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Expansion in the distribution of *Pauesia silana* Tremblay (Hymenoptera, Braconidae, Aphidiinae), across North Africa, a recent discovery in Tunisia

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ABSTRACT. The occurrence of *Pauesia silana* Tremblay, as an aphid parasitoid (Hym., Braconidae, Aphidiinae) in Tunisia is documented. The parasitoid was found in association with the Aleppo pine aphid, *Cinara palaestinensis* Hille Ris Lambers, infesting *Pinus halepensis*. Specimens were collected by the rearing of the mummified aphids from the colonies infested the pine trees in the Arboretum of the Institut Supérieur Agronomique Chott Mériem (ISA CM - Tunisia) during March–April 2021. A brief diagnosis is provided for the recorded parasitoid. This is the first record of a *Pauesia* species in Tunisia (out of the purposeful introduction of *Pauesia antennata* Mukerji). Two secondary parasitoids including *Asaphes vulgaris* Walker and *Pachyneuron aphidis* (Bouché) (Hym., Chalcidoidea, Pteromalidae) have also emerged from the mummified aphids. The known *Cinara* aphids and their associated parasitoids in the North African country are reviewed. Both Aleppo pine aphid and the newly detected parasitoid might be considered exotic species in North Africa, sourced from a recent accidental introduction inside the Mediterranean area, or a horizontal expansion across the North African countries.

Key words: Conifers, pest aphid, invasive species, biological control, parasitoids

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INTRODUCTION

The coniferous plants (Pinophyta, Pinopsida, Pinales), consist of eight families and about 630 species, among them, the genus *Pinus* (Pinaceae) represented the largest taxon with about 126 species mostly in the northern hemisphere (Farjon et al., 2019). *Pinus* spp. are known as very important elements of the

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environment, affecting the amount of CO2 (Quiroz Dahik et al., 2021), food webs (Filipiak, 2016), and wood production (Le Maitre, 1998). Among the common pine tree species in Tunisia, the Aleppo pine, *Pinus halepensis* Miller, has grown in a wide area of about 57% of the total forests and occupied the main forest element of Tunisia (Khouja, 1997).

Various species of the Lachninae aphids (Hemiptera, Aphididae) belonging to the genera *Eulachnus* Del Guercio, 1909 (Ben Halima Kamel et al., 2019) and *Cinara* Curtis, 1835 (Ben Halima Kamel, 2012) are known in association with Pine trees in Tunisia, as well as in the other North African countries (Sekkat, 1987; Boukhris-Bouhachem et al., 2007; Laamari et al., 2010, 2013; Ben Hamacha et al. 2017; Ayache et al., 2020). The aphids generally cause a considerable anti-aesthetic impact on the ornamental and shading pine trees in urban areas (Morris, 2006). Various strategies include chemical control (Insecticides) (Kearby & Bliss, 1969; Canakcioglu, 1970), as well as management of resistance (Wu et al., 2017), and getting benefits from the biological control agents (Cardoso & Lázzar, 2003; Kfir et al., 2003; Kairo & Murphy, 2005; Rosagro et al., 2020; Kamanga-Thole et al., 2021) were the matter of investigation to find a solution for reducing the aphid populations. Both predators and parasitoids were already recorded in association with the pine aphids in Tunisia (Ben Halima Kamel et al., 2018; Ben Jamâa et al., 2020) and some other African countries (Mills, 1990). *Cinara palaestinensis* Hille Ris Lambers, 1948, a widely distributed aphid, has been known in association with *Pinus halepensis* in Tunisia since several years ago (Ben Halima Kamel, 2012) with no record of the associated parasitoid.

The aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) are one of the most important biological control agents both in the course of conservation programs and purposeful introduction into the new areas for control of invasive pest aphids (Rakhshani & Stary, 2021). A rich assemblage of the parasitoids, associated with the Lachninae aphid mainly belonging to the genus *Pauesia* is known from the Mediterranean area (Stary, 1960; 1976; Mifsud & Stary, 2009), Southern (Tremblay, 1969; Stary et al., 1971; Tremblay, 1975) and Southeastern Europe (Kavallieratos et a., 2004), where large transportation is occurring into the North African countries, emphasizing on Tunisia. In comparison, the review of the aphid parasitoids in the Middle East and North African countries (Rakhshani et al., 2019) represents a few *Pauesia* species attacking *Cinara* species mainly the same Mediterranean elements. The majority of *Cinara* aphids have been considered invasive species imported along with the seedlings of their host plants. Considering the significance of the biological control programs against invasive aphids (Stary et al., 2005; Mdellel et al., 2015; Adouani et al., 2017), it is important to increase the knowledge about the distribution of their known parasitoids, area of origin and possible routes of the accidental introduction. Our recent surveys on the natural enemies of Lachninae aphids led to the discovery of a *Pauesia* species associated with *Cinara palaestinensis* on *Pinus halepensis*, representing the first parasitoid of *Cinara* aphids in Tunisia. The occurrence of this parasitoid species in Tunisia along with the other North African countries is highlighted. A brief diagnosis on the basis of the relevant morphological characters and the possible routes for its accidental or purposeful introduction into the new areas is also provided.

