, Ac-3 basal sclerites forming more elongated stalks, pre- and postfrontale separated. The specimens of *P. colligera* collected from Erzincan province differs in having shorter rostrum, P-3 with two setae (in European specimens P-3 with four setae - see Di Sabatino et al. (2010a)) and male pre- and postfrontale fused (Fig. 4A). In females, prefrontale is shown variability in absence to right or left side (Figs 4B, C). In addition, the pregenital sclerite fused with the anterior genital sclerite in females of *P. palustris* (Fig. 1C).

## Statement of ethics approval

Not applicable.

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## Conflict of interest

No potential conflict of interest was reported by the author.

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RESEARCH ARTICLE

# First record of the family Rotundabaloghiidae Kontschán, 2010 in Chile with the description of *Rotundabaloghia* (*Circobaloghia*) *chilensis* sp. nov. (Acari: Mesostigmata)

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**ASBTRACT:** *Rotundabaloghia* (*Circobaloghia*) *chilensis* **sp. nov.** is described based on one female specimen collected from soil in Osorno Coastal Range, Chile. The new species differs from the other species from the subgenus *Circobaloghia* in the shape of peritremes, the shape and length of the ventral and dorsal setae. This is the first record of this family in Chile and also the southernmost occurrence of the family.

**Keywords:** Mite, taxonomy, new species, South-America.
**Zoobank:** http://zoobank.org/304AA9D3-0846-4435-8891-F4637CF89E87

## INTRODUCTION

The subgenus *Circobaloghia* Kontschán, 2010 is a widely distributed pan-tropical mite group within the family Rotundabaloghiidae Kontschán, 2010. The members of this subgenus occur in the Neotropical, Afrotropical and Oriental realms (Kontschán, 2010). Contrary with the subgenus *Rotundabaloghia* Hirschmann, 1975, these species is well characterized by absence of the three or four pairs of short and needle-like setae in rows *j-J* on dorsal body among the lot of uniform setae. After monographic work of Kontschán (2010), only one rotundabaloghiid was described from the Neotropical realm, from Peru (Błoszyk et al., 2019). With this new species the 80 rotundabaloghiid mite species is known from this region (from south Mexico to southern part of Brazil).

During my one of last visit to Natural History Museum of Geneva (Switzerland), a few soil samples from Chile were investigated. One of the samples contained one specimen of rotundabaloghiid mites, described herein as a first species from this family from Chile.

## MATERIALS AND METHODS

The rotundabaloghiid mite specimen examined were cleared in lactic acid for a week and afterwards, the specimens were investigated on half-covered deep slides with a Leica 1000 microscope. Drawings were made with the aid of a drawing tube on a Leica 1000 microscope. The specimen is stored in 75% ethanol and deposited in the Natural History Museum in Geneva. Abbreviations: *v* = ventral setae, *st* = sternal setae, *ad* = adanal setae, *p* = pores, *lf* = lyriform fissures, e = epistome. All measurements and the scale bars of the figures are given in micrometres (µm).

## RESULTS

### Taxonomy

***Rotundabaloghia* (*Circobaloghia*) *chilensis* sp. nov.**

(http://zoobank.org/NomenclaturalActs/8338d123-5840-445d-9ccc-91e9b83f61c6)

(Figures 1-7).

Diagnosis. Surface of dorsal shield smooth, dorsal setae short and smooth. All ventral setae short, smooth and needle-like, setae *st1* and *st3* shorter st3 and st4. Peritremes question mark-shaped. Longitudinal groove between posterior end of pedofossae IV and posterior margin of ventral shield present.

Material examined. Holotype. female. Chili-84/8. Chile, Osorno Coastal Range, Pucatrihue; II. 1988; leg. L.E. Pena.

### Description

*Female* (*n=1*).

*Description.* Length of idiosoma 270, width 240. Shape circular, posterior margin rounded, color yellowish brown.

*Dorsal idiosoma* (Fig. 1). Marginal and dorsal shields fused. Majority of dorsal setae basally curved, smooth and ca 16–20 long. Three pairs of lyriform fissures situated on posterior-central area of dorsal shield. Surface of dorsal shield without sculptural pattern.

*Ventral idiosoma* (Fig. 2). Surface of sternal shield smooth. All sternal setae smooth and needle-like, *st1* and *st2* short (ca 4-5), *st3* and *st4* long (ca 7-9). Setae *st1* situated close to anterior margin of sternal shield, *st2* at level of central area of coxae II, *st3* at level of central area of coxae III, *st4* at level of anterior margin of coxae IV. All ventral setae short (ca 6-8 long), smooth and needle-like. Setae *v2* situated near basal edges of genital shield, *v7* and *v8* situated at level of setae *ad*. Setae *v6* situated close to *v2*. Setae *ad* placed lateral to anal opening, at level of its anterior margin. Ventral shield without sculptural pattern. One pair of lyriform fissures situated close to setae *v2*, other one pair

close to *v7*. Two pairs of pores placed close to setae *v2* and on pair close to setae *ad*. Peritremes short, poststigmatid part absent, prestigmatid part question mark-shaped. Stigmata situated between coxae II and III. Genital shield wide, linguliform (100 long and 56 wide at level of *st4*), without apical process and without sculptural pattern, only some longitudinal shallow grows visible on posterior part. Pedofossae deep, their surface smooth, separate furrows for tarsi IV present. Longitudinal groove between posterior end of pedofossae IV and posterior margin of ventral shield present.

Base of tritosternum narrow, vase-like, tritosternal laciniae smooth, subdivided into three smooth branches in its distal half (Fig. 3).

*Gnathosoma* (Fig. 3). Corniculi horn-like, internal malae smooth and as long as corniculi. All hypostomal setae smooth and needle-like, *h1* long (ca 11-12), *h2*, *h3* and *h4* shorter (ca 5-6). Epistome apically serrate. Palp with smooth and needle-like setae. Chelicerae not visible.

