First record of the nesting whitefly (*Paraleyrodes minei* Iaccarino, 1990) in Croatia

Prvi nalaz gnjezdastog štitastog moljca (Paraleyrodes minei Iaccarino, 1990) u Hrvatskoj

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Abstract

The nesting whitefly, *Paraleyrodes minei* Iaccarino, 1990 (Aleyrodidae: Aleurodicinae), was recorded for the first time in Croatia on leaves of mandarin and orange trees on the island of Vis in September 2023. Whitefly puparia collected from the infested leaves were morphologically identified as species *P. minei* in the laboratory. Currently, the whitefly fauna in Croatia comprises 37 species belonging to 22 genera. They all are classified in the subfamily Aleyrodinae. Therefore, the exotic Neotropical species *P. minei* is the first and, for now, the only member of the aleyrodid subfamily Aleuro-dicinae that has been found in Croatia. It is a polyphagous species feeding mostly on woody plants assigned to 19 families. It is most frequently recorded on genus *Citrus* (Rutaceae) plants, with a status of moderate to severe pest. Due to the intensive international trade of plants and global climatic changes, this invasive pest presents certain phytosanitary risks to citrus orchards, not only on geographically isolated island Vis, but potentially also on the mainland of the Adriatic part of Croatia. The aim of this paper is to present the first record of *P. minei* in Croatia and provide information about the species morphology, biology, and damages.

Keywords: Aleyrodidae, Aleurodicinae, Paraleyrodes minei, first record, citrus, Croatia

Sažetak

Gnjezdasti štitasti moljac *Paraleyrodes minei* Iaccarino, 1990 (Aleyrodidae: Aleurodicinae) zabilježen je po prvi put u Hrvatskoj na listovima mandarine i naranče na otoku Visu u rujnu 2023. Pupariji štitastog moljca prikupljeni na zaraženim listovima morfološki su identificirani u laboratoriju kao vrsta *P. minei*. Fauna štitastih moljaca trenutno u Hrvatskoj broji 37 vrsta iz 22 roda, isključivo iz potporodice Aleyrodinae. Stoga je egzotična, neotropikalna vrsta *P. minei* prva i jedina do sada zabilježena vrsta u Hrvatskoj iz potporodice štitastih moljaca Aleurodicinae. To je polifag koji se uglavnom hrani na drvenastim biljnim vrstama iz 19 porodica, a najčešće je nađen na agrumima (Rutaceae) na kojima ima status umjerenog do značajnog štetnika. Zbog intenzivne međunarodne trgovine biljem i globalnih klimatskih promjena, ovaj invazivni štetnik predstavlja određeni fitosanitarni rizik za nasade agruma, ne samo na geografski izoliranom otoku Visu već potencijalno i na kopnenom dijelu obale Jadrana. Cilj ovog rada prikazati je prvi nalaz vrste *P. minei* u Hrvatskoj te pružiti informacije o morfologiji i biologiji vrste te štetama.

Ključne riječi: Aleyrodidae, Aleurodicinae, Paraleyrodes minei, prvi nalaz, agrumi, Hrvatska