MATERIAL AND METHODS

Samples were collected from the Aleppo pine trees, *Pinus halepensis* in the Arboretum of the Institut Supérieur Agronomique Chott Mériem (ISA CM - Tunisia) from March-April 2021. The infested trees with colonies of *Cinara* aphids were inspected for the mummified individuals. Once detected, the mummies were carefully picked up and placed inside the gelatin capsules. The infested branches were also carefully cut at the point far from the aphid colonies using a gardening scissor, put inside the plastic bags and transferred to the laboratory. Few live aphids were separately collected and preserved in 70% ethanol for later identification. The cut branches were placed into the glass tube containing water and caged inside the mesh ventilated plexiglass containers (60 cm × 60 cm), at 21°C ± 1°C, a photoperiod of 16:8 (L:D) hours and relative humidity of 60 ± 10% (Mdellel et al., 2015). The gelatin

capsules containing the mummies and the caged plant material with living aphids were maintained for 3–5 weeks and inspected daily for the emergence of adult parasitoids and hyperparasitoids. The emerged parasitoids were carefully captured, using an aspirator and dropped into 75% ethanol. In order to examine the morphological details, two female parasitoid specimens were dissected and slide-mounted in Hoyer medium. The rest of the material were treated according to AXA protocol (van Achterberg, 2009), afterwards properly dried on a piece of blotter paper and glued on a triangular card. The slide specimens were studied under a Nikon Eclipse E200 microscope (Nikon Corporation, Japan). The identity of specimens was compared with the species keyed in Rakhshani et al. (2019). The morphological terminology used in this study follows that of Sharkey and Wharton (1997). Photographs were captured by a Canon EOS 700D camera (Canon Inc., Japan), mounted with an adapter on the Nikon Eclipse E200 microscope. A series of 40–60 multi-focused captured photographs of dried specimens were subsequently merged into a single in-focus image by using Helicon Focus image stacking software ver. 7.6.6. (Helicon Soft, Ltd). A distribution map for the parasitoid species is generated in SimpleMappr (Shorthouse, 2010) on the basis of data compiled from Yu et al. (2016) and subsequently published literature. Parasitoid specimens are deposited in the collection of the Department of Plant Protection, University of Zabol (DPPZ), Iran.

RESULTS

Rearing the *Cinara* aphid material led to the emergence of a primary parasitoid (Hymenoptera, Braconidae, Aphidiinae), which was identified as *Pauesia silana* Tremblay, 1969, representing a new species record from Tunisia.

Taxonomic account

Order Hymenoptera Linnaeus, 1758

Family Braconidae Nees, 1811

Subfamily Aphidiinae Haliday, 1833

Genus *Pauesia* Quilis, 1931:67. Type species: *Pauesia albuferensis* Quilis, 1931 by original designation.

Pauesia silana Tremblay, 1969

Pauesia silana Tremblay, 1969:153–160. Holotype ♀. – Italy

(Figs 1–2)

Material examined: 7♀♀ 4♂♂ (DPPZ), ex. *Cinara palaestinensis* Hille Ris Lambers, 1948, on *Pinus halepensis* Miller, Tunisia, ISA CM (35°54'58"N, 10°33'36"E), 1♀ 1♂, 25.03.2021; 1♀ (on slide) 1♂, 26.03.2021; 4♀♀ (1♀ on slide) 1♂, 22.04.2021; 1♀ 1♂ (partly damaged), 29.04.2021, leg. M. Ben Halima Kamel.

Diagnosis (Female – Fig. 1)**.** Body length 2.9–3.1 mm. Head (Fig. 1A) sparsely setose, wider than thorax at tegula. Eyes medium sized. Malar space 0.45–0.50 times longitudinal eye diameter. Maxillary palpi with four palpomeres, labial palpi with three palpomeres. Antenna (Fig. 1B) filiform, flagellum with 17–18 flagellomeres. Mesoscutum smooth and shiny, slightly punctulated at dorso-lateral areas. Notauli poorly developed, present on anterior part of mesoscutum, with slightly different sculpturing marking their posterior ends among the rows of sparse setae. Forewing (Fig. 1C) stigma distinct, wide triangular, its length 2.0–2.1 times its maximum width, and 0.88–0.90 time R1. Vein r long, 1.3–1.4 times 3RSa and 2.3–2.4 times as long as r-m vein. Propodeum (Fig. 1E) with anterolateral carinae at the extreme apex but the posterolateral area never bordered. Petiole (Fig. 1E) elongated, with constriction behind its spiracles, 3.40–3.55 times its width at spiracles. Gaster elongated, expanded toward the tip. Ovipositor sheath (Fig. 1F) short, 2.0–2.15 times its maximum width at base, apically truncated, dorsal and anterio-ventral sides of ovipositor sheath, each with three and two long and curved setae.