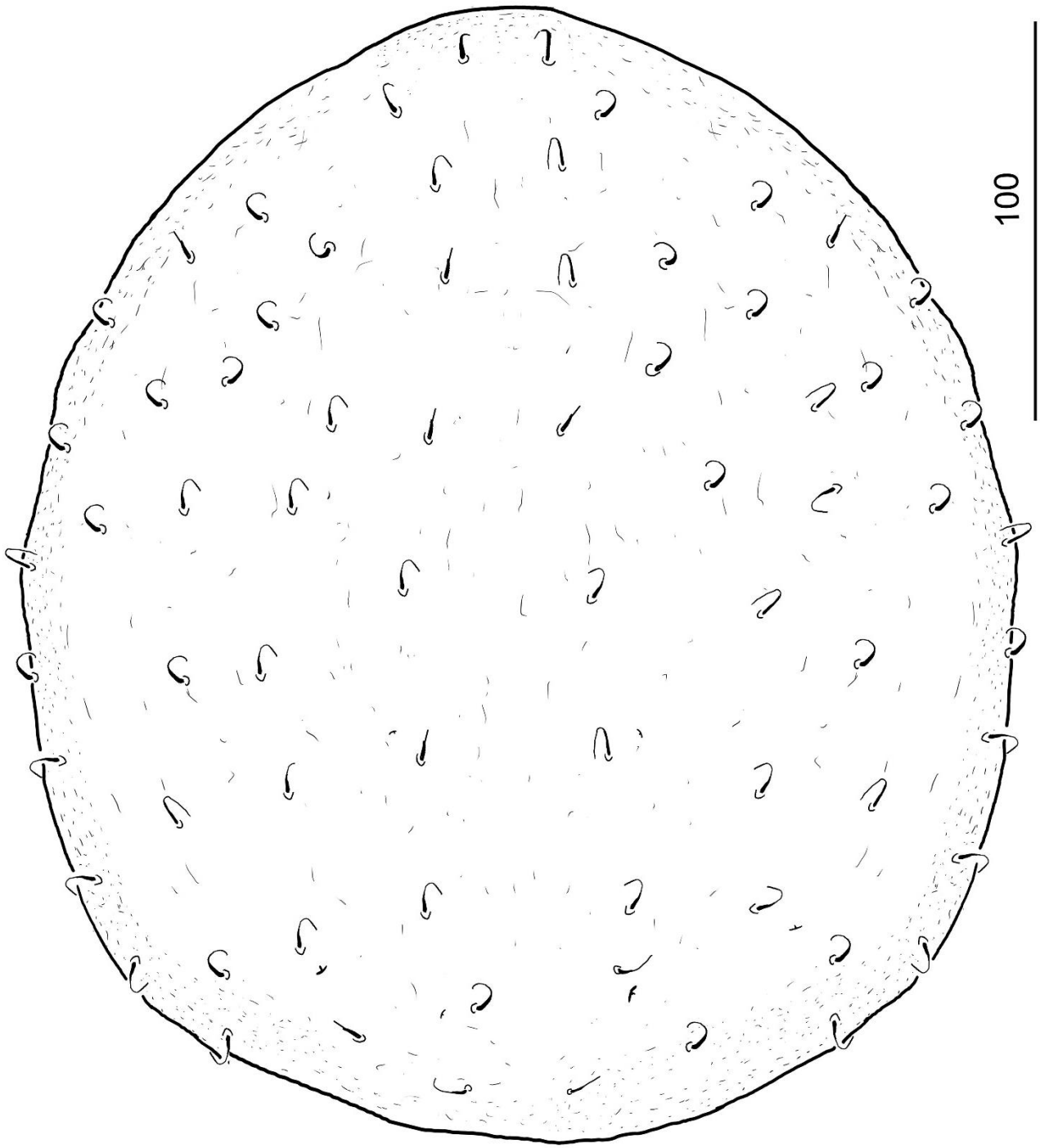



**Figure 1.** Dorsal view of *Rotundabaloghia* (*Circobaloghia*) *chilensis* **sp. nov.** female, holotype.

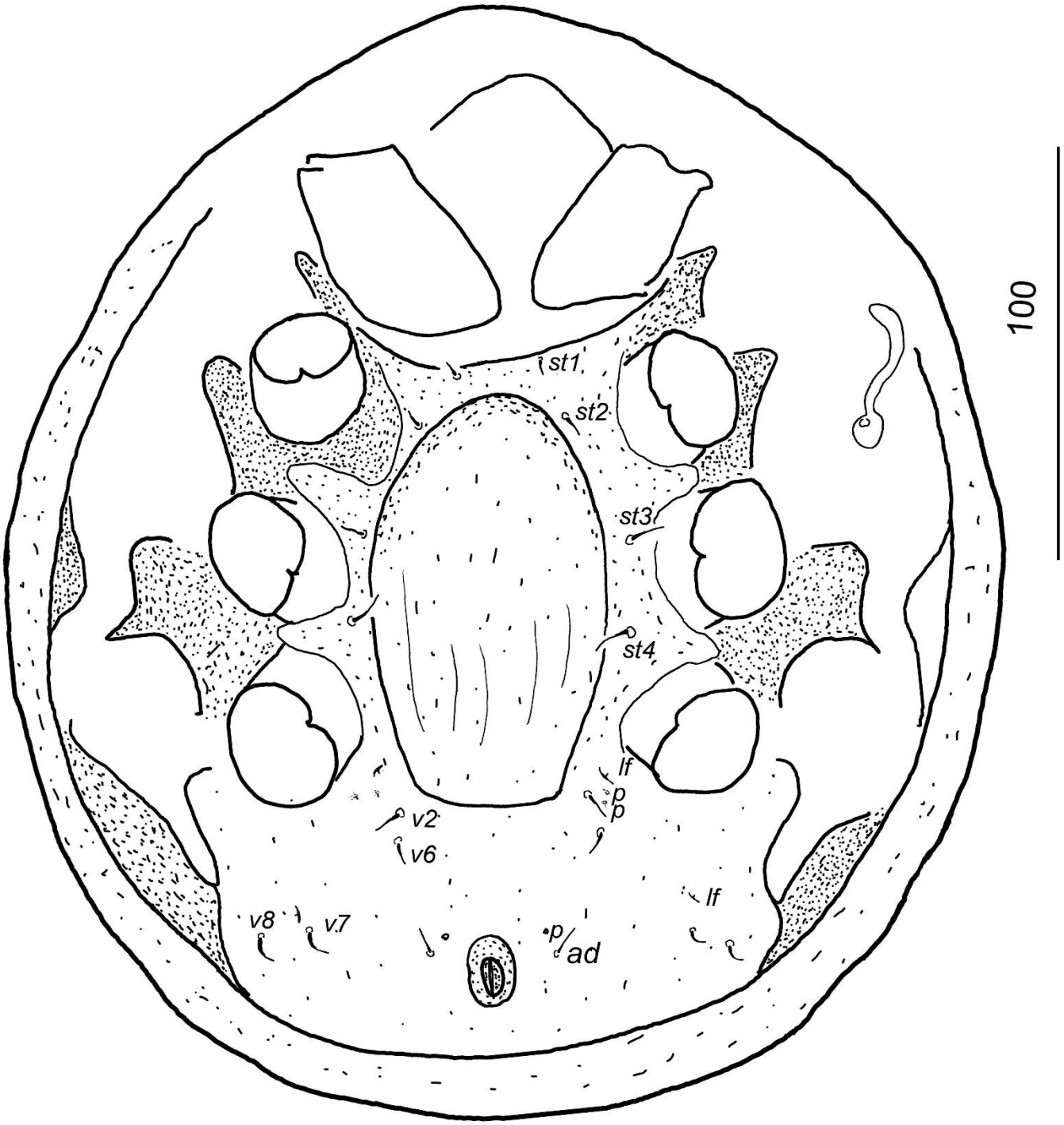


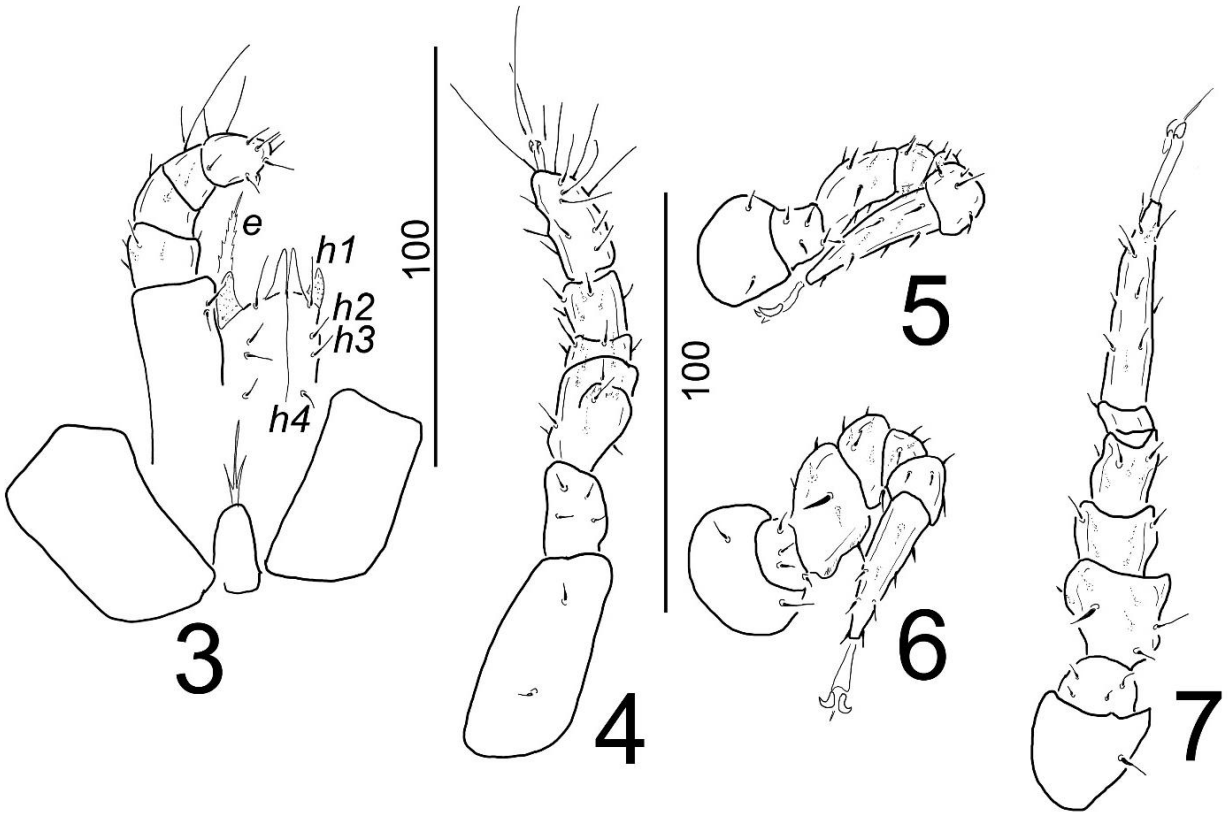

**Figure 2.** Ventral view of *Rotundabaloghia* (*Circobaloghia*) *chilensis* **sp. nov.** female, holotype.

*Legs* (Figs 4-7). All legs with smooth and needle-like setae, the claws on first leg present, but smaller than others. All femora bearing flap-like ventral processes. Leg I 140-142, leg II 108-109, leg III 100-101, leg IV 145-147.

**Male and immature stages.** Unknown.

**Etymology.** The name of the new species refers to the country where the new species was collected.

**Remark.** The new species has a unique character combination (short and smooth dorsal setae, short and smooth setae on ventral idiosoma, smooth surface of dorsal and ventral parts of idiosoma and the shape of peritremes, the longitudinal groove between posterior end of pedofossae IV and posterior margin of ventral shield present) which is not observable on other known rotundabaloghiid mites.




**Figures 3-7.** *Rotundabaloghia* (*Circobaloghia*) *chilensis* **sp. nov.** female, holotype. **3.** Ventral view of gnathosoma, palp, tritosternum, epistome (e) and coxae I, **4.** Leg I. in ventral view, **5.