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First record of *Diaeretus leucopterus* (Haliday) (Hymenoptera, Braconidae, Aphidiinae), the parasitoid of the aphid species, *Eulachnus agilis* (Kaltenbach) (Hemiptera, Aphididae) in North Africa

Monia Ben Halima Kamel¹, Nickolas G. Kavallieratos², Petr Stary³ and Ehsan Rakhshani^{4*}

Abstract

A survey of pine-associated aphids and their parasitoids was carried out in the arboretum of *Pinus halepensis* Miller in the Higher Agronomic Institute Chott Mariem (ISA CM, Tunisia) during 2010–2011 and 2018. The survey revealed the presence of the aphid parasitoid species, *Diaeretus leucopterus* (Haliday) (Hymenoptera, Braconidae, Aphidiinae). This is the first record of *D. leucopterus* from Tunisia and the African continent. The parasitoid emerged from the spotted green pine needle aphid, *Eulachnus agilis* (Kaltenbach) (Hemiptera, Aphididae, Lachninae), on *P. halepensis*. The generalist hyperparasitoid, *Asaphes suspensus* (Nees) (Hymenoptera, Pteromalidae), was also found attacking *D. leucopterus*. The diagnosis of the primary parasitoid, on the basis of relevant illustrations, was provided, and the possible routes of its transportation into North Africa were discussed.

Keywords: Pine aphids, *Eulachnus agilis*, *Diaeretus leucopterus*, Hyperparasitoids, Tunisia

Background

Pinus spp. are the largest genus of the coniferous plants in the northern hemisphere. They include many important species from the economic and ecologic point of views (Gernandt et al. 2005). Three pine tree species are common in Tunisia, including the Aleppo pine, *Pinus halepensis* Miller; the maritime pine, *P. pinaster* Aiton; and the stone pine, *P. pinea* L. The first two species are naturally grown in a wide area of Tunisia. *P. halepensis* consists of the main forest element of Tunisia that encompasses about 297,000 ha, i.e., 57% of the total forest area of the country (Khouja 1997). *P. pinea* forests are found along the coasts of Tunisia, in about 27,000 ha, after plantation of dunes between Tabarka and Kelibia.

Although several insect species are associated with the pine forests globally, some of them have been primarily raised as economic pests. Aphids of the genus *Eulachnus* del Guercio (Hemiptera, Aphididae, Lachninae) have a significant anti-aesthetic impact on the urban areas, where pine species are grown as ornamental and shading plants (Morris 2006). *Eulachnus* aphids are widely distributed outside their original sources, due to the massive transportation of infested pine stock through international trade (Richardson 1998). Although *Eulachnus* spp. have a Palae-arctic origin, 3 species (*Eulachnus agilis* (Kaltenbach), *E. brevopilosus* Börner, and *E. rileyi* (Williams)) have invaded North and South America (Blackman and Eastop 1994). The majority of these aphids are effectively controlled in their native areas of distribution mainly due to the activity of their natural enemies. However, they become important pests in the absence of their natural enemies in the areas

* Correspondence: rakhshani@uoz.ac.ir; erakhshani@gmail.com

⁴Department of Plant Protection, College of Agriculture, University of Zabol, P.O. Box 98615-538, Zabol, Islamic Republic of Iran
Full list of author information is available at the end of the article

of invasion. Therefore, once established, they cause serious damages to seedling and mature pine trees (Alford 2012).

Four *Eulachnus* species, i.e., *E. agilis*, *E. nigricola* (Pašek) (Ben Halima Kamel 2012; Kanturski et al. 2017), *E. rileyi* (Boukhris-Bouhachem et al. 2007), and *E. tuberculostemmatus* (Theobald) (Ben Halima Kamel et al. 2019) have been recorded in Tunisia. Although the complex of natural enemies of *Eulachnus* spp. is well studied in the Mediterranean countries (Núñez-Pérez and Tizado 1996; Kavallieratos et al. 2004), there are no data from Tunisia and other North African countries. Till now, only generalist coccinellids have been recorded that associated with *Eulachnus* spp. in this region (Ben Halima Kamel et al. 2018).