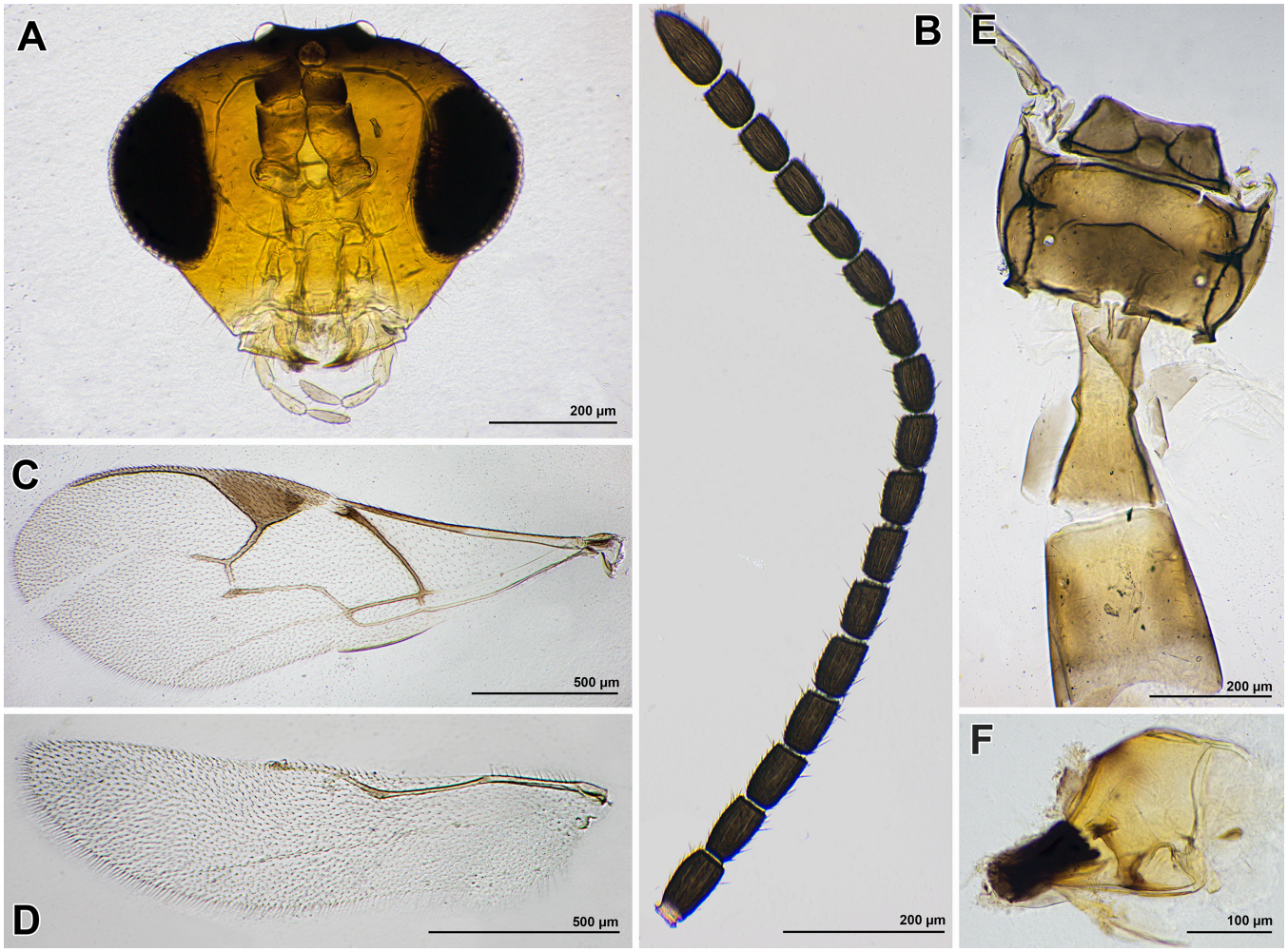

Figure 1. *Pauesia silana* Tremblay, 1969, female. **A.** Head, frontal view; **B.** Antenna; C. Forewing; **D.** Hindwing **E.** Propodeum and petiole, dorsal view; **F.** Genitalia, lateral view.