Introduction - Uvod

The insect family Aleyrodidae (Hemiptera), commonly known as whiteflies, consists of 1556 described species placed in 161 genera from three extant and one fossil (Bernaeinae) subfamilies. Most of the species are classified in the subfamilies Aleyrodinae or Aleurodicinae, while the history and status of the subfamily Udamoselinae are controversial (Martin and Mound 2007). The subfamily Aleyrodinae is worldwide in distribution and includes 1424 species in 148 genera. One hundred and eighteen species belonging to the subfamily Aleurodicinae are classified into 18 genera and distributed primarily in the New World (Evans 2007). All species of the subfamily Aleurodicinae are tropical, with the majority of them described from the Neotropical zoogeographical region (Mexico, Central and South America, Caribbean Islands). Some of them have extended their distribution range into the Nearctic and Western Palearctic regions (Suh and Evans 2012). In Europe and the Mediterranean region, the whitefly fauna comprises 57 native or naturalized species in 25 genera. The subfamily Aleurodicinae includes five recorded Neotropical species from the genera Aleurodicus Douglas, 1892 and Paraleyrodes Quaintance, 1909 (Martin et al. 2000). The last published checklist of whiteflies in Croatia comprises 31 species placed in 18 genera (Šimala and Masten Milek 2008). Six additional species were discovered afterward, making a total of 37 whitefly species recorded for Croatia so far. These are the following species: Aleurocanthus spiniferus Quaintance, 1903 (Šimala and Masten Milek 2013), Aleuroclava aucubae (Kuwana, 1911) (Šimala et al. 2014), Pealius quercus (Signoret, 1868) (Šimala and Masten Milek 2014), Parabemisia myricae (Kuwana 1927) (Šimala et al. 2016), Singhiella simplex (Singh, 1931) (Šimala et al. 2020) and Aleurocanthus camelliae Kanmiya & Kasai, 2011 (Šimala et al. 2023). All recorded aleyrodid species belong to the subfamily Aleyrodinae, none to Aleurodicinae. Paraleyrodes is a Neotropical genus represented by a total of 17 species (Martin and Mound 2007). Members of this genus are commonly called "nesting whiteflies", due to typical fluffy wax produced in the shape of a white "nest" by ovipositing females (Longo and Rapisarda 2014). Although the species Paraleyrodes minei Iaccarino, 1990 was first described from specimens collected in Syria in 1990 on Citrus aurantium L., it is an example of a whitefly species that was described outside of its native distribution range, following its introduction and establishment as a pest in California (Hernandez-Suarez et al. 2012). According to Evans (2007), P. minei is recorded in the Nearctic, Neotropical, Afrotropical, Oriental, and Palearctic regions and the Pacific. In the Palaearctic region, P. minei has been recorded in the Canary Islands, Iran, Israel, Italy, Lebanon, Madeira, Morocco, Spain, Syria, and Turkey (Longo and Rapisarda 2014). Since P. minei is the most geographically mobile species within the genus, its occurrence in the Old World may be due to introductions from the New World (Martin 2004). It is polyphagous on plants of numerous families and is reported as a moderate pest of citrus (Longo and Rapisarda 2014). Damage on host plants is similar to other species of whiteflies. Direct damage arises from adults and larvae sucking on plant sap from the leaves, which affects quantity and quality of yield. Indirect damage is a consequence of excreting of honeydew, on which sooty molds subsequently develop (Kalaitzaki et al. 2016). P. minei is the first recorded species from the aleyrodid subfamily Aleurodicinae in Croatia. Therefore, puparial and adult identification characteristics, detection methods, and biology, as well as pest's impact on citrus plants are presented and discussed below.

Materials and Methods - Materijali i metode

During a visual inspection of citrus plants on the island of Vis on September 20th and 21st 2023, unusual white, fluffy congregations of whitefly larvae were observed on the lower surface of inspected leaves. The suspect leaf samples with preimaginal and several adult whiteflies, collected at two locations, were placed and stored dry in envelopes until laboratory analysis (Martin 1987). In the laboratory, according to the modified preparation method by Watson and Chandler (1999), a few specimens of puparia and pupal cases were picked from the leaves with an entomological needle and transferred to cold 10 % KOH for 24 hours for maceration. Afterward, specimens were cleaned in 70 % ethanol, heated at 90 °C in 40 % lactic acid for 20 minutes, stained in a mixture of lignin pink, Essig's aphid fluid, and acid fuchsin, dehydrated in acetic acid and then placed in clove oil for about 15 minutes. Finally, specimens were slide-mounted in a few drops of Canada balsam diluted with benzyl alcohol, as permanent microscopic slides. Moreover, one specimen of a whitefly adult male was prepared as a permanent microscopic slide for additional analysis, in terms of species auxiliary identification. Collected specimens of whiteflies were identified to the species level based on morphological characters of puparium and pupal case and additionally based on male genitalia according to the key provided by Martin (1996). For accurate identification, a binocular dissecting microscope Olympus SZX 7 equipped with an Olympus LC 20 digital camera, and optical microscope Olympus BX 51 equipped with an Olympus DP 25 digital camera were used. Slide-mounted specimens labelled with the laboratory sample number (86/SM/23, 88/SM/23) were deposited, after drying for about two months, in the collection of the Laboratory for Zoology of the Centre for Plant Protection - CAAF.

Results and Discussion - Rezultati i rasprava

Whitefly species *P. minei*, was detected for the first time in Croatia on the leaves of mandarin and sweet orange in 2023 in town Vis on the island of Vis (Figure 1). Faunistic details about the analysed samples and the species finding are as follows:

Paraleyrodes minei Iaccarino, 1990

Material examined: Citrus reticulata Blanco, 1837, commercial orchard, Vis (43°3′51.0″N 16°10′59.0′′E), 20.9.2023; *Citrus sinensis* (L.) Osbeck, garden centre Vis (43°3′47.0″N 16°11′0″E), 21.9.2023.