In the present study and through a survey of the pine-associated aphids and their parasitoids carried out in Tunisia, the aphid parasitoid species, *Diaeretus leucopterus* (Haliday) (Hymenoptera, Braconidae, Aphidiinae), was recorded for the first time. Its diagnosis and the possible routes of its transportation into North Africa were discussed.

Material and methods

The materials were collected from the arboretum of *P. halepensis* in the Higher Agronomic Institute Chott Mariem (ISA CM, Tunisia) during spring (2010, 2011, 2018). Pine needles were inspected in situ for aphid colonies (*Eulachnus* spp.). Once detected, needles, that carried aphids, were carefully cut off and subdivided into plastic boxes with a dimension of 100 × 150 mm. Subsequently, boxes were covered by muslin to allow ventilation of the content. Few adult aphids were separately preserved in 70% ethanol for later identification. The collected materials were maintained for 2–3 weeks under laboratory conditions at 25 °C and inspected daily for the emergence of adult parasitoids. The remaining material was kept for 2 additional weeks at the same conditions to rear any secondary parasitoids. The emerged parasitoids were carefully captured, using an aspirator and dropped into 75% ethanol. Female parasitoids were dissected and slide-mounted in the Hoyer medium. The slide specimens were studied under a Nikon® Eclipse E200 microscope (Nikon Corporation, Japan). Photos from the slides were captured by a Canon® EOS 700D camera (Canon Inc., Japan) and used to prepare line drawings in the Adobe® Illustrator CS5. A card-mounted specimen was imaged by using the Canon® EOS 700D, mounted with an adapter on Hund® stereomicroscope (Wetzlar Inc., Germany). A series of 10–15 multi-focused captured photographs were subsequently merged into a single in-focus image by using the Zerene Stacker version 1.04 software (Zerene Systems LLC, 2017). The morphological terminology used in this study follows that of Sharkey and Wharton (1997) for

parasitoids and Blackman and Eastop (2016) for aphids. Parasitoid specimens are deposited in the collection of the Department of Plant Protection, University of Zabol (DPPZ), Iran.

Results and discussion

The aphid parasitoid species *D. leucopterus* emerged from mummies of *E. agilis*.

Taxonomic account

Order: Hymenoptera Linnaeus, 1758

Family: Braconidae Nees, 1811

Subfamily: Aphidiinae Haliday, 1833

***Diaeretus leucopterus* (Haliday, 1834)**

Aphidius leucopterus Haliday, 1834. *Entomological Magazine*, 2:93–106.

Material examined: 3♀ 1♂, ex. *Eulachnus agilis* (Kaltenbach), on *Pinus halepensis* Miller, during April 2010, 2011, and 2018, Tunisia, ISA CM (35° 54' 58" N, 10° 33' 36" E).

Diagnosis: (female—Fig. 1). Body length 1.7–1.9 mm. Head sparsely setose, wider than thorax at tegula. Eyes medium sized. Malar space 0.25–0.30 times longitudinal eye diameter. Tentorial index 0.6–0.7. Clypeus flat, with 4–6 long setae. Maxillary palpi four-segmented, labial palpi 2-segmented. Antenna filiform, 16-segmented. Mesoscutum (Fig. 2a) smooth and shiny with rows of sparse setae along the trace of effaced notaulices. Forewing (Fig. 2b) stigma triangular, its length 2.4–2.6 times its maximum width, and 1.9–2.1 times R1. Vein r&RS short, not reaching the end of stigma, its length 0.85–0.95 times R1. Propodeum (Fig. 2c) smooth with the anterolateral and central carinae which extended near the spiracles. Petiole (Fig. 2d) stout, slightly constricted after spiracles, then parallel sided, 3 times its width at spiracles. Gaster lanceolate. Ovipositor sheath (Fig. 2e) stout, sub quadrate and apically truncated. Recorded diagnostic characters agree with that reported by Kaliuzhna (2014).