Coloration. (Fig. 2A) Generally yellowish-brown, dorsal surface of scape and pedicel, vertex and hind legs except for coxae brown; the patches around ocelli and whole flagellomeres dark brown to black. Head and mesosoma black, gaster dark brown. Legs light brown with yellow patches at the tip of segments.

Male (Fig. 2B)**.** Similar to female, body length 1.9–2.1 mm, body generally dark brown, the lower part of the face and moth parts pale yellow, mesosoma, except posterior part of mesoscutum to propodeum and lower part of mesopleuron, yellow.

Mummified aphids (Fig. 3)**.** Concolorous with healthy aphids in early stages (Figs 3A–B) migrating outside the colony on the same branch, before final mummification. Winged mummies with head and thorax black (Fig. 3C), wingless mummies brown (Fig. 3D). Parasitoids emerged 5–9 days (average 6.7 days) post-collection by cutting a circular hole at the posterior part of the mummy, at the area between cornicles and the cauda (Fig. 3E).

Zoogeographical Distribution: Western Palaearctic (Europe, North Africa) into the border of Eastern Palaearctic (South-central Russia) (Fig. 5).

Secondary parasitoids

Few mummies were hatched lately, which led to the emergence of two secondary parasitoids (Hym., Chalcidoidea, Pteromalidae). The hyperparasites emerged 12–25 days (average 20.4) post-collection by

Figure 2. *Pauesia silana* Tremblay, 1969. General habitus, lateral view: **A.** Female; **B.** Male.

