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RESEARCH ARTICLE

Two parasitic mite species on Phlebotominae sand flies (Diptera: Psychodidae) from Türkiye: *Biskratrombium persicum* (Microtrombidiidae) and *Eustigmaeus johnstoni* (Stigmaeidae)

Metin PEKAĞIRBAŞ[1,6], Mehmet KARAKUŞ[2], Ayda YILMAZ[3], Özge ERİŞÖZ KASAP[3], Sevgi SEVSAY[4], Yusuf ÖZBEL[5], Seray TÖZ[5], Salih DOĞAN[4]

[1]*Department of Parasitology, Faculty of Veterinary, Aydın Adnan Menderes University, Aydın, Türkiye*
[2]*Department of Medical Microbiology, Hamidiye Faculty of Medicine, University of Health Sciences, Istanbul, Türkiye*
[3]*Department of Biology, Ecology Section, Faculty of Science, VERG Laboratories, Hacettepe University, Ankara, Türkiye*
[4]*Department of Biology, Faculty of Arts and Sciences, Erzincan Binali Yıldırım University, Erzincan, Türkiye*
[5]*Department of Parasitology, Faculty of Medicine, Ege University, İzmir, Türkiye*
[6]Corresponding author: metin.pekagirbas@adu.edu.tr

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ABSTRACT: Sand flies (Diptera: Psychodidae) are biological vectors for *Leishmania* species, which are health problems for humans and animals, bacteria such as *Bartonella baciliformis*, and various viruses belonging to the arboviruses such as Phlebovirus, the families Flaviviridae and Reoviridae. They are also known to be in contact with entomoparasites, such as various nematodes, mites and fungi. For a faunistic study, sand flies were collected by CDC light traps set up in various localities within the scope of two separate field studies carried out in Muğla and Adana provinces, Türkiye. During this study, two ectoparasitic mite species, *Eustigmaeus johnstoni* Zhang and Gerson on *Phlebotomus tobbi* Adler, Theodor and Lourie in Muğla province and *Biskratrombium persicum* Majidi, Hahiqanbar and Saboori (Microtrombidiidae) on *Phlebotomus alexandri* (Sinton, 1928) in Adana province, were observed from Türkiye. More detailed studies are needed since there are not enough studies about parasitic mites belonging to different families that can infest sand flies, their hosts, and the damage caused by these mites.

Keywords: Acari, country record, host record, parasitic association, Prostigmata.
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INTRODUCTION

Sand flies (Diptera: Psychodidae) are biological vectors for the species of *Leishmania* which have a health concern for humans and animals, bacteria such as *Bartonella baciliformis*, and various viruses belonging to the arboviruses such as Phlebovirus, Flaviviridae and Reoviridae families (Ready, 2013). They are also known to be in associated with entomoparasites such as various nematodes, mites and fungi (Karakuş et al., 2013; Dinesh et al., 2014). So far, 15 different families of mites have been identified on sand flies, including Microtrombidiidae and Stigmaeidae which are usually attached to the exoskeleton of adult flies and their presence can be determined from visible scars (Majidi et al., 2020). Some of the aforementioned organisms have lethal effects on sand flies, reducing their lifespan or reproductive capacity. There are not enough studies on the mites that affect the life span of sand flies and cause this condition, which can also be defined as superparasitism (Lewis and MacFarlane, 1982; Gerson and Smiley, 1990).

The mite family Stigmaeidae is represented by more than 600 species in the world (Fan et al., 2016, 2019; Beron, 2020). So far, 28 species of the genus *Eustigmaeus* Berlese, 1910 have been reported in Türkiye (Doğan, 2019; Doğan and Doğan, 2020). It has been reported that some species of *Eustigmaeus* only parasitize sand flies (Badakhshan et al., 2013). Currently, six species of *Eustigmaeus*, *E. johnstoni* Zhang and Gerson, 1995, *E. gorgasi* (Chaudhri, 1965), *E. gammus* (Chaudhri, 1965), *E. parasiticus* (Chaudhri, 1965), *E. dyemkoumai* (Abonnenc, 1970), *E. lirellus* (Summers and Price, 1961), have been detected on sand flies in different studies conducted around the world. *Eustigmaeus johnstoni*, whose presence in sand flies was also reported in Türkiye in the previous studies (Özbel et al., 1999; Pekağırbaş et al., 2017), has been reported to parasitize only *Phlebotomus papatasi*, *P. alexandri* and *P. longicuspis* in the world (Shehata and Baker, 1996, Badakhshan et al., 2013., Majidi et al., 2019). In this study, *E. johnstoni* was detected for the first time on a host, an adult female of *P. tobbi*.