** Leg II in ventral view, **6.** Leg III in ventral view, **7.** Leg IV in ventral view.

## DISCUSSION

The new species is described based on only a single specimen. The new species differs several characters from the congeners that confirming that this is a unique, separated, and easy to recognize species, on other hand, this type locality is lying very far from other localities (Fig. 8). Our knowledge about biodiversity would be very poor without the information about the very rare species, especially in tropical areas. Therefore, the description of the new species based on a single specimen is very important and it seems to be a bad way to not describe these ones. On the other hand, the description of a new species based on a single specimen is not a rare phenomenon in the invertebrate taxonomy, where 30% of the known species is described based on a single type specimen (see Lim et al., 2012). Naturally, several researchers reject this practice (see Dayrat, 2005), because the intraspecific variability is not observable in this case.

## Statement of ethics approval

Not applicable.

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## Conflict of interest

No potential conflict of interest was reported by the author.

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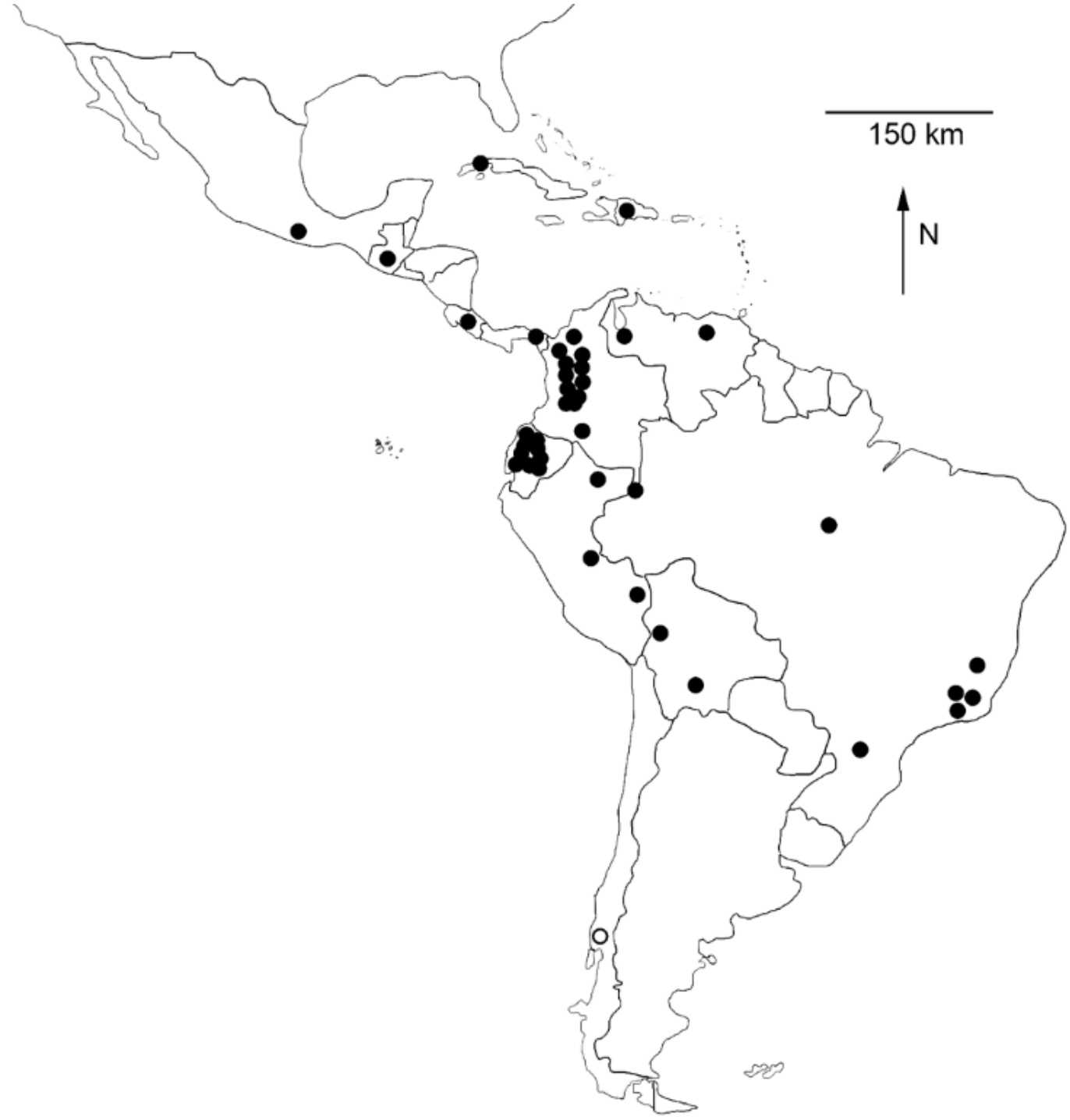