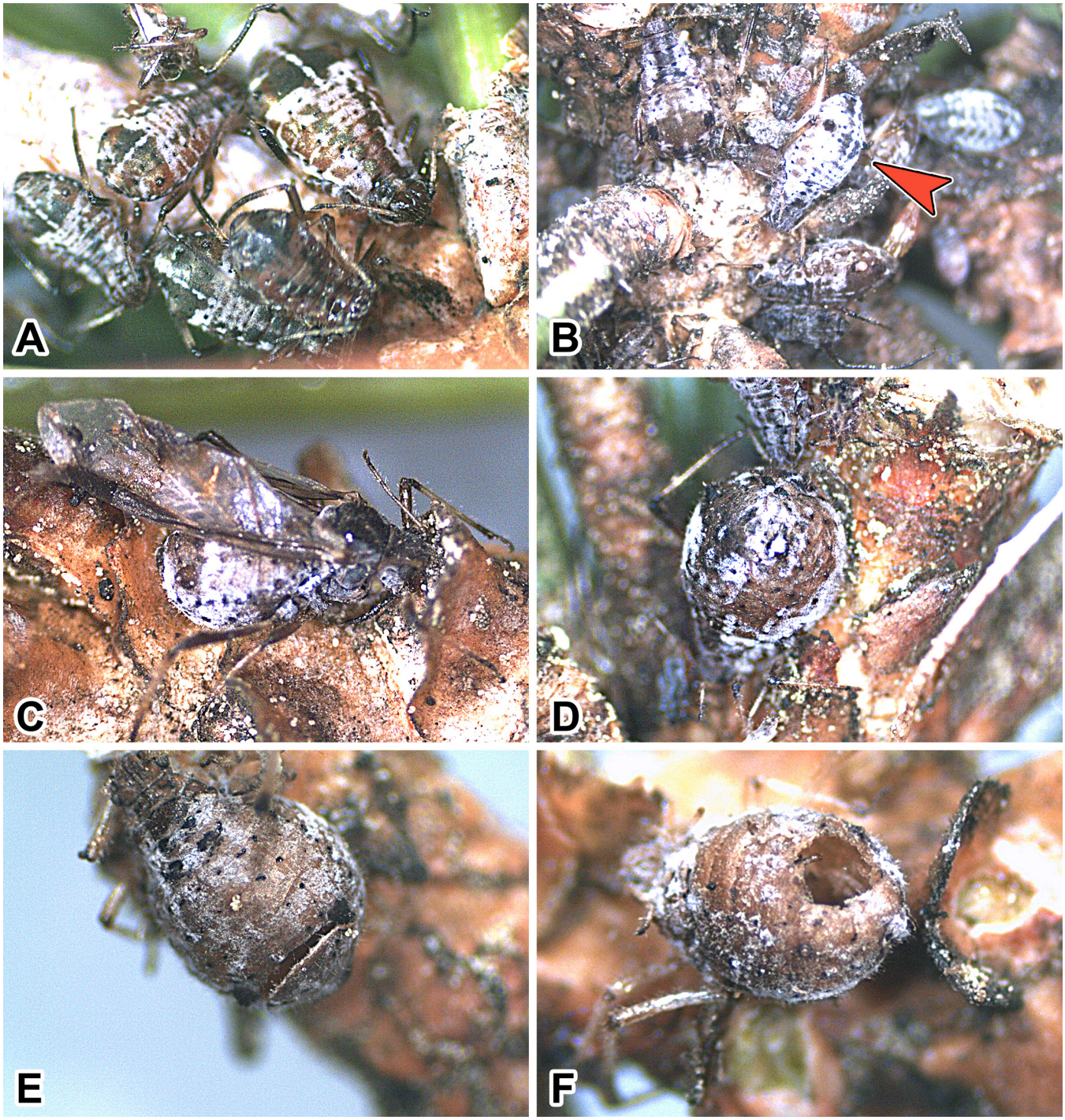

Figure 3. Branch of *Pinus halepensis* infested with the Aleppo pine aphid, *Cinara palaestinensis* Hille Ris Lambers, 1948 parasitized by *Pauesia silana* Tremblay, 1969. **A.** Small colony of the healthy nymphs; **B.** Parasitized aphids starting mummification; **C**. Winged parasitized aphids; **D.** Wingless mature mummy; **E.** Mummified aphid with emergence hole by the adult parasitoid at the postero-dorsal area; **F.** Irregular emergence hole of the hyperparasitoid.

cutting an irregular hole at the dorsal or the posterior-dorsal part of the mummy (Fig. 3F). The emerged hyperparasites were as follows: *Asaphes vulgaris* Walker, 1834 (Fig. 4A), 1♀, 26.03.2021; 1♀, 01.04.2021; 1♀, 04.04.2021; 1♀, 28.04.2021; *Pachyneuron aphidis* (Bouché, 1834) (Fig. 4B), 1♀, 22.04.2021, ex. mummies of *Cinara palaestinensis* Hille Ris Lambers, on *Pinus halepensis* Miller, parasitized by *Pauesia silana* Tremblay, Tunisia, ISA CM (35°54'58"N, 10°33'36"E).

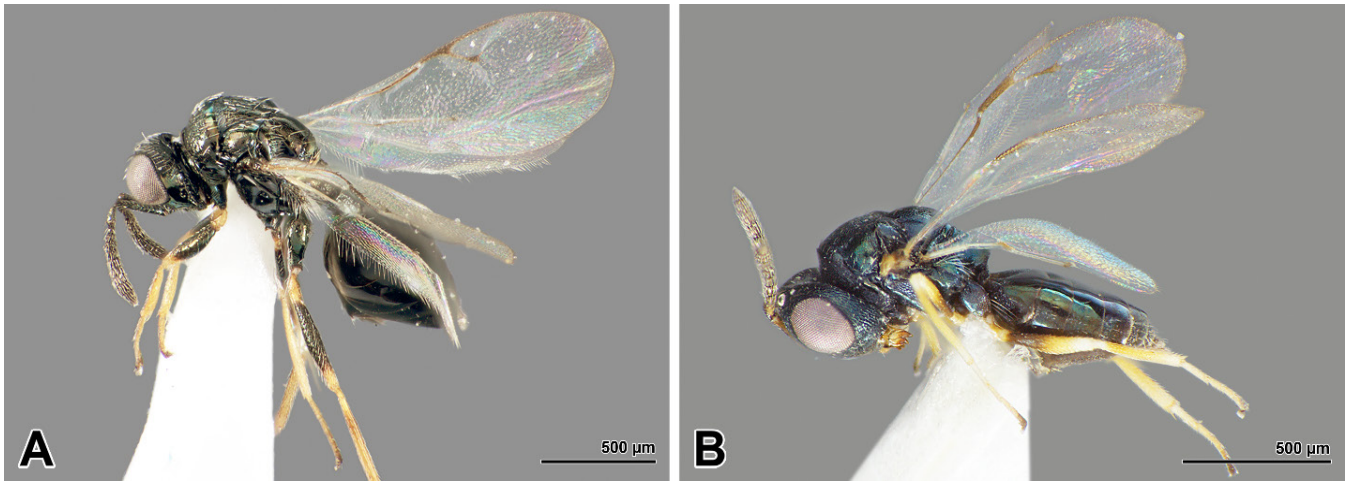

Figure 4. The secondary parasitoids (Hymenoptera, Chalcidoidea, Pteromalidae) emerged from the mummies of *Cinara palaestinensis* Hille Ris Lambers, 1948, parasitized by *Pauesia silana* Tremblay, 1969. **A.** *Asaphes vulgaris* Walker, 1834 (female); **B.** *Pachyneuron aphidis* (Bouché, 1834) (female).

