Characterization of *Platynaspis luteorubra*, a Ladybird Associated with Aphid Colonies on Ornamental Trees in Tunis Region (Tunisia)

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ABSTRACT

Boukhris-Bouhachem, S. and Souissi, R. 2025. Characterization of *Platynaspis luteorubra*, a ladybird associated with aphid colonies on ornamental trees in Tunis region (Tunisia). Tunisian Journal of Plant Protection 20 (1): 17-27.

Platynaspis luteorubra was identified in the Tunis region for the first time. This species was collected in July 2024 from a colony of aphids on *Cestrum nocturnum* and *Nerium oleander* in the National Agricultural Research Institute of Tunisia (INRAT) garden feeding on *Aphis spiraecola*, *Aphis gossypii* and *Aphis nerii*. An illustration and brief description are provided, including notes on its world distribution, biology, and host prey. Additionally, a fragment of mitochondrial DNA from the cytochrome c oxidase 1 (COI) gene was analyzed for species confirmation.

Keywords: Aphids, biocontrol, biodiversity, ladybird beetle, *Platynaspis luteorubra*

Ladybirds (Coleoptera, Coccinellidae) are among the most studied and paradigmatic groups of insect predators. Approximately 90% of the 6,000 known species of ladybirds worldwide are predatory, primarily feeding on sap-sucking insects such as scale insects, aphids, whiteflies, and psyllids (Hemiptera, Sternorrhyncha) (Hodek and Honek 1996, Iperti 1999). Recent reviews of the diversity of predatory Coccinellid beetles offer an inventory and a description of the ladybird species (Teja et al. 2023, Wargé et al. 2021). Previously, Kovář (1996) divided Coccinellidae into six subfamilies, which are still broadly recognizable today: Sticholotinae, Scymninae, Chilocorinae, Coccidulinae, Coccinellinae, and Epilachninae.

Within coccinellids, each taxonomic group is associated with a group of prey. The tribe Scyminini, Aspiridimerini, Hyperaspini, and Platynaspini feed mostly on aphids (Hodek and Honek 1996, Hodek and Honek 2009).

Regardless of the diversity of habitat and prey preference among

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ladybirds, this group occupied most terrestrial regions, including tundra, forest, grassland, and agroecosystems. Furthermore, some species periodically show movements from meadows to forests or valleys to mountains (Iperti 1999). This widespread distribution of ladybirds in most land ecosystems makes these species highly noticeable as thriving key elements of many ecosystems (Iperti 1999).

Coccinellids are holometabolous insects with four larval instars. The complete life cycle lasts from 2 weeks to 2 months, depending on the ladybird species thermal conditions and feeding guild (Iperti 1999). Usually, adults only mate and reproduce after feeding on their prey, which also assures prey availability for neonate larvae (Hodek and Honek 1996). Most ladybird species show reproductive diapause (Hodek 2012). This diapause may be facultative when induced or interrupted by environmental signals or food availability, or obligatory and depends on genetic factors (Hodek 2002, Hodek 2012). Sometimes, individuals from different populations within the same species may present different diapause types, as with *Coccinella septempunctata* (Hodek and Okuda 1993).

The genus *Platynaspis* described by (Poorani 2023) is widespread, including countries such as Spain, Portugal, France, Luxembourg, Italy, Algeria, Turkey, Greece, Palestine, England, Netherlands, Sweden, Belgium, Austria, Poland, Bulgaria, Czechia, Slovenia, Belarus, Russian Federation, Lithuania, Switzerland, Hungary, Denmark, Estonia, Norway, Ukraine, Croatia, India, Georgia, and Germany (The Global Biodiversity Information Facility (GBIF) documents the global distribution of this genus; https://www.gbif.org).

Platynaspis luteorubra (Goeze, 1777) or Ant-nest Ladybird, is a Palearctic species belonging to the family Coccinellidae, subfamily Coccinellinae, and tribe Platynaspini. *P. luteorubra* is a rare ladybird beetle primarily found in Europe (from the UK to Russia). It is particularly abundant in Poland and Belgium, with observations also reported in France, Spain, Italy, Portugal, UK and North Africa: Algeria and Morocco (GBIF). *P. luteorubra*, an aphidophagous, xerothermophilic, and myrmecophilic ladybird, thrives in open sunny environments with sparse vegetation. Both adults and larvae are aphidophagous, preying on aphids and potentially other soft-bodied pests like mites, scale insects and whiteflies. The species plays a functional role in reducing aphid populations, particularly in habitats where ants protect aphids (Rondoni et al. 2012). *P. luteorubra* is often found in anthropogenic habitats such as quarries, gravel pits, wastelands, and along railway tracks (Adriaens 2015, San Martin et al. 2006).