**Figure 8.** Occurrences of rotundabaloghiid mites in Neotropical realm (full circles: known species, empty circle: new species).

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RESEARCH ARTICLE

# Karyotype analysis of two oribatid mite species (Acari: Oribatida)

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**ABSTRACT:** The chromosomal parameters and karyotypic relationships may provide very valuable information about speciation and karyotype evolution. In the order Oribatida, the chromosomal data are limited to a few reports. In the present study, the chromosomal data of two species are provided for the first time. The diploid chromosome numbers are 2*n* = 14 in *Oribotritia hermanni* Grandjean, 1967 (Oribatida: Oribotritiidae) and 2*n* = 22 in *Hermanniella gibber* Kulijev, 1979 (Oribatida: Hermanniellidae) and chromosomes are small holocentric chromosomes. The smallest and largest chromosome sizes are 0.38 µm and 1.08 µm in *O. hermanni*, respectively. The total haploid chromosome length is 4.88 µm, in *O. hermanni*, and a higher value of 6.98 µm is recorded in *H. gibber*. The sex chromosomes could not be identified, because the oribatid mites show weak sexual dimorphism. In this respect, the results of the study provide important contributions to the cytotaxonomy of oribatid mites.

**Keywords:** Acari; Oribatida, karyotype, holocentric chromosome, Turkey.
**Zoobank:** http://zoobank.org/7F0A897D-E77E-4965-BFB9-4E6B4653EAD9

## INTRODUCTION

Soil, which hosts more than a quarter of all living species in the world, is an extremely complex system. It also comprises a large variety of small invertebrates, such as nematodes, pauropods, pseudoscorpions, myriapods, springtails and mites, other invertebrates or microorganisms (Schuppenhaurer et al., 2019). Acari, commonly known as mites and ticks is a member of the class Arachnida and may be among the most species-rich groups of animals, after insects and possibly nematodes (Dabert, 2005). Oribatid mites form one of the most dominant arthropod groups of the soil fauna, particularly abundant and diverse in moist forest floors. They feed on decaying plant remains and fungi (Heethoff et al., 2007; Bezci and Baran, 2016; Norton and Franklin, 2018).

So far, there are approximately 11332 species of oribatid mites in 1306 genera belonging to 162 families identified (Subías, 2004). However, in a very small number of species, the chromosome number has been reported. In addition, oribatid mites are a valuable model for holocentric chromosomes in cytogenetic studies. Generally, the number of diploid chromosomes in oribatid mites is 2*n* = 18, though some have 2*n* = 16 and 30 (Oliver, 1977; Norton et al., 1993; Eroğlu and Per, 2016; Gümüş et al., 2018). Although there are numerous morphological and systematic studies on *Hermanniella gibber* Kulijev and *Oribotritia hermanni* Grandjean, there is no information about their karyotype and chromosome number. The present study aimed to investigate the chromosomal data and karyotype analyses of *H. gibber* and *O. hermanni*.

## MATERIALS AND METHODS

### Oribatid mites sampling and locations

Specimens of *Hermanniella gibber* and *Oribotritia hermanni* were collected from Turkey's Sakarya (Kılıçkaya Hill) and Çorum (Laçin district) provinces. The collecting data of *H. gibber* is: Çorum: Laçin, Laçin district, 40°46.278' N, 34°52.793' E, 779 m, soil, 19 October 2014, 35 exs.; 40°46.215' N, 34°52.848' E, 830 m, moss on rock, 19 October 2014, 1 ex.; 40°46.247' N, 34°52.868' E, 788 m, soil, 19 October 2014, 13 exs.; 40°46.224' N, 34°53.009' E, 813 m, moss on *Pinus* sp., 19 October 2014, 16 exs. (3 exs mounted on aluminum stubs and gold-coated for scanning electron microscopy); 40°46.284' N, 34°52.654' E, 817 m, soil, 30 October 2014, 4 exs.; 40°46.269' N, 34°52.500'E, 834 m, moss on *Pinus* sp., 30 October 2014, 3 exs.; 40°46.283' N, 34°52.476' E, 813 m, soil, 30 October 2014, 5 exs.; 40°46.293' N, 34°52.378' E, 800 m, moss on *Pinus* sp., 30 October 2014, 9 exs.; 40°46.090' N, 34°52.757' E, 882 m, soil, 29 October 2015, 1 ex.; 40°46.215' N, 34°53.170' E, 748 m, lichen on rock, 07 November 2015, 3 exs.; 40°46.112' N, 34°53.225' E, 822 m, soil, 07 November 2015, 2 exs.; 40°46.025' N, 34°53.241' E, 882 m, moss, 07 November 2015, 2 exs.; 40°45.698' N, 34°52.182' E, 987 m, soil, 17 April 2016, 6 exs.; 40°45.631' N, 34°52.054' E, 960 m, moss, 17 April 2016, 1 ex.; 40°45.862' N, 34°52.818' E, 995 m, soil, 01 August 2016, 3 exs.; 40°45.929' N, 34°52.680' E, 995 m, soil, 01 August 2016, 1 ex.; 40°45.903' N, 34°52.687' E, 1001 m, soil, 01 August 2016, 3 exs.; 40°45.641' N, 34°52.848' E, 1070 m, soil, 01 August 2016, 4 exs.; 40°45.612' N, 34°52.900' N, 1067 m, soil, 01 July 2016, 6 exs. The collecting data of *O. hermanni* specimens is: Sakarya, Kılıckaya Hill, 40° 29.066' N, 30° 26.464' E, 1082 m, in soil under *Quercus*

sp., 09 June 2015, 14 exs (3 exs mounted on aluminum stubs and gold-coated for scanning electron microscopy); 40° 28.900' N, 30° 23.510' E, 598 m, in lichen on *Pinus* sp., 06 November 2015, 1 ex. All materials were collected by Sedat Per.

