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Original scientific paper

Bionomy of *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), a new species in the fauna of Serbia

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SUMMARY

Citricola scale, *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae) was for the first time registered in Serbia in 2015 in the area of Belgrade on *Celtis occidentalis* L. *C. pseudomagnoliarum* develops one generation annually and overwinters as the second-instar nymph on host twigs. It forms numerous colonies on infested plants, and symptoms of its feeding appear in the form of drying leaves and twigs. In addition, large amounts of honeydew that this scale secretes reduce photosynthesis and transpiration in plants, which accelerates their decay. Citricola scale attracts many entomophagous insects which are able to reduce pest population. The parasitoid wasps *Coccophagus lycimnia* (Walker), *Coccophagus piceae* Erdos, *Coccophagus scutellaris* (Dalman), *Coccophagus shillongensis* (Hayat and Singh) (Aphelinidae), *Cheiloneurus claviger* Thomson and *Metaphycus stanleyi* Compere (Encyrtidae) were reared. *C. piceae* and *M. stanleyi* are new species in the fauna of Serbia. *C. pseudomagnoliarum* is a new host for the species *M. stanleyi*. The predators *Coccinella septempunctata* L., *Exochomus quadripustulatus* (L.) (Coccinellidae) and *Chrysoperla carnea* (Stephens) (Chrysopidae) were found in scale colonies. The most efficient natural enemy of *C. pseudomagnoliarum* nymphs was *C. lycimnia*, reducing scale populations by 11-26%.

Keywords: citricola scale, Coccidae, natural enemies, Serbia

INTRODUCTION

Citricola scale, *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), originates from Asia, and is now considered a cosmopolitan species. In Europe, it is most widespread in Mediterranean countries, where it causes significant damage to citrus fruits, especially lemons, oranges, tangerines, and grapefruits (Barbagallo, 1974; Tena & Garcia-Marí, 2008a; Kapranas, 2012; García-Morales et al., 2016). In addition, it was found on pomegranate (*Punica granatum* L.), walnut (*Juglans regia* L.), as well as many ornamental plants in the genera *Berberis*, *Celtis*, *Pinus*, *Tamarix* and *Ulmus* (Japoshvili et al., 2008; Batsankalashvili et al., 2017).

In the former Yugoslavia, *C. pseudomagnoliarum* was first registered in the 1970s on citrus trees in Bar, Tivat and Herceg Novi (Velimirović, 1985).

The pest causes considerable damage to infested plants, especially citrus species, by feeding on plant sap, and thereby causing a decline in growth, and physiological weakening of plants. Indirect damage is caused by honeydew excretion as it covers the aboveground plant organs, which enables sooty mold to settle. All infested plant parts turn black, resulting in reduced photosynthesis and transpiration. Infested fruits are smaller, stunted and unsightly, and their quality and market value are lower (Masten-Milek et al., 2017; Kondo, 2022).

Citricola scale populations are controlled by a number of natural enemies. Fourty-five of such parasitoid species belonging to the families Aphelinidae, Encyrtidae, Eulophidae and Pteromalidae (Hymenoptera: Chalcidoidea) have been registered (Noyes, 2019). Predators from the families Anthocoridae, Geocoridae (Hemiptera), Coccinellidae (Coleoptera), Noctuidae and Pyralidae (Lepidoptera) have also been identified (Öncüer, 1977; Lázaro-Castellanos, 2022; Saleh, 2022).

In 2015, *Coccus pseudomagnoliarum* was detected for the first time in Serbia on *Celtis occidentalis* L. at the Ušće location in Belgrade (Graora et al., 2016), which led to more detailed subsequent studies. This paper provides data on the presence, infestation intensity and harmfulness of citricola scale, as well as information on its morphology, development cycle, and presence of its natural enemies.

MATERIALS AND METHODS

The study of citricola scale bionomy was carried out on *Celtis occidentalis* L. at three locations in Belgrade (Ušće, New Belgrade, Zemun) and in Požarevac over the period 2015-2017.

The presence, distribution, intensity of *C. pseudomagnoliarum* infestation and symptoms of damage were determined by visual examination of plants and sampling of infested plant material. The intensity of infestation was determined using the Borchsenius scale (1963). Plant material was sampled every 7-10 days during the vegetative period, and once a month during plant dormancy. Five one- or two-year-old twigs, 20 cm long were sampled from each infested plant.