In this study, we recorded the presence of the coccinellid larvae feeding on aphids *Aphis spiraecola* and *Aphis gossypii* colonizing *Cestrum nocturnum* and *Aphis nerii* on *Nerium oleander*. Morphological examination suggests that the specimen belongs to *P. luteorubra*. This species was previously reported on citrus in the Cap Bon region (North Tunisia) (Limem-Sellami et al. 2017). However, details on its identification were limited. Here, we provide a detailed description of *P. luteorubra* and confirm its identification by molecular tools, and report some ecological characteristics.

MATERIAL AND METHODS

Collect site.

Coccinellid larvae feeding on aphids colonizing *C. nocturnum* and *N. oleander* plants were collected from the garden of the National Agricultural

Research Institute of Tunisia (INRAT) in July 2024 (36.844748N, 10.192160E). The garden is a collection of ornamental trees: *Nerium oleander, Pistacia lentiscus, P. terebintus, Cestrum nocturnum, Pittosporum tobira, Rosa canina, Melia azedarach, Hibiscus*… and Citrus trees, with limited management. Larvae were identified according to Klausnitzer's (2007) descriptions. Collected larvae were reared on aphids until the adults were emerged in the laboratory to confirm the identification.

Morphological identification.

Morphological identification was conducted using a Leica MC205 dissecting stereomicroscope. For laboratory preparation of the lady beetle genitalia for slide mounting, the cut abdomen was placed in a 40% KOH bath for one hour. The internal genitalia were manually extracted under a dissecting microscope using insect pins. The extracted genitalia were transferred to a 10% KOH solution on a hotplate at 90°C for 5 min. Afterward, the genitalia were removed from the KOH solution and transferred to a clean Petri dish with distilled water and washed for 5 min. The genitalia are removed placed on a slide with a drop of glycerin and covered with a slip for microscopic examination. Ladybird beetle identification was based on the keys of Fürsch (1990). A voucher specimen of this ladybird beetle was stored in the author's collection in the Entomology Laboratory of the National Agricultural Research Institute of Tunisia (INRAT).

Molecular identification.

DNA barcoding was done to complete the species identification. Total DNA was extracted from a single adult or larva using the Cetyltrimethyl Ammonium Bromide (CTAB) protocol. Barcoding was performed based on 800-bp fragment of the mitochondrial gene for cytochrome c oxidase subunit I (COI) which was amplified by polymerase chain reaction (PCR) using the primers (Simon et al. 1994) C1-J-2195 (5′-TTGATTTTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5′-TCCAATGCACTAATCTGCCATATTA-3'). PCR was performed on the thermocycler of Applied Biosystems in a total volume of 75 µl. The PCR program consisted of an initial denaturation for 2 min at 94°C, followed by 35 cycles for amplification reactions of 30 s at 94°C, 40 s at 50°C, 60 s at 72°C, a final 7 min extension at 72°C and storage at 10°C. RAN BioLinks SARL (Tunis, Tunisia) performed sequencing of the PCR products. The sequencing method used is Sanger sequencing with an automatic sequencer (ABI Biosystem, USA).

Sequencing and alignment.

The sequence was compared to homologous sequences in the GenBank database using BLAST (Basic Local Alignment Search Tool). The results were expressed as the percentage of similarity between the species to be identified and the closest species. The sequence was then trimmed and submitted in GenBank (Table 1) to get an accession number.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 12.524 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsentein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamara et al. 2004)

and are in the units of the number of base substitutions per site. The analytical procedure encompassed 10 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions. The pairwise deletion option was applied to all ambiguous positions for each sequence pair, resulting in a final data set comprising 11 119 positions. Evolutionary analyses were conducted in MEGA12 (Kumar et al. 2024) utilizing up to 7 parallel computing threads.

RESULTS

Morphological identification.

The collected larvae and adults feeding on aphids were identified as *Platynaspis luteorubra* (Fig. 1a). Our observations, notably, concern *C. nocturnum* (Mesk-Elil) and *Nerium oleander*, whose stems and leaves were colonized by aphid colonies *A. spiraecola*, *A. gossypii* and *A. nerii* respectively hosting numerous ants.