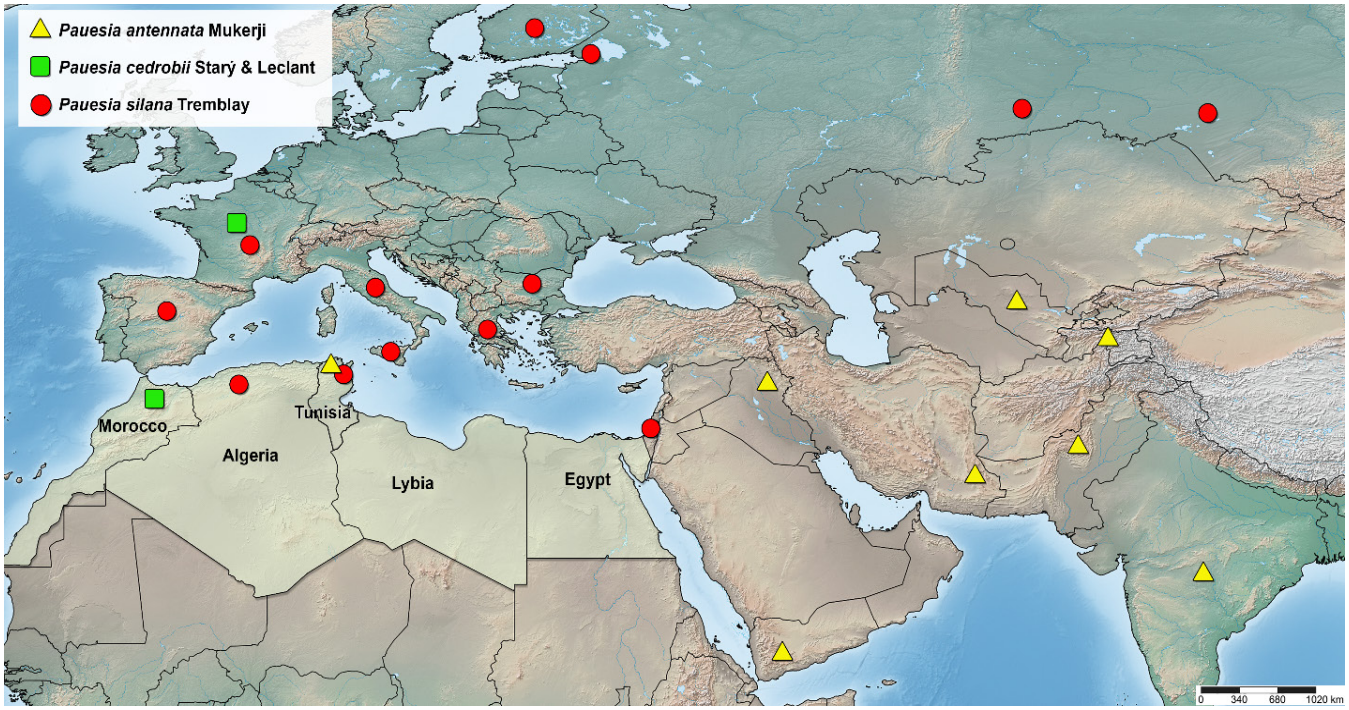

Figure 5. Map of distribution for three species of *Pauesia* Quilis, 1931 of North Africa, in the Palaearctic (and a part of Oriental) region.

Seasonal occurrence of *Cinara palaestinensis* in Tunisia

The first colonies of *Cinara* are observed on the young shoots in March. The activity of aphids reached a maximum in mid-April. Thereafter a regression of population was kept until the annihilation of the colonies at the end of May. The presence of mummies was detected till the end of March. The parasitoid remains active until the end of April. During this survey, we obtain twenty-four mummies from which 11 parasitoid and five hyperparasitoid specimens emerged.

Table 1. Updated list of Lachninae aphids and their parasitoids in the North African countries.