In the laboratory, we examined the sampled plant material, reared and made permanent microscopic slides of citricola scale, and identified the scale and its natural enemies.

To analyze the morphological characteristics of the scale, permanent microscopic slides of females were made following a method of Kosztarab & Kozár (1988), and species identification was performed using the identification keys of Gill (1988) and Kosztarab & Kozár (1988).

For rearing purposes, the sampled twigs with scale colonies were placed in glass cylinders covered with dense synthetic meshes. The time of oviposition, number of eggs laid, and duration of embryonic and postembryonic development of scales were monitored by daily examination of the twigs. The average number of eggs laid by females was determined by counting the eggs of 10 females.

In addition, plant material was examined under binoculars to determine scale parasitism. The percentage of parasitism was calculated using the formula $P = B \times 100 / a$, where P – parasitism percentage, B – the number of parasitized scales, and a – the total number of examined scales in all samples (Hadzibeyli, 1983). Plant material with scale colonies was then placed in glass cylinders for rearing parasitoids. Examination was performed daily to determine the time and number of eclosed parasitoid specimens. Wasps were collected using an aspirator, killed with ethyl acetate and stored in gelatin capsules; they were then mounted on cards and identified by the second author. The mounted specimens are preserved in the Laboratory for Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia.

Predator larvae collected with scales were reared individually in petri dishes in order to prevent cannibalism. Eclosed adults mounted on cards, and predatory ladybugs were identified using the Bieńkowski (2018) key.

RESULTS

The conducted analysis of morphological characteristics of female specimens led to conclusive identification of the species as *Coccus pseudomagnoliarum* (Kuwana, 1914).

Taxonomic status

Coccus pseudomagnoliarum belongs to the order Hemiptera, family Coccidae, subfamily Coccinae, tribe Coccini, genus Coccus (Choi & Lee, 2020).

Synonyms: *Lecanium (Eulecanium) pseudomagnoliarum*, Kuwana, 1914; *Coccus citricola*, Campbell, 1914; *Coccus aegaeus*, De Lotto, 1973a; *Coccus magnoliarum* (Kuwana, 1914) (García-Morales et al., 2016).

Morphological characteristics of *C. pseudomagnoliarum* female

The female has an elongated oval, convex body shape, 2-7 mm long. The color varies from gray to brown, with a central pale longitudinal ridge and two transverse pale bands extending from the stigmas. There are brown spots on the integument that merge with the edge of the female, so the scale takes on a marble pattern (Figure 1). The female antennae are 8-segmented. There are three setae in each group in the stigmatic depressions, and the central one is much longer than the lateral ones and curved at the top. Discoid pores are scattered throughout the body. Simple discoid pores form a transverse band on the abdomen. Quinquelocular pores are present in the area of stigmas. Multilocular pores are located in the area around anal plates.

C. pseudomagnoliarum life cycle

In the course of research, the species was found to reproduce by parthenogenesis and develop one generation annually. Its second-instar nymphs overwinter on twigs of *C. occidentalis*. In the spring, during March and April, nymphs continue feeding, and after molting they form females (Figure 1). The first appearance of females was recorded at the end of April in 2015 and 2017 and at the beginning of May in 2016 (Table 1). Females feed intensively on plant sap over the following three to four weeks, and then start laying eggs at the end of May. *C. pseudomagnoliarum* females are ovoviviparous. They lay eggs individually under their body. The duration of embryonic development depends primarily on weather conditions. When the weather is warm, nymphs hatch within an hour, while in cold and rainy weather they hatch after a day. The oviposition period is quite long and lasts from the end of May to the end of June. During that period, eggs laid and already hatched nymphs are located under the female scales. The average number of eggs laid per female is 799.6±3.3. The hatched nymphs migrate to leaves to feed there during the summer months, mostly on the reverse side of leaf along its main veins (Figure 2). Due to the extended egg-laying period, first-instar nymphs are present on leaves almost until mid-September, when they molt, forming second-instar nymphs (Figure 3). Second-instar nymphs continue to feed on leaves until October, when they descend to thicker twigs to overwinter (Figure 4).

Figure 1. Female of *C. pseudomagnoliarum* (orig.)