Description: The *P. luteorubra* nymph is yellowish to brownish and has a broad, oval body covered in white hairs (Figs. 1b-c). It is moderately convex and densely pubescent. The younger stages resemble the final stage but are smaller. Adult sizes range from 3 to 3.5 mm. The adult features an oval, shiny black pubescent body ornamented with dark brown hairs (Figs. 1d-e). Its head is black, and the antennae are very short, ending in a faintly marked three-section club (Fig. 2b). The pronotum is dark brown and has two lateral light yellow-orange spots (Figs. 1d-f). Each elytron exhibits two spots: one located in the anterior half just before the middle, and the posterior spot at the end of the body before the apical margin, where a mix of short, yellowish hairs are visible in the pubescence. The ventral side of the abdomen is brown, featuring five symmetrical yellow spots along each lateral margin (Fig. 2a). The species has a rectangular-shaped extension in the postcoxal line (Fig. 2a). The hind legs have brown femora with yellow tibial apices (Fig. 2c). The male genitalia, illustrated in Figs. 2d-e, show the penis, viewed ventrally (Fig. 2d), is elongated and lanceolate in shape, progressively broadening just beyond the midpoint. The apical part is triangular, gradually narrowing to a rounded apex, with a prominent, broad basal capsule. The tegmen is in lateral view (Fig. 2e).

Molecular analysis.

Bar-coding.

For molecular confirmation of the taxonomy, a larva and two adult specimens of the ladybird *P. luteorubra* were successfully sequenced for the mitochondrial COI gene. The obtained 801-bp COI sequence from the ladybird larva was compared to reference sequences in GenBank. BLASTN analysis (35) revealed a high level of sequence similarity (≥ 99%) with two accessions from Toulouse (France) and London (UK) (Table 1), indicating strong sequence conservation. The accession from Toulouse, submitted to GenBank, showed particularly close genetic proximity to our specimen. These findings highlight the effectiveness of DNA barcoding as a powerful tool for accurately identifying newly encountered specimens at the species level.

Table 1. Nucleotide sequence identities of Tunisian species compared to reference sequences from the GenBank

Accession No. PV076945.1 Tunisia	GU073913.1 Toulouse	HQ164711.1 UK
Identity with sequence (%)	99.87	99.74