Coloration: Generally dark, head and mesosoma black, gaster dark brown. Antenna brown, with dark brown scape and pedicel. Legs light brown with yellow patches at tip of segments. Mummified aphids of yellowish brown color were mainly attached to the underside of the needles (Figs. 3a, b). Parasitoids were emerged 7 days post-collection by cutting a circular hole at the posterior part of the mummy above the cauda (Fig. 3c).

The hyperparasitoid *Asaphes suspensus* (Nees) (Hymenoptera, Pteromalidae) was also emerged from the mummified aphids (2♀ 3♂, same data as for the parasitoid, April 2010 and 2011). This species was already recorded in North Africa in Morocco (Vago 2002). It is a generalist hyperparasitoid of numerous aphid species excluding pine aphids (Gibson and Vikberg 1998). The emergence hole of the adult hyperparasitoid, with irregular margins, is distinctly smaller than the corresponding

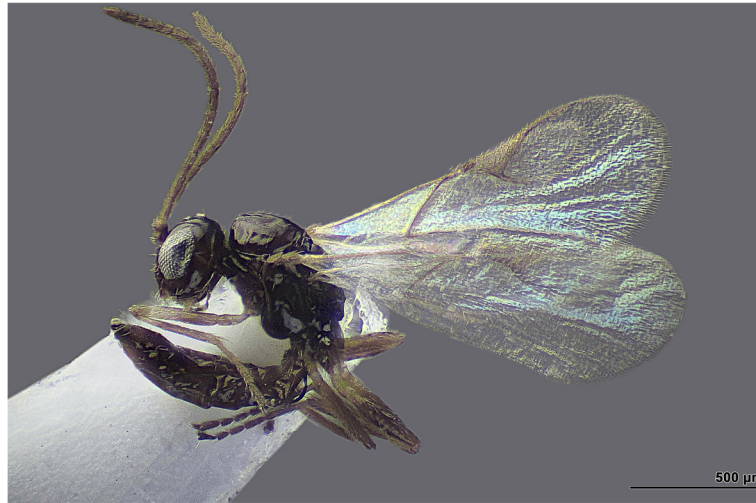


Fig. 1 *Diaeretus leucopterus* (Haliday), female, lateral aspect of habitus

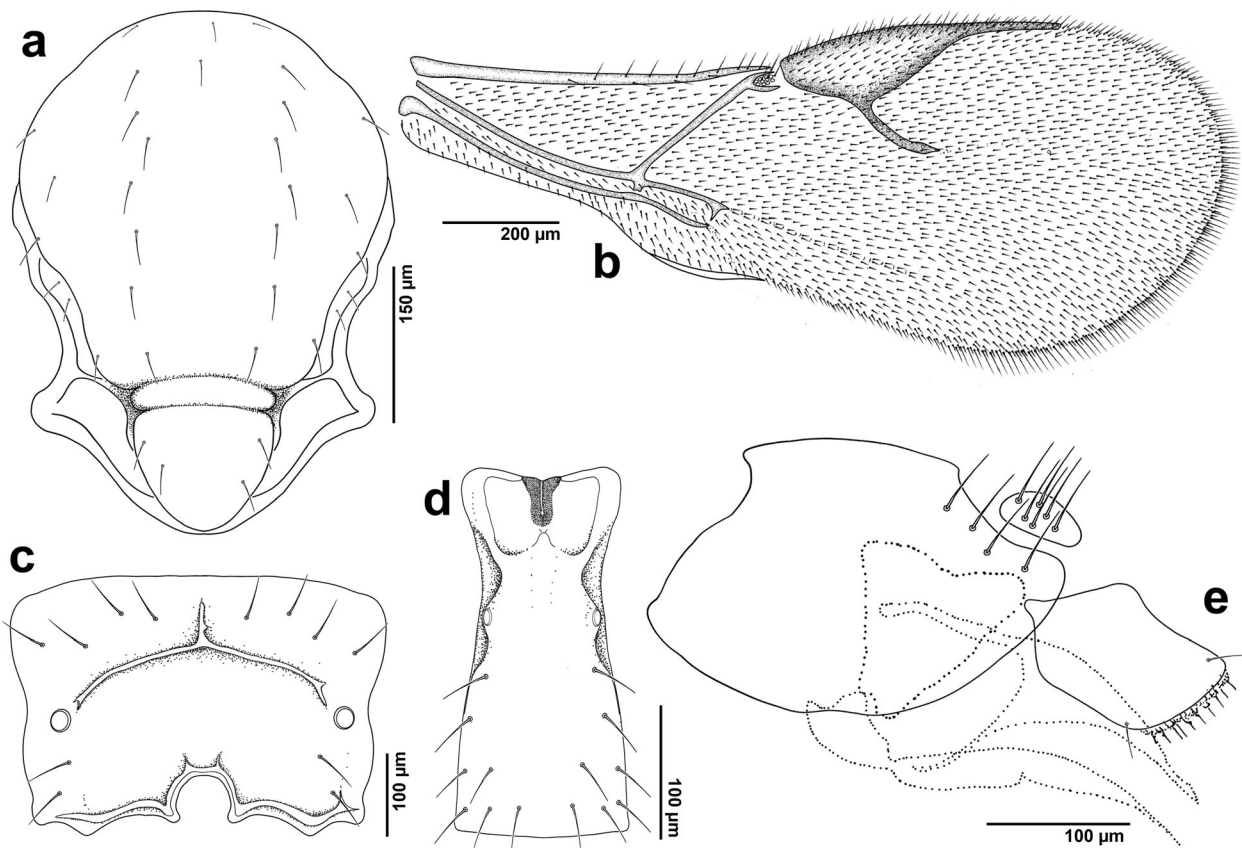


Fig. 2 *Diaeretus leucopterus* (Haliday), female. **a** Mesonotum, dorsal aspect. **b** Forewing. **c** Propodeum, dorsal aspect. **d** Petiole, dorsal aspect. **e** Genitalia, lateral aspect. (After Rakhshani et al. 2019)



Fig. 3 Parasitized individuals of *Eulachnus agilis* (Kaltenbach). **a, b** Matured mummified aphid parasitized by *Diaeretus leucopterus* (Haliday). **c** Mummified aphid with emergence hole by the adult parasitoid at the postero-dorsal area. **d** Emergence hole of a hyperparasitoid, *Asaphes suspensus* (Nees), at the antero-dorsal area of the mummy