Figure 2. First-instar nymphs of *C. pseudomagnoliarum* (orig.)

Figure 3. Second-instar nymphs of *C. pseudomagnoliarum* (orig.)

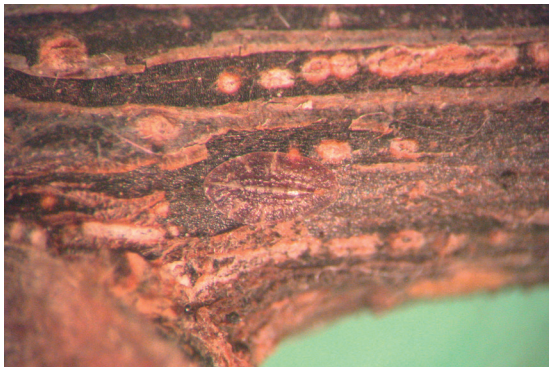

Figure 4. Overwintering second-instar nymphs (orig.)

Table 1. The life cycle of *Coccus pseudomagnoliarum* on *Celtis occidentalis*

Development stage	Year		
	2015	2016	2017
female	27.04.	04.05.	24.04.
egg	28.05.	25.05.	22.05.
N_1	29.05.	26.05.	22.05.
N_2	18.09.	15.09.	10.09.

N_1 – first-instar „crawler"
N_2 – second-instar

Figure 5. Colony of *C. pseudomagnoliarum* on *C. occidentalis* (orig.)

Figure 6. *C. occidentalis* covered with honeydew (orig.)

C. pseudomagnoliarum distribution, infestation intensity and damage symptoms

C. pseudomagnoliarum was found on *C. occidentalis* at three locations in Belgrade (Ušće, New Belgrade, Zemun) and a location in Požarevac. The highest intensities of infestation at all locations in Belgrade and Požarevac were level 3 and 4, so that the aboveground plant parts were covered with dense scale colonies (Figure 5). *C. pseudomagnoliarum* nymphs generally cluster on leaves along their main nerves, while females inhabit the twigs of host plants. As a result of feeding, the observed symptoms include chlorotic spots and lesions, discoloration and premature leaf fall, and drying of individual twigs. Apart from direct damage caused by feeding, *C. pseudomagnoliarum* also produces large amounts of honeydew (Figure 6), which is a suitable substrate for development of sooty mold that reduces photosynthesis and transpiration, devestating plant aesthetics.

Natural enemies of *C. pseudomagnoliarum*

During the research, 6 species of parasitoid wasps (Hymenoptera: Chalcidoidea) were found and reared from *C. pseudomagnoliarum* colonies. Four species were identified as belonging to the genus *Coccophagus* (family Aphelinidae): *C. lycimnia* (Walker), *C. piceae* Erdos, *C. scutellaris* (Dalman), and *C. shillongensis* (Hayat and Singh). Another two species, *Cheiloneurus claviger* Thomson and *Metaphycus stanleyi* Compere of the family Encyrtidae, were also observed (Table 2). In this study, two parasitoid wasps, *C. piceae* and *M. stanleyi*, were detected for the first time in the fauna of Serbia. For the species *C. piceae*, citrus scale is a new host. In the family Aphelinidae, the most numerous and efficient natural enemy was *C. lycimnia*, whose parasitism in second-instar nymphs of citricola scale was 11-26%. In the family Encyrtidae, the newly-identified species *M. stanleyi* was the most numerous as a gregarious parasitoid.

Three distinct predator species were successfully reared and identified during the study: *Coccinella septempunctata* L., *Exochomus quadripustulatus* (L.) (Coleoptera, Coccinellidae) and *Chrysoperla carnea* (Neuroptera, Chrysopidae). These species were individually present in *C. pseudomagnoliarum* colonies (Table 2). Larvae and adults of these predators fed on all developmental stages of citricola scale.