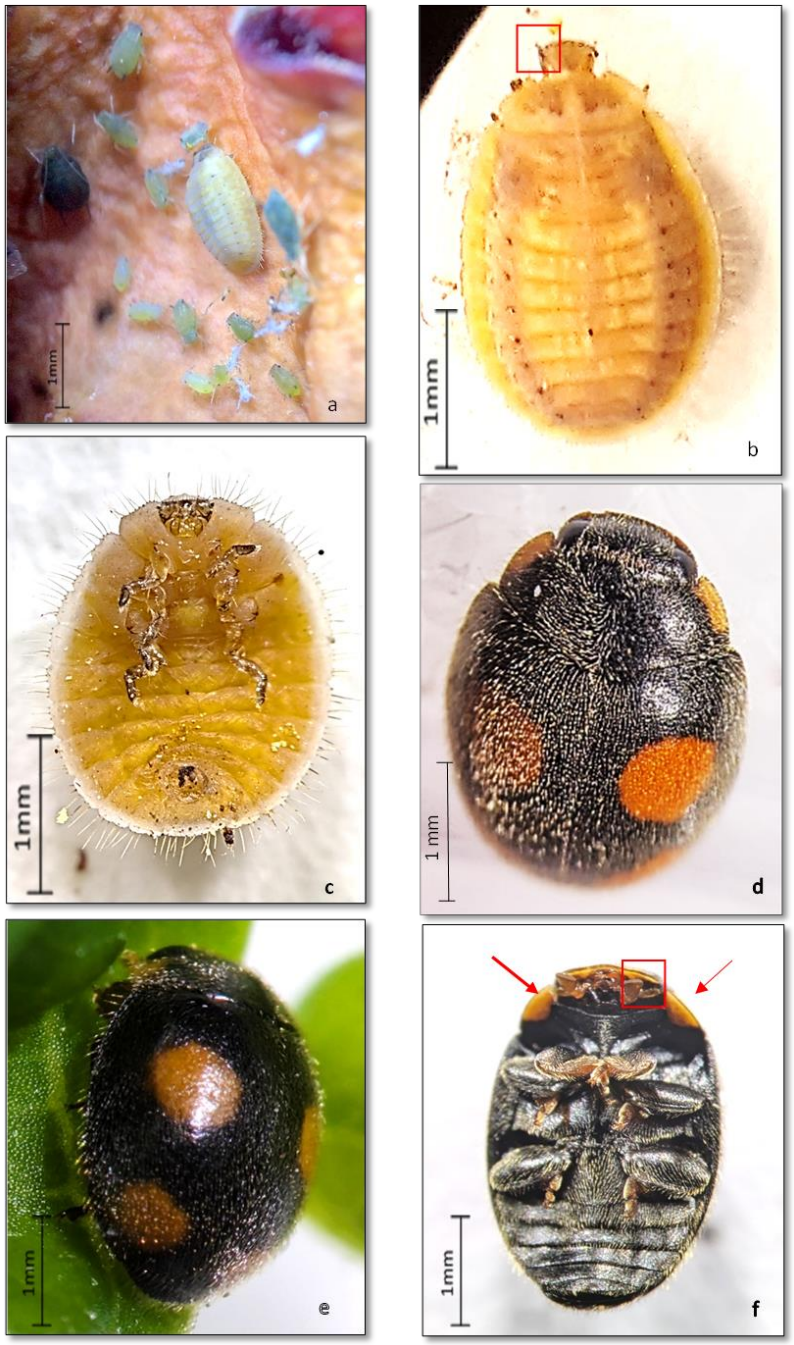

Fig. 1. *Platynaspis luteorubra*. a: larva in presence of an aphid colony 2024, b: details of larva in dorsal view, c: details of larva in ventral view; d-e, adult, dorsal view, f: adult, ventral view (© S. Bouhachem).