hole of the primary parasitoid and cutted at the antero-dorsal area of the mummy (Fig. 3d). Another species, *Asaphes vulgaris* Walker (Hymenoptera, Pteromalidae), was also recorded as a hyperparasitoid of *D. leucopterus* via *E. rileyi* (Suay Cano et al. 1998). Further records regarding the associations of the generalist hyperparasitoids of the genus *Dendrocerus* Ratzeburg (Hymenoptera, Megaspilidae) with *D. leucopterus*, both in Eastern (Takada 1973) and Western (Starý 1977) Palaearctics, indicate the presence of diverse food webs rooted on *Eulachnus* spp. to be explored by subsequent studies.

Diaeretus leucopterus was recorded for the first time not only in Tunisia but also in the African continent in association with *E. agilis* on *P. halepensis*. *Diaeretus leucopterus* is a widely distributed species in the Western Palaearctic, including some European countries (Mackauer and Starý 1967; Kavallieratos et al. 2004; Kaliuzhna 2014) and some countries of the Eastern Palaearctic (Starý and Schlinger 1967; Takada 1968; Chen and Shi 2001; Starý et al. 2010) or Oriental (Starý and Ghosh 1983). Several Mediterranean countries, including France, Greece, and Italy, have been recorded as common areas of distribution of *D. leucopterus*, from where it might be accidentally introduced into the North African area (Tunisia). According to Sghaier and Clautriaux (2015), *P. halepensis* is of mixed origin since some *P. halepensis* forests are assumed to originate

from the nearby countries, i.e., Africa (Morocco), Southern Europe (Italy), and Western Asia (Israel).