Table 2. Natural enemies of *C. pseudomagnoliarum*

Order	Family	Species	Location	Total eclosed individuals
Hymenoptera	Aphelinidae	*Coccophagus lycimnia*	New Belgrade	37
			Ušće	37
			Zemun	1
		*Coccophagus piceae**	Ušće	5
		Coccophagus scutellaris	New Belgrade	1
		Coccophagus schillongensis	New Belgrade	2
			Ušće	5
	Encyrtidae	*Cheiloneurus claviger*	Ušće	3
		*Metaphycus stanleyi**	New Belgrade	20
			Ušće	383
Coleoptera	Coccinelidae	*Coccinella septempunctata*	Ušće	2
		Exochomus quadripustulatus	Ušće	1
			New Belgrade	2
Neuroptera	Chrysopidae	*Chrysoperla carnea*	Ušće	2

*new species in Serbia

DISCUSSION

After its first detection in Serbia in 2015 on *C. occidentalis* at the location Ušće (Belgrade), *C. pseudomagnoliarum* was found at two additional locations in the territory of Belgrade (New Belgrade and Zemun), and also in Požarevac. Significant citricola scale infestations were observed at all of these locations, forming numerous colonies on infested plants. Extensive feeding of numerous nymphs and females caused leaves to dry out and fall off, and individual twigs to dry out.

C. pseudomagnoliarum has been detected on over 30 host plants from 16 families worldwide (García-Morales et al., 2016) but it most commonly causes high damage with economic impact in citrus-growing areas (Velimirović, 1985; Bernal et al., 2001; Mohamed et al., 2012). In Croatia, it is a widespread pest that inflicts significant damage on citrus fruits. Through its sap-sucking activities, the pest induces substantial weakening of host plants, leading to their decay (Masten-Milek, 2007; Masten-Milek et al., 2017). In California, it has been found to cause a 43% decrease in orange yields (Grafton-Cardwell et al., 2022). In addition, an intense infestation by this pest was detected in a commercial citrus orchard in Croatia, where neither agrotechnical nor chemical control measures had been applied. It is hypothesized that the lush canopy of fruit trees along with high humidity in the canopy, create favourable conditions for intensive reproduction of this species (Markotić, 2023).

In Serbia, *C. pseudomagnoliarum* develops one generation per annum and reproduces by parthenogenesis, which is consistent with data from Israel (Ben-Dov, 1980), Montenegro (Velimirović, 1985), Hungary (Fetykó et al., 2013), Croatia (Masten-Milek et al., 2017), Greece (Stathas & Karipidis, 2020) and California (Gill, 1988). Its females are ovoviviparous. Oviposition was observed from the end of May to the end of June, and females were found to lay an average of about 800 eggs each. In Hungary, a female lays between 250 and 280 eggs from mid-May to mid-July (Fetykó et al., 2013). Its nymphs hatch within a few hours and promptly migrate to plant leaves, where they feed until the end of summer. After this period they molt and form second-instar nymphs, which descend onto twigs to prepare for overwintering there. Typically, the species overwinters as the second-instar nymph, while young overwintering females were reported in Italy by Barbagallo (1974).

The abundance of *C. pseudomagnoliarum* is regulated by numerous natural enemies. In this study, 6 species of parasitoid wasps and 3 species of predators were reared from colonies of this scale. Among the identified species of parasitoids are four species from the genus *Coccophagus* (family Aphelinidae). This genus includes about 200 described species that are parasitoids of scale insects, most commonly from the family Coccidae, but they have also been found to be parasitoids of Diaspididae, Pseudococcidae, Eriococcidae, and Kermesidae (Hayat, 1997; Noyes, 2019). Females of the genus *Coccophagus* are endoparasitoids of scales, while males can also be

secondary hyperparasitoids (Kapranas et al., 2007). Most species are polyphagous and parasitize different hosts, and some species are described as potential biological agents for scale insect control (Schweizer et al., 2003). During our survey, *C. lycimnia* demonstrated the highest efficiency as a parasitoid targeting second-instar nymphs, with parasitation levels reaching 11-26%.