Aphid species	Host plant	Countries	Parasitoids	References
Cinara cedri Mimeur, 1936	*Cedrus atlantica*	Algeria	-	Laamari et al. (2013)
	Cedrus atlantica	Morocco	-	Remaudière (1954)
Cinara costata (Zetterstedt, 1828) *	-	Tunisia	-	Boukhris-Bouhachem et al. (2007)
Cinara cupressi (Bukton, 1881)	*Thuja* sp.	Tunisia	-	Boukhris-Bouhachem et al. (2007)
	Populus nigra (?)			
Cinara escherichi (Börner, 1959) *	-	Algeria	-	Laamari et al. (2010)
Cinara ferrisi (Swain, 1918)	*Thuja* sp.	Tunisia	-	Ben Halima Kamel (2012)
	Populus nigra (?)			
Cinara juniperi (de Geer, 1773)	*Juniperus oxycedrus*	Algeria	-	Laamari et al. (2013)
	Juniperus oxycedrus	Morocco	-	Sekkat (1987)
Cinara laportei (Remaudière, 1954)	*Cedrus atlantica*	Algeria	-	Laamari et al. (2010)
		Morocco	*Pauesia cedrobii* Starý & Leclant	Stary & Leclant (1977)
Cinara magrebica (Mimeur, 1934)	*Pinus halepensis*	Morocco	-	Sekkat (1987)
	Pinus halepensis	Tunisia	-	Ben Halima Kamel (2012)
Cinara pinimaritimae (Dufour, 1833)	*Pinus halepensis*	Tunisia	-	Ben Halima Kamel (2012)
	Pinus maritima			
Cinara palaestinensis Hille Ris Lambers, 1948	*Pinus halepensis*	Tunisia	-	Ben Halima Kamel (2012)
Cinara pinea (Mordivilko, 1895)	*Pinus halepensis*	Tunisia	-	Ben Halima Kamel (2012)
Cinara pini (Linnaeus, 1758)	*Pinus halepensis*	Algeria	-	Laamari et al. (2010)
	Pinus nigra		*Pauesia silana* Tremblay	Ben Hamacha et al. (2017)
Cinara tellenica Binazzi & Strangi, 2020	*Cedrus atlantica*	Algeria	-	Ayache et al. (2020)
Cinara tujafilina (Del Guercio, 1909)	*Cupressus sempervirens*	Tunisia	-	Ben Halima Kamel (2012)
	Thuja orientalis	Egypt	-	Theobald (1922)
Eulachnus nigricola (Pašek, 1953)	*Pinus halepensis*	Tunisia	-	Ben Halima Kamel (2012)
Eulachnus rileyi (Williams, 1911) *	-	Tunisia	-	Boukhris-Bouhachem et al. (2007)
Eulachnus agilis (Kaltenbach, 1843)	*Pinus halepensis*	Tunisia	*Diaeretus leucopterus* (Haliday)	Ben Halima Kamel et al. (2020)
Eulachnus tuberculostemmatus (Theobald, 1915)	*Pinus halepensis*	Tunisia	-	Ben Halima Kamel et al. (2019)
	Pinus halepensis (?)	Egypt	-	Theobald (1915)
Pterochloroides persicae Chlodkovsky, 1899	*Prunus persica*	Tunisia	*Pauesia antennata* Mukerji	Mdellel et al. (2015)
				Adouani et al. (2017, 2021)
Siphonatrophia cupressi (Swain, 1918)	*Cupressus sempervirens*	Tunisia	Unidentified	Ben Halima Kamel & Mdellel (2017)
Tuberolachnus salignus (Gmelin, 1790)	*Salix* sp.	Egypt	-	Theobald (1915)

* Caught in yellow pan-trap

DISCUSSION

The genus *Pauesia* in Tunisia was represented by a single species, *Pauesia antennata* Mukerji, 1950 that was purposefully introduced from Iran for biological control of Brown Peach Aphid, *Pterochloroides persicae* Chlodkovsky, 1899 (Hemiptera: Aphididae) (Mdellel et al., 2015; Adouani et al., 2017, 2021). *Pauesia silana* is the second species that is now first recorded from Tunisia, by which the total number of Aphidiinae parasitoids raised to 18 species (see Rakhshani et al., 2019; Ben Halima Kamel et al., 2020). This species has recently been recorded from Algeria in association with *Cinara pini* (Linnaeus, 1758) on *Pinus nigra* (Ben Hamacha et al., 2017). New evidence from Tunisia would suggest a recent accidental introduction of parasitoids to both countries, or a horizontal expansion across North Africa. The Lachninae aphids associated with coniferous trees in the North African countries comprise species of various genera (*Cinara* Curtis, *Eulachnus* Del Guercio, *Siphonatrophia* Swain, 1918 and *Tuberolachnus* Mordvilko, 1909) (Table 1), for many of them, no parasitoid species are recorded. The updated data indicated the occurrence of only three parasitoid species (Fig. 5) associated with these aphids in the North African countries, i.e. *Diaeretus leucopterus* (Haliday, 1834) (Tunisia – Ben Halima Kamel et al., 2020), *Pauesia cedrobii* Starý & Leclant, 1977 (Morocco - Starý & Leclant 1977; Fabre & Rabasse, 1987) and