Trombidioidea constitutes the largest subgroup of terrestrial Parasitengona. Microtrombidiidae belongs to this superfamily (Wohltman et al., 2006). Some larval members of the family Microtrombidiidae have previously been observed as parasites on sand flies (Majidi et al., 2020). Active postlarval stages (deutonymph and adult) of this family are predators and feed on various arthropods, so they may have economic importance (Robaux, 1974; Welbourn, 1983; Sevsay and Buğa, 2018). Until now, 13 genera belonging to the family Microtrombidiidae have been reported in Türkiye (Sevsay, 2017; Buğa and Sevsay, 2020). The genus *Biskratrombium* is known only from the larva (Fan and Baker, 1993; Majidi et., 2020). These larvae were reported as ectoparasites only from phlebotomine sand flies. Two species belonging to this genus; *Biskratrombium coineaui* larvae were reported as ectoparasites on the adults of *Phlebotomus papatasi* from Algeria and another species *B. persicum* larvae were observed as ectoparasites on the adults of *Phlebotomus*

alexandri, *P. papatasi* and *Sergentomyia mervynae* from Iran (Fain and Izri, 1993; Majidi et al., 2020).

This study was prepared on the parasitic mites observed during a study carried out to show the presence of *Leishmania* parasites by the microscopic and molecular methods in sand flies collected from Adana and Muğla provinces.

MATERIALS AND METHODS

The main material of the study constitutes the mite specimens detected on the sand flies belonging to the genus *Phlebotomus*, *Eustigmaeus johnstoni* and *Biskratrombium persicum*. Ethics committee approval was not required for this study.

Collection and examination of sand flies

CDC (John W. Hock Co., Gainesville, FL, USA) light traps were set up in various localities to collect sand flies as part of two separate field studies carried out in Muğla (Dalaman-Kayadibi- 36°49'15.8"N 28°55'18.3"E) and Adana (İmamoğlu-Otluk- 37° 18' 4.75'' N, 35° 30' 59.83'' E). Light traps were set in the evening (6:00-8:00 PM) and were collected the following morning (07:00-09:30 AM). Sand flies caught alive were taken from the traps with the help of mouth aspirators and transferred to separate resting bowls according to their collection localities. Head and genitalia of the captured sand flies were cut and prepared for species identification. Sand fly specimens were identified according to available morphological identification keys (Lewis, 1982; Killick-Kendrick et al., 1991). Midgut dissection of female sand flies was performed under a stereo microscope (Olympus Co., Japan).

Examination of mite specimens

During the study, the observed mites were removed from the sand fly body with a fine needle and washed in a penicillin-streptomycin solution. The slide-mounting was done in Swan solution (chloral hydrate-50 gr/acetic acid-5 ml/Arabic gum-15 gr/glucose-3 gr, water-20 ml) (Arserim et al., 2017). The mites detected on the sand flies during midgut dissection were also identified according to their morphological characters. Identification of the specimens was carried out as described by Fain and Izri (1993), Zhang and Gerson (1995), and Majidi et al. (2020).