### Cytogenetic procedure

The cytogenetic method developed by Imai et al. (1988) and later modified by Gokhman and Quicke (1995) was used. The chromosome spreads were prepared from whole specimens of unknown sex. The samples were pretreated and crushed in a hypotonic solution (1% sodium citrate) containing colchicine (0.005%) (Sigma Aldrich, Germany). Then, the material was incubated in the hypotonic solution for 20 min, fixed in fixative series with fixative 1 (glacial acetic acid: absolute alcohol: distilled water - 3:3:4, v:v:v), fixative 2 (glacial acetic acid: absolute alcohol - 1:1, v:v), and fixative 3 (glacial acetic acid); transferred onto pre-cleaned glass slides, air-dried and stained in 5% Giemsa (Sigma Aldrich, Germany).

Ten metaphase plates were selected and evaluated for each species. The photos were photographed with a DP72 digital camera mounted on an Olympus BX-53 microscope and analyzed with KaryoType software loaded on a personal computer. The following parameters were evaluated: CL referring to chromosome length, THL referring to total haploid length, MHL referring to mean haploid length, and RL = CL / THL × 100 referring to relative length. Finally, the monoploid ideograms were drawn based on chromosome lengths.

## RESULTS

The chromosome records of two species are herein provided (Fig. 1), which are reported for the first time. The diploid chromosome numbers are 2*n* = 14 in *Oribotritia hermanni* and 2*n* = 22 in *Hermanniella gibber* and chromosomes are holocentric type (Table 1). The smallest and largest chromosome sizes are 0.38 µm and 1.08 µm in *O. hermanni*, respectively (Table 2). The total haploid chromosome length is 4.88 µm, in *O. hermanni* and a higher value of 6.98 µm is recorded in *H. gibber*. The mean haploid length is 0.63 µm, in *H. gibber*, and a higher value of 0.70 µm in *O. hermanni*. The relative lengths range from 5.59 µm to 12.89 µm in *H. gibber* and from 7.79 µm to 22.13 µm in *O. hermanni*. The monoploid ideograms generated by *x* = 7 and 11 are given in Figure 2.

**Table 1.** The comparison of chromosomal data of the species.

| | *Hermanniella gibber* | *Oribotritia hermanni* |
|---|---|---|
| Chromosome type | Holocentric | Holocentric |
| *x* (basic number) | 11 | 7 |
| 2*n* (diploid number) | 22 | 14 |
| Karyotype formula | - | - |
| THL (total haploid length, µm) | 6.98 | 4.88 |
| MHL (mean haploid length, µm) | 0.63 | 0.70 |
| Karyotype asymmetry | - | - |

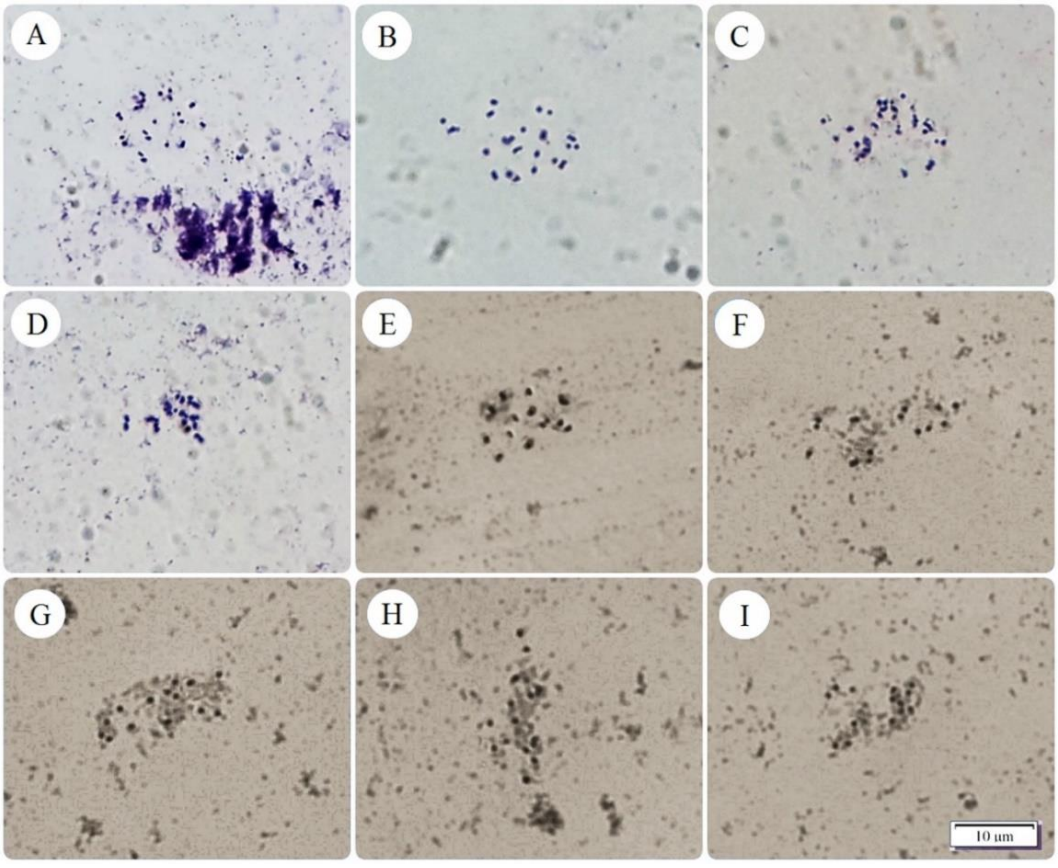


**Figure 1.** Photomicrograph of mitotic metaphase chromosomes, **A-D.** *Hermanniella gibber*, **E-I.** *Oribotritia hermanni*

**Table 2.** The total and relative chromosome lengths of *Hermanniella gibber* and *Oribotritia hermanni*.

| *Hermanniella gibber* | | | *Oribotritia hermanni* | | |
|---|---|---|---|---|---|
| Chromosome Pair | Length (µm) | Relative length (%) | Chromosome pair | Length (µm) | Relative length (%) |
| 1 | 0.90 | 12.89 | 1 | 1.08 | 22.13 |
| 2 | 0.84 | 12.03 | 2 | 0.91 | 18.65 |
| 3 | 0.81 | 11.60 | 3 | 0.73 | 14.96 |
| 4 | 0.72 | 10.32 | 4 | 0.61 | 12.50 |
| 5 | 0.67 | 9.60 | 5 | 0.60 | 12.30 |
| 6 | 0.61 | 8.74 | 6 | 0.57 | 11.68 |
| 7 | 0.57 | 8.17 | 7 | 0.38 | 7.79 |
| 8 | 0.54 | 7.74 | | | |
| 9 | 0.48 | 6.88 | | | |
| 10 | 0.45 | 6.45 | | | |
| 11 | 0.39 | 5.59 | | | |