Pauesia silana (Algeria - Ben Hamacha et al. 2017, and Tunisia). An unidentified parasitoid was also collected in association with *Siphonatrophia cupressi* (Swain, 1918) in Tunisia (Ben Halima Kamel & Mdellel, 2017). None of the above-mentioned aphids is recorded from Egypt and Libya. Subsequent surveys are needed to reveal the whole complex of Lachninae aphid parasitoids in the pine forest of Tunisia. *Pauesia silana* has been recorded frequently as a parasitoid of *Cinara palaestinensis* (Mescheloff & Rosen, 1990; Ben Hamacha et al., 2017; Kavallieratos et al., 2001, 2004; Mifsud & Stary, 2009), but it was also found in association with other *Cinara* aphids, including *Cinara acutirostris* Hille Ris Lambers, 1956 (Tremblay, 1969), *Cinara maghrebica* Mimeur, 1934 (Starý et al., 1973; Michelena Saval & Gonzalez Funes, 1988) and *Cinara pini* (Starý, 1976). So the further investigation is needed to find the possible association of *P. silana* with other Pine feeding *Cinara* aphids in Tunisia (Table 1).

According to the current survey, no outbreak was observed in the population of *Cinara palaestinensis* in Tunisia. It should be further investigated which factors affect the aphid populations. Both native natural enemies, including the newly recorded parasitoid and the climatic condition, can have significant controlling effects on the population of aphids in Tunisia. In the case of *Cinara laportei* (Remaudière, 1954) in Algeria, the existence of a specific parasitoid and unfavourable climatic conditions are reported to cause a severe reduction in the aphid population (Mouna & Fabre, 2005). Many coniferous plants including the pine trees are endemic to some parts of the Mediterranean basin (Watson et al., 1999), which is an area with a complex of the species/biotypes that have been commercially distributed over the world or imported vis versa. The associated aphids, mainly of the *Cinara* species often transported together with their host plants. The current research targeting the parasitoids of *Cinara* aphids in Tunisia has also yielded an originally Mediterranean species, *Pauesia silana*, which represents a potentially useful biocontrol agent which can be found in association with other *Cinara* aphids inside or outside of its known host range (Starý et al., 1973, Starý, 1976; Michelena Saval & Gonzalez Funes, 1988). On the other hand, Many *Pauesia* species are known from the Mediterranean region (Starý, 1960, 1976; Mescheloff & Rosen, 1990; Sanchis et al., 2001; Michelena Saval et al., 2005) that can be found/transported in the target areas.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution in the paper as follows: M. Ben Halima Kamel and S. Zouari reared the specimens and collected the data. H. Barahoei sorted the specimens, prepared the microscopic slides and made an early comparison of specimens. E. Rakhshani identified the parasitoid and hyperparasitoids, prepared the illustrations and primary draft of the manuscript. All authors read and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

All the reference specimens are deposited in and accessible from the collection of the Department of Plant Protection, University of Zabol (DPPZ), Iran.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

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

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گسترش دامنه انتشار زنبور *Pauesia silana* Tremblay (Hymenoptera, Braconidae, Aphidiinae) در شمال آفریقا، یافته جدید در تونس

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چکیـده: انتشار زنبور پارازیتویید شته به نام *Pauesia silana* Tremblay (Hym., Braconidae, Aphidiinae) در کشور تونس گزارش و ثبت شد. این گونه پارازیتویید، مرتبط با شته *Cinara palaestinensis* Hille Ris Lambers از روی کاج حلب، *Pinus halepensis* جمع‌آوری شد. نمونه‌های زنبور پارازیتویید از طریق پرورش شته‌های مومیایی شده در بین کلونی‌های شته مستقر روی درختان کاج موسسه عالی کشاورزی شط مریم تونس طی ماه‌های مارس تا آوریل سال ۲۰۲۱ بدست آمدند. توصیف افتراقی مختصر برای گونه پارازیتویید گزارش شده، ارایه شد. این اولین گزارش انتشار گونه‌ای از جنس *Pauesia* از کشور تونس (جدای از گونه انتقال یافته *Pauesia antennata* Mukerji محسوب می‌شود. همچنین دو گونه پارازیتویید ثانویه شامل *Asaphes vulgaris* Walker و *Pachyneuron aphidis* (Bouché) (Hym., Chalcidoidea, Pteromalidae) نیز از شته‌های مومیایی شده خارج شدند. گونه‌های شناخته شده شته‌های جنس *Cinara* و پارازیتوییدهای مرتبط با آنها در کشورهای شمال آفریقا نیز مرور شد. بر اساس یافته‌های موجود، هم شته کاج حلب و هم پارازیتویید گزارش شده، جزو گونه‌های غیر بومی در شمال آفریقا محسوب می‌شوند که اخیرا به طور تصادفی از طریق نقل و انتقالات در منطقه مدیترانه‌ای وارد شده و یا به تدریج در حال گسترش دامنه انتشار خود در امتداد کشورهای شمال آفریقا هستند.

واژگـان کلیدی: سوزنی‌برگان، شته آفت، گونهٔ مهاجم، مهار زیستی، پارازیتویید