RESULTS AND DISCUSSION

Order Trombidiformes Reuter, 1909

Suborder Prostigmata Kramer, 1877

Superfamily Raphignathoidea Kramer, 1877

Family Stigmaeidae Oudemans, 1931

Genus *Eustigmaeus* Berlese, 1910

***Eustigmaeus johnstoni* Zhang and Gerson, 1995**

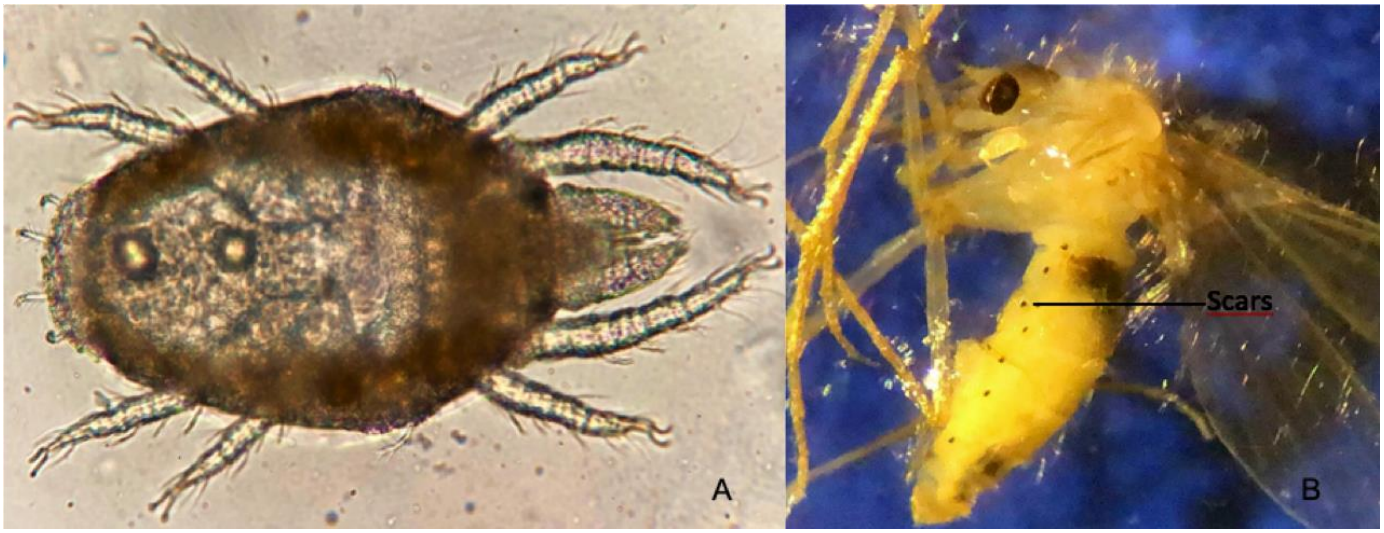

Figure 1. A. Dorsal view of *Eustigmaeus johnstoni* (female), 400x, **B.** Scars on the lateral sides of the abdominal sternites belongs to a *Phlebotomus tobbi* individual, 40x.

Sand flies collected during field studies in Adana were dissected for species identification. During the dissection, mite specimens were detected on some sand flies and were examined. Accordingly, three adult females of *Eustigmaeus johnstoni* (Fig. 1A) were identified on the thorax of the adult female *Phlebotomus tobbi* during the field study in Adana. The scars caused by *E. johnstoni* on the lateral side of the abdominal tergites belongs to *P. tobbi* individual were viewed under a stereo microscope (Fig. 1B).

The morphological features of the specimens from Türkiye are completely similar to those of the known specimens of *E. johnstoni*. Since the description of the species is well and it has been previously recorded and described in Türkiye, the description has not been repeated here.

Host information

Phlebotomus papatasi, *S. magna*, *S. dreyfussi*, *S. africana*, *P. longicuspis*, *P. bergeroti*, *P. sergenti*, *Sergentomyia clydei*, *S. sintoni* (Zhang and Gerson, 1995; Fan et al., 2016), and newly recorded host *P. tobbi* (present study).

Distribution

Cyprus, Egypt, India, Iran, Israel, Pakistan, Saudi Arabia, Spain, Tunisia, Türkiye and Yemen (Zhang and Gerson, 1995; Shehata and Baker, 1996; Özbel et al., 1999; Badakhshan et al., 2013; Pekağırbaş et al., 2017; Doğan, 2019; Majidi et al., 2019).

Order Trombidiformes Reuter, 1909

Suborder Prostigmata Kramer, 1877

Superfamily Trombidioidea Leach, 1815

Family Microtrombidiidae Thor, 1935

Genus *Biskratrombium* Fain and Izri, 1993

***Biskratrombium persicum* Majidi, Hajiqanbar and Saboori, 2020**

In the study conducted in Muğla, the presence of a larva of *Biskratrombium persicum* (Fig. 2) was determined as ectoparasitic on adult female *Phlebotomus alexandri.* The specimen form Türkiye is identified as *Biskratrombium persicum* by the presence of eupathidia on palpal tarsus; the longest seta on palpal tarsus is not a solenidion; scutum, scutellum and platelets smooth; setae on scutellum placed in the middle. Scutum's lateral borders slightly concave.

Figure 2. *Biskratrombium persicum* (larva).

Short description

Cheliceral blade curved and simple. Palpfemur and palpgenu without seta. Eyes absent. Scutum trapezoidal in shape; lateral borders slightly concave, posterior border convex, slightly concave at anterior border. Scutum with three pairs of non-sensillary (*AM*, *AL* and *PL*) and one pair of sensillary setae (*S*). The width of scutellum less than the scutum, bearing one pair of barbed setae c_1. Legs segmentation formula: 6–6–6. Tarsi I and II each bearing only two trifurcate claws, while tarsus III with two trifurcate claws and a claw-like empodium.