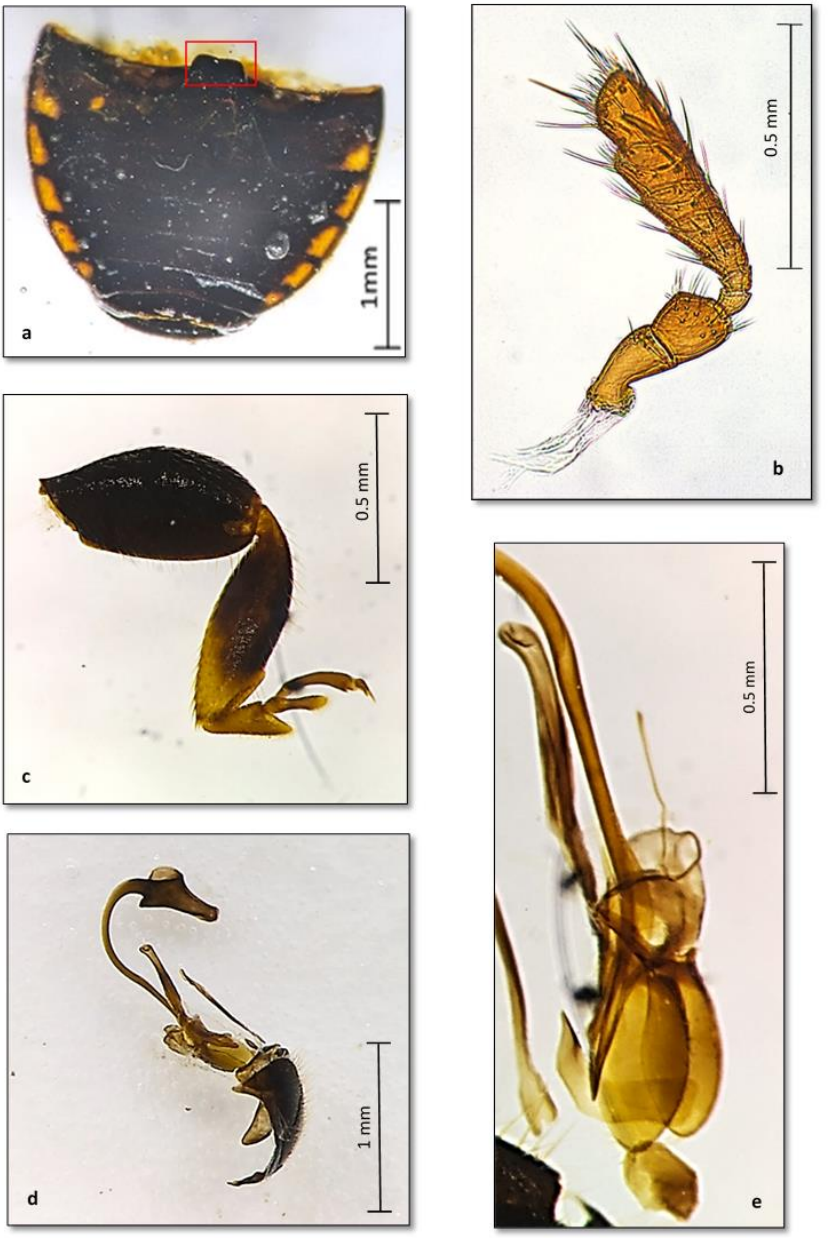

Fig. 2. Characteristic features of *Platynaspis luteorubra*. a, abdominal postcoxal line; b, antennae; c, hind tibia and tarsus, d, penis ventral view, e, tegmen lateral view (© S. Bouhachem).

Phylogenetic analysis.

Phylogenetic analysis has demonstrated to offer reliable results in clustering *Platynaspis* species from all over the world. It also showed that *P. luteorubra* sequences were split in the tree between two groups but seemed very close to GU073913 from France with a bootstrap value of 97% (Fig. 3).

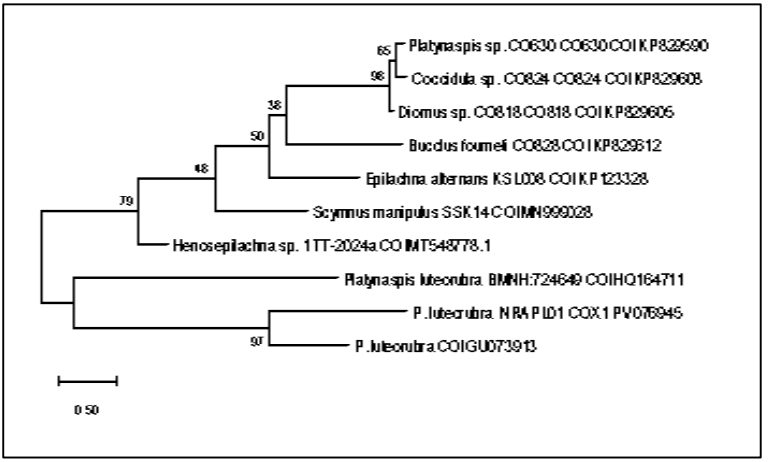

Fig. 3. Phylogenetic relationship of *Platynaspis* species based on the nucleotides, alignment using ClustalW through MEGA-X software. The numbers below the joining lines are bootstrapping values. The Tunisian specimen is *P. luteorubra* INRAPL01.

Ecological characteristics.

This species is observed from July to October at INRAT garden on Cestrum. In the laboratory, nymphs were maintained on Cestrum young shoots colonized by *A. spiraecola* in a controlled room temperature (23°C, 16h:8h, 65% HR). Nymphs take about 8-22 days to become adults according to their developmental stage. Adults begin reproducing about 1.5 days after emergence. The female lays about 14 to 31 eggs (average on 11 females). The eggs hatched after 3-4 days.

DISCUSSION

The species *P. luteorubra* is identified based on morphological and molecular methods. The details of morphological features were shown in Figs 1-2. DNA barcoding confirmed the species identity. It was revealed that barcodes can be valuable in routine identifications of unidentified Coccinellidae specimens in collections.

This species described here was feeding on aphid colonies that host numerous ants. Indeed, it has been reported that *P. luteorubra* feeds on aphids tended by *Lasius niger* (Klaunitzer 2007, Wargé 2022). The ladybird was current from July until October, while larvae are reported to be found in vegetation from May to September (Fürsch 1967). Khalil (2006) observed *P. luteorubra* on pine, apple, pomegranate, maize and rose plants

in south Syria. The beetle was reported to prey on *Aphis fabae* and other common aphids in grassland and meadow habitats (Völkl 1995). *P. luteorubra* is also commonly observed on *Cirsium arvense* where it feeds on *Aphis fabae* cirsii acanthoides tended by ant *L. niger*. Mymecophily affects the abundance of resources by prolonging the life span of aphid colonies (Flatt & Weisser 2000). Recently, *P. luteorubra* was firstly identified in the Republic of Moldova in alfalfa fields (Burdujaand Bușmachiu 2023).