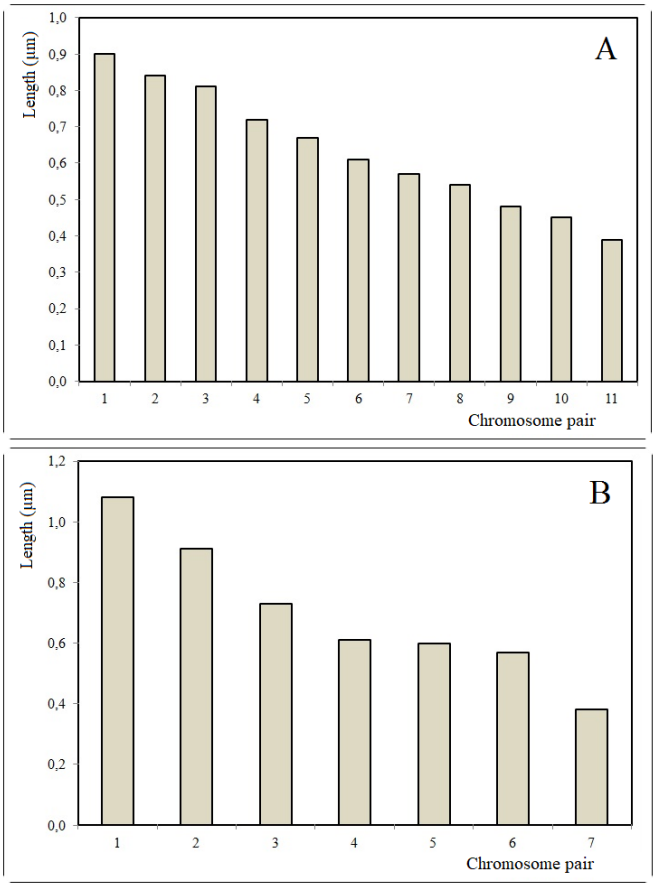


**Figure 2.** The monoploid ideograms of *Hermanniella gibber* (A) and *Oribotritia hermanni* (B).

## DISCUSSION

The chromosomal data, which are basic chromosome number, diploid chromosome number, total haploid length, karyotype formula, centromeric index, and karyotype asymmetry etc., are important parameters for understanding the relationships among the taxa and karyotype evolution (Peruzzi and Eroğlu, 2013; Eroğlu, 2015). The diploid numbers are reported here for the first time for two oribatid mites, namely *Oribotritia hermanni* ($2n$ = 14) and *Hermanniella gibber* ($2n$ = 22). The common diploid number is $2n$ = 18 in oribatid mites (Norton et al., 1993; Heethoff et al., 2006). In the present and previous relevant studies, various chromosome numbers were recorded. Eroğlu and Per (2016) reported that the chromosome number is $2n$ = 30 in *Zygoribatula cognata* Oudemans, 1902. In addition, the diploid number is $2n$ = 12 in *Phauloppia lucorum* Koch, 1841 (Gümüş et al., 2018).

The chromosomes of *H.* gibber and *O. hermanni* are small holocentric chromosomes. The holocentric chromosomes have multiple kinetochores that cover an important area along their length, rather than a single centromere-specific to monocentric chromosomes. Melters et al. (2012) assumed that the holocentric chromosomes arose at least six times overall in arthropod evolution. The holocentric chromosomes vary from 0.5 to 2.0 µm (Wrensch et al., 1994). *H. gibber* and *O. hermanni* have small holocentric chromosomes (range 0.38-1.38 µm). In Arthropoda, holocentric chromosomes are found in many orders, which are Hemiptera, Lepidoptera, Trichoptera, Dermaptera, Zoraptera, Odonata, Ephemeroptera and Oribatida (White, 1973; Heethoff et al., 2006; Melters et al., 2012). In Oribatida, Eroğlu and Per (2016) reported that *Z. cognata* has small holocentric chromosomes (range 0.46-1.30 µm). In addition, holocentric chromosomes vary from 0.91 to 1.67 µm in *P. lucorum* (Gümüş et al., 2018). Holocentric chromosomes have some advantages. For example, butterflies have holocentric chromosomes, so they are lower sensitivity to radiation infertility than other insect groups (North, 1967). Following radiation application, all the parts separated from the holocentric chromosome do not disappear in the anaphase phase, as they act as separate chromosomes (Lachange, 1967). In addition, adaptation to holocentric chromosomes requires different meiotic adaptations for organisms. These adaptations are asymmetric meiosis, inverted meiosis, and restriction of kinetochore activity (Melters et al., 2012).

The sex chromosomes could not be identified in *H. gibber* and *O. hermanni*. In addition, the oribatid mites show weak sexual dimorphism, which is the condition where the two sexes of the same species exhibit different characteristics beyond the differences in their sexual organs (Behan-Pelletier, 2015). The karyotype formulae and karyotype asymmetries could not be detected, too, because the holocentric chromosomes do not contain long and short arms.

In the present study, new chromosomal data are recorded for two species of the order Oribatida. In this respect, the results of the study provide important contributions to the cytotaxonomy of oribatid mites. Because studies on oribatid mites are very few and limited to a few reports (Norton et al., 1993; Heethoff et al., 2006; Eroğlu and Per, 2016; Gümüş et al., 2018). We think that the main reasons for the limited number of such studies are due to the organism and the method. It is difficult to work with an organism that is small and shows weak sexual dimorphism. Also, although we do have a useful method, we do not have clearer protocols like those of higher plants and animals. In addition, the increase in such studies is important in terms of understanding interspecies relations and karyotype evolution.

## Authors' contributions

**Nisa Gümüş:** Investigation, analysis, formal analysis, visualization, writing - original draft. **Halil Erhan Eroğlu:** Investigation, analysis, conceptualization, data curation methodology, visualization, software, writing - original draft. **Sedat Per:** Project administration, investigation, supervision, methodology, identification, writing - review and editing.

## Statement of ethics approval

Not applicable.


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## Conflict of interest

None.


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RESEARCH NOTE

# Abnormal morphology in *Amblyomma coelebs* and *Amblyomma* cf. *oblongoguttatum* (Acari: Ixodidae) collected on free-roaming Central American Tapir (*Tapirus bairdii*) from Nicaragua

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**ABSTRACT:** In this work we describe abnormalities in *Amblyomma coelebs* and *Amblyomma* cf. *oblongoguttatum* adults collected from *Tapirus bairdii*. The observed anomalies in adults corresponded to general (asymmetry of scutum and idiosoma), and local malformation (atrophy and ectromely). This is the first record of morphological anomalies in *A. coelebs* and also it increases the observations of malformations in ticks from Nicaragua and Central America.

**Keywords:** Ixodidae, *Amblyomma*, abnormalities, Nicaragua.
**Zoobank:** http://zoobank.org/F1DBDB9B-07A4-4F1A-8F38-27041752FD19

Ixodidae comprise 760 species of hard ticks, which are studied for their relevance in public health (Guglielmone and Robbins, 2018; Guglielmone et al., 2021). However, other aspects related to its biology may go unnoticed or be less well known. One of these aspects is the malformations or abnormalities in its morphology, phenomena that have been reported in the genera *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus* (Kar et al., 2015). Abnormalities in the idiosoma are considering as rare and can be observed in any stage (Nuttal, 1914; Guglielmone et al., 1999; Keskin, 2018). In recent years, these phenomena have been reported increasingly, and effort has been made to understand the origin of these malformations, or to assess whether they affect the ability of these ticks to feed and transmit pathogens (Nuttal, 1914; Kar et al., 2015; Keskin et al., 2016).