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Figure 1 Finding places of *P. minei* (yellow marks) on island Vis in 2023 (Google Earth Pro)Slika 1. Lokaliteti nalaza *P. minei* (žute oznake) na otoku Visu u 2023. godini (Google Earth Pro)

At first, visual symptoms of infestation on the lower surface of inspected citrus leaves (Figure 2) were referred to another whitefly species often present on citrus, the woolly whitefly [Aleurothrixus floccosus (Maskell, 1896)]. According to the literature data, whitefly species that are often found in mixed populations with P. minei on citrus plants in the Mediterranean Region include A. floccosus and citrus whitefly [Dialeurodes citri (Ashmead, 1885)]. The puparia of P. minei can be distinguished from the puparia of two other whitefly species by the presence of long wax rods (Figure 3). These lack completely in D. citri, while A. floccosus puparia produce a flocculent mass of short and curly wax filaments which form a dense white mat over the colony (Figure 4) (Malumphy and Mifsud 2016). Both species were detected in mixed colonies of whitefly populations in a sample of leaves collected on sweet orange trees, on which P. minei, as well as A. spiniferus, were also found. Therefore, the activities that were conducted within the official survey of quarantine Aleurocanthus Quaintance and Baker, 1914 whitefly species on citrus plants on the island of Vis resulted in a positive finding of a quarantine harmful organism and additionally in the first record of a whitefly species from subfamily Aleurodicinae which is new to the fauna of Croatia. P. minei may be distinguished in the field from 5 other whitefly species recorded so far in citrus plantations in Croatia (Šimala et al. 2020) by the characteristic "nests" of long wax filaments and, fluffy wax forms around the ovipositing females (Figure 5) and long wax rods that form around the fourth larval instar, hence the common name of "nesting whitefly" (Malumphy and Mifsud 2016).



Figure 2 *P. minei* infestation on mandarin leafSlika 2. Napad *P. minei* na listu mandarine



Figure 3 Waxy secretions produced by species *P. minei* on the lower surface of mandarin leafSlika 3. Voštane izlučevine vrste *P. minei* na naličju lista mandarine



Figure 4 Waxy secretions produced by larvae of species *A. floccosus* on the lower surface of mandarin leafSlika 4. Voštane izlučevine ličinki vrste *A. floccosus* na naličju lista mandarine

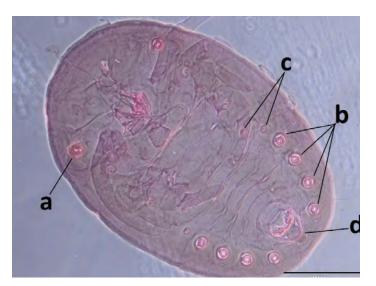


Figure 5 *P. minei* adult female and eggs **Slika 5.** Odrasla ženka i jaja *P. minei*

The source of infestation of citrus plants on the island of Vis by *P. minei* is unknown. Considering that the species was found in a garden centre, we can assume that it was introduced with imported plant material from Italy, since a large number of plants, especially ornamental cultivars of citrus, is imported from different parts of Italy, including Sicily, where *P. minei* was recently discovered (Longo and Rapisarda 2014). The pest has likely spread further into the nearby mandarin orchard actively by flight of adults. The infestation of citrus plants by *P. minei* was low to moderate. Infested plants did not show any significant damage symptoms. Honeydew and black sooty moulds were present sporadically on the leaves and twigs of infested citrus trees. Since a simultaneous mixed infestation with several whitefly species and also a brown soft scale (*Coccus hesperidum* L., 1758) was established, it is difficult to determine its causative agent.

The taxonomy and diagnosis of whiteflies is almost exclusively based on the microscopic morphological characteristics of the last (fourth) developmental stage of the larva from which adult emerges. The term "puparium" or "pupal case" is used for this stage. The morphology of the puparia/pupal cases of species from the subfamily Aleurodicinae is more complex than that of the subfamily Aleyrodinae, due to the presence of large dorsal complex pores that secrete wax. In Aleurodicinae species, the lingula is large and tongue-shaped, extending beyond posterior margin of vasiform orifice and bearing 4 long setae. Legs are without adhesion pads but each bears an apical claw. Puparia of Aleyrodinae have no dorsal compound pores, the lingula is much smaller bearing only 2 setae and the legs are without claws, but usually with adhesion pads. The adults of most Aleurodicinae species have a slightly more complex wing venation and sometimes their genitalia possess more characters than are to be found in members of the Aleyrodinae. Accordingly, morphological characters of adult Aleurodicinae are used taxonomically, particularly at the generic level. Adult males of the genus Paraleyrodes have extremely distinctive appendages on the aedeagal apex. Accordingly, adult males are required to confirm identifications based on morphological characteristics of puparium in most species (Martin 1996).

Based on the literature description (Iaccarino et al. 2011; Longo and Rapisarda 2014; Malumphy and Mifsud 2016; Mohan et al. 2019) and own microscopic observations, the main morphological characters of *P. minei* puparium (Figure 6) important for the identification are: length about 0,9 mm, width 0,6 mm, which is smaller than the most other genera of subfamily Aleurodicinae, subelliptic in shape; submargin with 14 pairs of long setae; dorsum with a single pair of cephalo-thoracic and four pairs of large abdominal compound pores (located at 5th to 8th abdominal segments), each of them secreting single long translucent wax rods upwards, breaking and depositing all around puparium forming the "nest-like" structures (Figure 7); outer ring of compound pores with ovoid cellular facets gives the appearance of stylized flower petals; two pairs of small compound pores (half of the size of large abdominal pores) situated dorsally at 3rd and 4th abdominal segments; operculum partially covers lingula and vasiform orifice; lingula spatulate-globose with two pairs of stout apical setae, extending beyond the posterior margin of the vasiform orifice.