Siphonatrophia cupressi (Swain) (Hemiptera, Aphididae, Aphidinae) has been recently recorded in Tunisia on *Cupressus sempervirens* L. parasitized by an unknown parasitoid species (Ben Halima Kamel and Mdellel 2017). Since both aphids (*E. agilis* and *S. cupressi*) and their host plants (*P. halepensis*, *C. sempervirens*) were found in the same area (ISA CM), the association of *D. leucopterus* with *S. cupressi* seems expectable. It should be noted that the 2 aphid genera, *Eulachnus* and *Schizolachnus* Mordvilko (Hemiptera, Aphididae, Lachninae), were recorded as hosts of *D. leucopterus* in the Mediterranean area (Kavallieratos et al. 2004). However, further field efforts are necessary to confirm this hypothesis. Few host records of *D. leucopterus* including *Mindarus abietinus* Koch (Hemiptera, Aphididae, Mindarinae) (Čapek 1960) and *Brachycaudus cardui* (L.) (Hemiptera, Aphididae, Aphidinae) (Fulmek 1968) look doubtful and need verification. Although some other coniferous-associated aphid species, mainly of the genus *Cinara* Curtis are present in Tunisia (Ben Halima Kamel 2012; Ben Halima Kamel et al. 2019), they have not been found parasitized yet (Rakhshani et al. 2019). One other European parasitoid of *Eulachnus* aphids, *Praon bicolor* Mackauer (Hymenoptera, Braconidae, Aphidiinae) (Kavallieratos et al. 2004), was

found in the Mediterranean counties (Tremblay 1975; Núñez-Perez and Tizado 1996). It is an uncommon species, while its occurrence in North Africa has not been documented yet. The Nearctic aphids of the genus *Essigella* del Guercio are biogeographically associated with pine trees in North America (Blackman and Eastop 1994; Théry et al. 2018). *Diaeretus essigellae* Starý & Zuparko (Hymenoptera, Braconidae, Aphidiinae) was described from *Essigella californica* (Essig) (Hemiptera, Aphididae, Lachninae) on *Pinus* spp. in California (Starý and Zuparko 2002). It is closely related to *D. leucopterus*, but it is immediately separated by the absence of propodeal carinae. However, it is rather unlikely to occur in Tunisia, since pine seedlings are not transported from North America to Tunisia. A comparison of both *Diaeretus* species, using DNA sequences, can provide further data about the identity of these taxa and their associated host aphids.

Conclusion

Both *Eulachnus* aphids and their parasitoid, *D. leucopterus*, seem to be invasive species in North Africa (Tunisia). Considering the economic importance of these pest aphids, further investigation in the neighboring countries is needed to reveal the expansion of the parasitoid and to confirm its possible establishment.

Abbreviations

DPPZ: Department of Plant Protection, University of Zabol; ISA CM: Institut Supérieur Agronomique Chott Mériem

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Authors' contributions

MKBH reared the specimens and identified the aphid specimens. NGK identified the aphid parasitoid specimens, revised the structure and manuscript and did the English grammar editing. PS identified the parasitoid specimens. ER identified the aphid parasitoid and hyperparasitoid specimens, and he was the major contributor in writing the manuscript and preparation of illustrations. The authors read and approved the final manuscript.

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Availability of data and materials

All the mentioned specimens are deposited in the DPPZ collection and are available.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Cultures maraichères Biologiques et conventionnelles, Institut Supérieur Agronomique, Université de Sousse, 4042 Chott Mariem, Sousse, Tunisia.

²Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos str., 11855 Athens, Attica, Greece. ³Laboratory of Aphidology, Institute of Entomology, Biology Center AVCR, Branišovska 31, 370 05, České Budějovice, Czech Republic.

⁴Department of Plant Protection, College of Agriculture, University of Zabol, P.O. Box 98615-538, Zabol, Islamic Republic of Iran.

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