In Neotropical region, 137 species are known (Guglielmone et al., 2021), in 27 of which abnormalities have been reported (Domínguez et al., 2021). To the best of our knowledge in the genus *Amblyomma* close to 345 cases of malformations have been found in 20 species, specifically in *A. auricularium*, *A. calcaratum*, *A. cajennense* complex, *A. dissimile*, *A. fuscum*, *A. mixtum*, *A. naponense*, *A. neumannii*, *A. oblongoguttatum*, *A.* cf. *oblongoguttatum*, *A. ovale*, *A. parkeri*, *A. parvitarsum*, *A. parvum*, *A. rotundatum*, *A. sabanerae*, *A. tapirellum*, *A. testudinis*, *A. tigrinum*, and *A. varium* (Beaurepaire-Aragao, 1912; Joan, 1919; Brumpt, 1934; Fonseca, 1935; Aguirre et al., 1999; Guglielmone et al., 1999; Labruna et al., 2000, 2002, 2009; Muñoz-Leal et al., 2017; Rivera-Páez et al., 2017; Dantas-Torres et al., 2019; Domínguez and Bermúdez, 2020; Domínguez et al., 2020, 2021). In Central America, abnormalities in Ixodidae were reported from Panama, Costa Rica, and Nicaragua (Domínguez and Bermúdez, 2020; Domínguez et al., 2020, 2021).

**Figure 1.** Dorsal view of festoon malformation in a *Amblyomma coelebs* male.

**Figure 2.** Ventral view of festoon malformation in a *Amblyomma coelebs* male.


**Figure 3.** Dorsal view of *Amblyomma* cf. *oblongoguttatum* female with atrophy on the left 3 (arrow).


**Figure 4.** Ventral view of *Amblyomma* cf. *oblongoguttatum* female with atrophy on the left 3 (arrow).

In this study we report the abnormalities in two species of *Amblyomma* from Nicaragua collected from wild tapirs. Earlier, Arana et al. (2021) collected 91 ticks from seven tapirs (36 *Amblyomma coelebs*, 38 *A.* cf. *oblongoguttatum*, and 17 *A. ovale*). Abnormalities were observed in one *A. coelebs* male and three *A*. cf. *oblongoguttatum* females, and were classified according to Campana-Rouget (1959a,b). The photographs were taken using an Amscope SE306-A stereomicroscope and Amscope 2 mp MD200 USB digital camera (Figs 1 and 2), and a stereomicroscope (Leica M205 A) with digital camera (Leica MC170 HD) (Figs 3-8). The ticks were deposited in the Institute of Natural Resources Environment and Sustainable Development of the University of the Autonomous Regions of the Nicaraguan Caribbean Coast, Nicaragua, and in the Ectoparasites Collection of the "Dr. Eustorgio Méndez" Zoological Collection of the Gorgas Memorial Institute for Health Studies.

A male of *A. coelebs* had festoons abnormalities (Figs 1 and 2). This type of malformation is among the most common (Campana-Roget, 1959b). According to Campana-Roget (1959b) irregularities in the shape, and size of the festoons, and their reduction in their number by fusion and absence, may be due to the atrophy of the posterior part of the idiosoma. Irregular and fused festoons have been reported in *Amblyomma marmoreum*, bumps at the caudal tip in *Rhipicephalus longiceps*, partial absence of festoons in *Rhipicephalus sanguineus* s.l., and *Haemaphysalis leachi* (Campana-Roget, 1959a,b). In Neotropical ticks, these types of irregularities have been reported in species such as *A. mixtum*, *A*. cf. *oblongoguttatum,* and *A. tapirellum* (Nuttal, 1914; Domínguez et al., 2021).


**Figure 5.** Asymmetry of the scutum of *Amblyomma* cf. *oblongoguttatum* female.

Regarding *A*. cf. *oblongoguttatum*, one female presented atrophy of the left leg 3 (Figs 3-5); while that another female exhibited ectromely of the left leg 4 (Figs 6-8). Idiosoma asymmetry is one of the anomalies that occurs most frequently in ticks and is often associated with other anomalies such as ectromely (loss of legs or leg segment) (Robison, 1920; Campana-Rouget, 1959a,b). According to these authors the aforementioned anomalies appear to be caused by uneven distension during the act of engorgement or may be related to an injury during the previous developmental stages. Other explanations indicate that abnormalities are probably the result of abnormal embryonic development, unfavorable molting conditions, and development under high humidity, injury, and abnormal regeneration of tick legs (Beaurepaire-Aragao, 1912; Robinson, 1920; Dergousoff and Chilton, 2007; Keskin et al., 2016).

In conclusion this work presents the first observation of abnormalities in *A. coelebs* by increasing the information of malformations to 30 tick species from Neotropical Regions, 17 of which from Central America and new reports for Nicaragua (Domínguez and Bermúdez, 2020; Domínguez et al., 2020, 2021).

## Authors' contributions

**Lillian Domínguez:** Conceptualization (equal), data curation, visualization (equal), review and editing (lead). **Jeffrey Arana-Espinoza:** Methodology, resources, review and editing (supporting). **Sergio Bermúdez Castillero:** Conceptualization (equal), supervision, visualization (equal), writing - original draft.

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**Figure 6.** Dorsal view of *Amblyomma* cf. *oblongoguttatum* female with ectromely and asymmetry of idiosoma.


**Figure 7.** Ventral view of *Amblyomma* cf. *oblongoguttatum* female with ectromely (arrow) and asymmetry of idiosoma.


**Figure 8.** Asymmetry of the scutum of *Amblyomma* cf. *oblongoguttatum* female.

## Statement of ethics approval

Not applicable.


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## Conflict of interest

The authors declare that they have no conflict of interest regarding this paper.

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RESEARCH NOTE

# Massive infestation of *Tyrophagus putrescentiae* (Astigmata: Acaridae) inside an office in City of Panama, Panama

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**ABSTRACT:** We report the presence of unusual overpopulation of the storage mite *Tyrophagus putrescentiae* in an office workplace, an environment that often does not provide the ideal conditions for the development of these mites. The infestation source was identified as two damaged sachets of spoiled sweetener found under the furniture of the office used for preparing and consuming refreshments.

**Keywords:** Storage mite, cleaning, human buildings.
**Zoobank:** http://zoobank.org/3C74F344-63F7-4BAD-A122-F52F507E1709

The storage mites inhabit human dwellings and habitats associated with food production. They thrive on storage products (hams, cheeses, seeds, dried eggs and fish meal); they mostly belong to the families Acaridae, Glycyphagidae and Chortoglyphidae (Colloff, 2009). Prolonged exposure to their allergens is a risk factor for the appearance of occupational allergy (Sánchez-Borges et al., 2017). These mites cause deterioration in the quality of stored products and can invade laboratory and animal husbandry environments as pests (Fernández-Duro et al., 2014; Silva et al., 2021).

*Tyrophagus putrescentiae* (Schrank, 1781) is a cosmopolitan storage mite that can also be found in soil, vertebrate nests, carrion, barns and in house dust (Fan and Zhang, 2007). It is a medically significant species that can cause skin and respiratory allergies and oral mite anaphylaxis (after intake of food contaminated with mites) (Matsumoto et al., 1996; Sánchez-Borges et al., 2017; Mullen and OConnor, 2019). The aim of this report is to describe a massive infestation of *T. putrescentiae* in a kitchen area in an administrative office in City of Panama, Panama.