- Figure 6 Microscopic slide of *P. minei* pupal case: a, cephalo-thoracic large compound pore; b, abdominal large compound pores; c, abdominal small compound pores; d, lingula
- Slika 6. Mikroskopski preparat egzuvija *P. minei*: a, cephalo-thorakalne velike složene pore; b, abdominalne velike složene pore; c, abdominalne male složene pore; d, jezičac



Figure 7 Puparium of *P. minei* with long upward wax rods exuded through compound poresSlika 7. Puparij *P. minei* s uspravnim, dugim štapićastim voštanim izlučevinama iz složenih pora

Adults of species of *Paraleyrodes* are considerably smaller than most other members of the Aleurodicinae and resemble whiteflies from the subfamily Aleyrodinae. They are distinguishable from other genera of subfamily Aleurodicinae in having unbranched radial veins on wings, reduced number of antennal segments (four segments in the females and three segments in the males), and complex aedeagal apices (Dubey 2019). Adults are yellow to pale orange, about 1,2 mm in length, with two pairs of wings covered with a mealy wax, held relatively flattish over the body (Malumphy and Mifsud 2016). By the additional microscopic analysis of the male genitalia and comparison with the drawings of Martin (1996), the identification of the sampled specimens as a species *P. minei*, based on morphological characteristics of puparium, was confirmed. As is the case in all members of the genus *Paraleyrodes*, male adults of *P. minei* can be recognized by the shape of aedeagus, whose apex, according to Longo and Rapisarda (2014), bears three dorsal and a single ventral horn, plus a pair of long ventral spines (Figure 8).

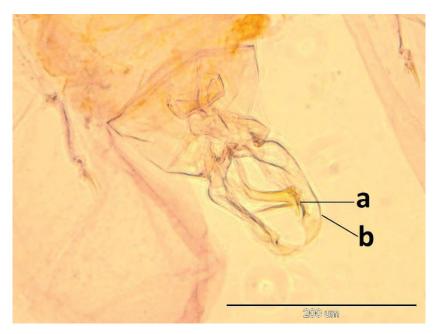


Figure 8 Microscopic slide of *P. minei* adult male genitalia (lateral view): a, aedeagus; b, parameresSlika 8. Mikroskopski preparat genitalija odraslog mužjaka *P. minei* (bočni prikaz): a, aedeagus; b, paramere

P. minei is a polyphagous pest species, feeding on mostly woody plants of numerous families. It is known and reported as a moderate pest of all main cultivated species of citrus (Longo and Rapisarda 2014). On the island of Vis, *P. minei* was detected only on mandarin and sweet orange trees. In Hong Kong, a small colony of *P. minei* was found on a most unusual host for any Aleurodicinae whitefly species – *Michantus sinensis* Andersson (Poaceae) (Hernandez-Suarez et al. 2012). However, the significance of feeding on grasses and other herbaceous plants is unclear. It is also a moderate pest of kaki (*Diospyros kaki* L.) (Malumphy and Mifsud 2016). In Spain, "alternative hosts" to citrus, apple, laurel, and tomato were recorded (Martin 1996). In the Canary Islands, *P. minei* is reported as a pest of ornamental palms (Longo and Rapisarda 2014). In California, it is a pest in commercial avocado plantations (Bellows, JR et al. 1998). Similar to other whitefly species, *P. minei* causes direct damage to infected plants by sucking plant sap and injecting toxic saliva. Sooty mould fungi grow on large amounts of excreted honeydew, subsequently affecting plant metabolism and lowering the production both in quantity and quality (Argov et al. 2012).

P. minei is a multivoltine whitefly species that develops three generations per year in laboratory conditions in Spain and four generations on *C. aurantium* in California. In Indian weather conditions it breeds throughout the year and completes more than three generations annually (Dubey 2019). Considering climatic conditions and the availability of host plants, it is assumed that in citrus plantations in the coastal part of Croatia, this species could develop several overlapping generations during the year.

Conclusion - Zaključci

P. minei is the last species discovered in the whitefly fauna of Croatia and so far the first from the subfamily Aleurodicinae. It is estimated that in the near future this new invasive pest will spread and cause additional phytosanitary problems in protection of citrus trees, next to the complex of five species (*D. citri, A. floccosus, P. myricae, A. aucubae* and *A. spiniferus*) already previously recorded in Croatia.

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