Host information

Phlebotomus papatasi, *P. alexandri* and *Sergentomyia mervynae* (Majidi et al., 2020).

Distribution

Iran (Majidi et al., 2020) and Türkiye (present study).

DISCUSSION

Various studies have been carried out on the distribution of the genus *Eustigmaeus* in the world (Fan et al., 2016, 2019; Beron, 2020). To date, *E. dyemkoumai*, *E. gamma*, *E. gorgosi*, *E. parasitica*, *E. johnstoni* and *E. lirella* species have been detected on sand flies. In previous studies, it has been reported that *Phlebotomus alexandri*, *P. papatasi*, *P. bergeroti*, *P. longicuspis* and *P. sergenti* were hosts for *E. johnstoni* (Chaudri, 1965; Abonnenc, 1970; Lewis and Macfarlane, 1982; Zhang and Gerson, 1995; Ozbel et al., 1999; Badakhshan et al., 2013; Pekağırbaş et al., 2017; Majidi et al., 2019). In this study, the presence of *E. johnstoni* was reported for the first time on adult female of *Phlebotomus tobbi*, which is known to be the proven vector of *L. infantum* in Türkiye and shows a high attraction for sucking blood from humans (Svobodova et al., 2009). *Eustigmaeus* species have been detected on proven vector sand fly species of different *Leishmania* parasites such as *P. alexandri*, *P. papatasi*, *P. sergenti*, and *P. longicuspis* (Zhang and Gerson, 1995; Yaghoobi-Ershadi et al., 2005; Azizi et al., 2006, Berdjane-Brouk et al., 2012; Fan et al., 2016), and in this study showing the presence of *Eustigmaeus johnstoni* on *P. tobbi* that is also proven vector species of *L. infantum*. These findings suggest the possibility that *Eustigmaeus* species may show tropism to sand flies that are vectors for *Leishmania* parasites.

Sand flies spend most of their lives in dark and damp areas such as animal shelters, wall cracks and crevices, and rodent burrows (Dinesh et al., 2014). Since immature *Eustigmaeus* has not been reported on sand flies so far, it is thought that the development and mating of the abovementioned mite took place in these habitats. The relationship between the mite-fly pair encountered in these habitats is supported by few publications showing the scars found on the sand flies' bodies (Badakhshan et al., 2013; Dinesh et al., 2014). Although there are different opinions about whether the relationship between *Eustigmaeus johnstoni* and sand flies is parasitic or phoretic (Badakhshan et al., 2013), the scars on the bodies of the sand flies shown in the present study indicate parasitism.

Parasitic association between the parasitengone mites and the sand flies is little reported probably due to low frequency of the phenomenon (Majidi et al., 2020). Although five microtrombidiids species, *Microtrombidium hindustanicum*, *M. nigeriense*, *M. lewisi*, *Kenyatrombium macfarlanei*, *Biskratrombium coineaui*, as ectoparasites have been detected on the sand flies, only one species belonging to the genus *Biskratrombium* has been detected so far in the Phlebotominae sand flies around the world (Hirst, 1926; Fain and Baker, 1993; Fain and Izri, 1993). *Biskratrombium persicum* was described by Majidi et al. (2020) as ectoparasite on *P. alexandri*. In this study, the presence of a larval stage, ectoparasitic of adult female *P.*

alexandri which is most third prevalent *Phlebotomus* species in Muğla (Pekağırbaş et al., 2021) belonging to the genus *Biskratrombium* was reported for the first time in Türkiye.

There is a need for more detailed studies on issues that lack information, such as the damage caused by parasitic mites from different families infesting sand flies in different parts of the world and their relations with the host.

Authors' contributions

Metin Pekağırbaş: Field studies, dissection for sand flies, writing-original draft. **Mehmet Karakuş:** Field studies, dissection for sand flies writing-review & editing. **Ayla Yılmaz:** Field studies, dissection of sand flies, identification of sand flies, writing-review. **Özge Erişöz Kasap:** Field studies, dissection of sand flies, identification of sand flies, writing-review. **Sevgi Sevsay:** Identification of the mites, writing-review & editing. **Seray Töz:** Field studies, dissection of sand flies, identification of sand flies, writing-review. **Yusuf Özbel:** Identification of sand flies and editing of manuscript. **Salih Doğan:** Identification of the mites, writing-review & editing.

Statement of ethics approval

Not applicable.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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