P. luteorubra is stated as a rare species in many regions and is considered to have a protected status (San Martin et al. 2006). Little is known about its biology and ecology. Hence, this highly specialized beetle species is recognized as being more vulnerable to extinction as compared to generalists because they are less adaptable to changing conditions (Davies et al. 2004). According to Rondoni et al. (2012), the low survival of *P. luteorubra* when paired with both *Adalia bipunctata* and *Coccinella septempunctata* indicates that these species may be important predators of *P. luteorubra* inhabiting both trees (e.g. *Prunus avium*) and weeds (*Arctium lappa*). The relatively higher survival of *P. luteorubra* paired with *Harmonia axyridis* is unexpected and suggest that its sticky reflex blood, coccid-like shape and ability to seek refuge in the plant sprouts could be important in its ability to avoid predation. *H. axyridis* and *C. septempunctata* could probably become co-dominants in the same habitats. Moreover, a decline in diversity has been reported for several arthropod taxa worldwide in recent years (Akhtar et al. 2024, Hallmann et al. 2017, Öckinger et al. 2012). Faunistic impoverishment results from intense land use (deforestation, urbanization), agricultural intensification (monocultures, insecticide use, cultural practices), and climate change (Wagner 2022). The decrease in insect species raises concerns due to the reduction of specialized species (Habel et al. 2016, Nolte et al. 2019) in favor of generalist ones (Thomas 2016), leading to ecological imbalance in nature. This makes *P. luteorubra* more important for conservation.

Ladybird beetles (Coccinellidae) are important predators widely used in biological control program. Oligophagous species are known to be the most successful in biological control. They can regulate the aphid population due to their voracity, search efficiency, predation capacity, and reproduction rate (Dixon 2000, Dixon et al. 1997, Kindlmann et al. 2015, Magro et al. 2002). Thus, ladybird beetles as *P. luteorubra*, can be a good candidate agent for biological control and IPM in ornamental trees and Citrus because they feed on *A. spiraecola.*

Beyond their role as predators, coccinellids play a vital role in maintaining ecological balance by regulating pest densities, ultimately reducing the dependence on chemical pesticides in agriculture. Thus, underlying the need for continued research to unlock the full potential of these remarkable insects in sustainable agriculture. In future work, it will be interesting to analyze the association between prey specializations of *P. luteorubra* and several life and ecological traits (body length, fecundity, voltinism), habitat, and plant specialization of the prey.

RESUME

Boukhris-Bouhachem S. et Souissi R. 2025. Caractérisation de *Platynaspis luteorubra*, une coccinelle associée aux colonies de pucerons sur les arbustes ornementaux dans la région de Tunis (Tunisie). Tunisian Journal of Plant Protection 20 (1): 17-27.

Platynaspis luteorubra a été identifiée pour la première fois dans la région de Tunis. Cette espèce a été collectée en juillet 2024 à partir d'une colonie de pucerons sur *Cestrum nocturnum* et *Nerium oleander* dans le jardin de l'Institut National de la Recherche Agronomique de Tunisie (INRAT). Une illustration et une brève description, avec des notes sur sa distribution, biologie et proies sont présentées. De plus, un fragment de l'ADN mitochondrial du gène du cytochrome c oxydase 1 (COI) a été utilisé pour la confirmation de l'espèce.

Mots-clés : Biodiversité, coccinelle, *Platynaspis luteorubra*, pucerons, lutte biologique

ملخص

بوخريص-بوهاشم، سنية ورابحة السويسي. 2025. توصيف الدعسوقة *Platynaspis luteorubra* المرتبطة بمستعمرات المن على أشجار زينة في منطقة تونس العاصمة (تونس).

Tunisian Journal of Plant Protection 20 (1): 17-27.

تم تشخيص الدعسوقة *Platynaspis luteorubra* لأول مرة في منطقة تونس. تم جمع العينات في يوليو 2024 من مستعمرة لحشرات المن على نباتات *Cestrum nocturnum* و *Nerium oleander* في حديقة المعهد الوطني للبحوث الزراعية بتونس. تم تقديم رسم توضيحي ووصف موجز مع ملاحظات حول الانتشار الجغرافي، والبيولوجيا، والفرائس. بالإضافة إلى ذلك، تم اعتماد جزء من الحمض النووي للميتوكوندريا من جين سيتوكروم سي أوكسيداز 1 (COI) لتأكيد تشخيص النوع.

كلمات مفتاحية: تنوع بيولوجي، دعسوقة، من، مكافحة بيولوجية، *Platynaspis luteorubra*

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