In July 2021, the employees at a City of Panama office reported "whitish dust" on a furniture used as a coffee table and a stand for a microwave oven. Biological contamination was suspected after it was observed that the "dust" was moving on its own. The Gorgas Memorial Institute for Health Studies (MED-GMI) Medical Entomology Department was contacted to determine the nature of the infestation.

During the initial inspection, the "bugs" were detected not only on the pantry furniture and on the microwave oven, but also, they were observed on the telephone, an umbrella handle, cup of coffee, a coffee package, and a bottle of alcohol (Figs 1-2). The samples were collected with fine brushes and deposited in vials with 70% ethanol. Through microscopic observation (Nikon SMZ1500, Leica DM750) and photography (Leica ICC50E), it was determined that they were larvae, nymphs, and adults of *T. putrescentiae* (Fig. 3). The specific determination of this species was made by reviewing the characters mentioned by Fan and Zhang (2007) and Klimov and OConnor (2009), through checking the shape of coxal sclerotization II with posterior margin almost straight.

Two days later, another collection was made due to the persistence of these mites in the office, despite efforts to eliminate them. During a third inspection of the infested area, two damaged sachets of spoiled sweetener were found under the kitchen area pantry furniture. After laboratory analysis, all stages of *T. putrescentiae* from eggs to adults were found inside the sachets.

Initially, the affected areas were cleaned with towels moistened with 10% chlorine and 70% alcohol; however, the mites persisted. Subsequently, fumigation by means of spraying with a broad-spectrum insecticide was carried out to control the mite population in the affected kitchen area and their surroundings.

Generally, the chemical control is effective for mites, they have been used acaricides (Cypermethrin), natural botanical extract (Azadirachtin) or biological control with predatory mites in stored food, also heat treatment and moisture levels low for control of storage mite populations (Collins, 2006).

A week later, the area was checked, and no live mites were found. During the following weeks, reviews were conducted and kept in contact with office staff looking for re-established populations but without the success of finding them.

*Tyrophagus putrescentiae* disperses actively or by air currents to new environments and does not form heteromorphic deutonymphs for dispersal by phoresy (Colloff, 2009). Previously, our hypotheses about the overpopulation of this mite could be by more favourable localized conditions, for example, the presence of an insect carcass or small vertebrate (gecko or mouse), the fallen food par-

ticles inside of pantry or on the floor, being a substrate for the micro-fungi on which *T. putrescentiae* feeds (Fig. 1D). This species can complete the development of a new generation in a period of 2 to 3 weeks with food available and optimal conditions of relative humidity (higher than 65%), and a temperature between 25 to 30 °C (Fan and Zhang, 2007; Collins, 2012; Mullen and OConnor, 2019). Mite-infested surfaces sometimes appear to move when viewed with the naked eye due to massive population growth (Zhao et al., 2016).

Generally, it shows low abundance in indoor environments compared with other dust-inhabiting mites such as *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus* and *Blomia tropicalis* (Ree et al., 1997; Arias et al., 2005; Lezcano et al., 2020). In Panama, on rare occasions, it accounts for more than 1% of the total species inside house dust (Estribi et al., 2018).

Until now, there has been no report of this mite causing infestations inside of an administrative office in Panama. There is not enough food or the environmental conditions are suboptimal for mite populations to proliferate in these places. The presence of this species in the office we studied is likely to have been directly related to the accidental deposition of food in areas where effective cleaning is not given.

So, it is necessary to regularly and thoroughly clean areas used for food and drink consumption to avoid the health risks associated with overpopulation and infestations of storage mites.


**Figure 1.** Infestation of *Tyrophagus putrescentiae* in furniture (A) bottle with alcohol 70% (B), cup of coffee (C), and inside pantry furniture (D) in administrative office in City of Panama, Panama.

## Authors' contributions

**Juan José Lezcano:** Visualization (lead), writing - original draft (lead), writing - review and editing (supporting), validation of the species (supporting). **Lyska Castillo:** Visualization (supporting), investigation, methodology (supporting). **Ingrid Lorena Murgas:** Project administration, supervision, writing - original draft (supporting). **Roberto J. Miranda:** Writing - original draft (supporting), methodology (lead), writing - review and editing (lead), validation of the species (lead).

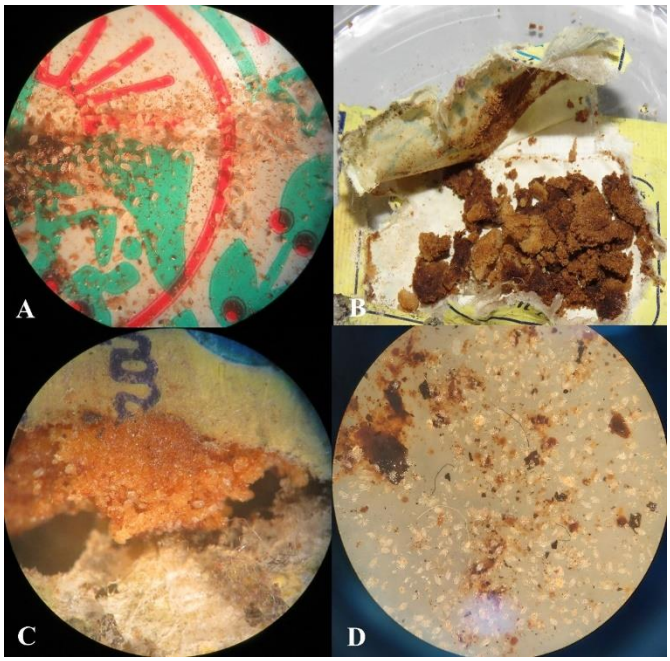


**Figure 2.** Coffee package (A) and sweetener sachets collected in the office (B-D) infested with *Tyrophagus putrescentiae*. Stereomicroscope magnification = 7.5X (A, C and D).

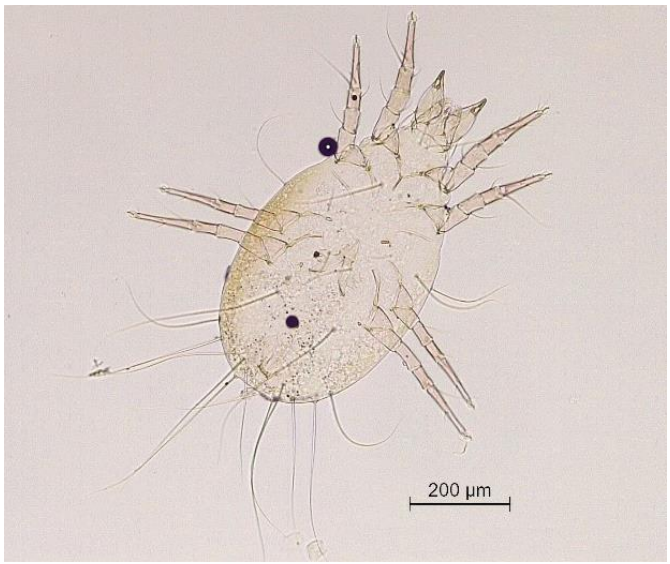


**Figure 3.** *Tyrophagus putrescentiae*, ventral view of female specimen. Scale bar = 200 µm.

## Statement of ethics approval

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## Conflict of interest

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