Among the established species of this genus, *C. piceae* is new to the fauna of Serbia. According to literature data, *C. piceae* has been identified on *Physokermes piceae* (Schrank), *Pulvinaria vitis* (L.), *Pulvinaria* sp. and *Didesmococcus unifasciatus* (Archangelskaya) (Ülgentürk, 2001; Bolu, 2012; Noyes, 2019). Notably, during this study, *C. pseudomagnoliarum* was determined as a new host for this parasitoid. Furthermore, the discovery of the species *C. shillongensis*, which was registered for the first time in Serbia in 2016, is also of significant importance. At the time, it was found on five other new hosts besides *C. pseudomagnoliarum* (Dervišević et al., 2021). The first finding of the species *M. stanleyi* in the genus *Metaphycus* was also reported. Up to 20 specimens of this gregarious parasitoid wasp were observed to hatch from a single *C. pseudomagnoliarum* female. Generally, one or more larvae of this parasitoid may develop in a female scale, depending largely on body size of the host (Bernal et al., 1999). *M. stanleyi* is the primary endoparasitoid for over 30 species of the Coccidae family (Noyes, 2019). As a biological control agent, it was introduced from South Africa to California in 1937 to control *Saissetia oleae* (Olivier) (Bartlett, 1978).

Along the Montenegrin coast, the species *C. lycimnia* and *Metaphycus flavus* (Howard) have significantly reduced the number of citricola scale populations (Velimirović, 1994). In Greece, two species of parasitoids, *Coccophagus shillongensis* and *Metaphycus dispar* (Mercet), reached a parasitism rate of 35% (Stathas & Karipidis, 2020), while in Spain the species *Metaphycus helvolus* (Compere) exhibited a noteworthy parasitism rate of 50% (Tena & García-Marí, 2008b).

Besides, the predators *Coccinella septempunctata*, *Exochomus quadripustulatus* (Coleoptera, Coccinellidae) and *Chrysoperla carnea* (Neuroptera, Chrysopidae) were also reared. However, all predatory species were present in relatively low numbers in scale insect colonies. Numerous predators of *C. pseudomagnoliarum* have been recorded worldwide, including the ladybugs *Chilocorus bipustulatus* (L.), *C. renipustulatus* (Scriba), *Cryptolaemus montrouzieri* (Mulsant), *Exochomus quadripustulatus*, *Serangium parcesetosum* (Sicard), and *Rhyzobius lophanthae* (Blaisdell) (Deeb et al., 2017; Basheer et al., 2022; Saleh, 2022). *C. bipustulatus* and *S. parcesetosum* were found to reduce the number of scale nymphs under controlled conditions by 97.8 and 99.2%, respectively (Deeb et al., 2017).

CONCLUSION

The data in the present study provide information concerning the biology of and damage caused by *C. pseudomagnoliarum,* which is considered a new pest in Serbia. Although the scale is currently found only on *C. occidentalis*, it may be considered as a potentially serious threat after being reported as an important pest of citrus and ornamental plants in many parts of the world. Also, data on the diversity and number of natural enemies are significant for the control of *C. pseudomagnoliarum,* specifically in urban areas, where chemical pest control measures are rarely applied.

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Bionomija *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), nove vrste u fauni Srbije

REZIME

Tokom 2015. godine, u Srbiji je prvi put registrovana štitasta vaš citrusa, *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), na *Celtis occidentalis* L. u Beogradu. *C. pseudomagnoliarum* tokom godine razvija jednu generaciju i prezimljava u stadijumu larve drugog stupnja na grančicama domaćina. Na infestiranim biljkama obrazuje brojne kolonije, usled čije ishrane se javljaju simptomi u vidu sušenja listova i grančica. Osim toga, velika količina medene rose koju ova vaš luči, smanjuje fotosintezu i transpiraciju biljaka što ubrzava njihovo propadanje. Štitasta vaš citrusa privlači brojne entomofagne insekte koji mogu redukovati brojnost njenih populacija. Odgajene su parazitoidne osice *Coccophagus lycimnia* (Walker), *Coccophagus piceae* Erdos, *Coccophagus scutellaris* (Dalman), *Coccophagus shillongensis* (Hayat and Singh) (Aphelinidae), *Cheiloneurus claviger* Thomson i *Metaphycus stanleyi* Compere (Encyrtidae). *C. piceae* i *M. stanleyi* su nove vrste u fauni Srbije. *C. pseudomagnoliarum* je novi domaćin za vrstu *M. stanleyi*. Od predatora, utvrđene su vrste *Coccinella septempunctata* L., *Exochomus quadripustulatus* (L.) (Coccinellidae) i *Chrysoperla carnea* (Stephens) (Chrysopidae). Najefikasniji prirodni neprijatelj larvi *C. pseudomagnoliarum*, bila je *C. lycimnia*, redukujući brojnost populacija za 11-26%.

Ključne reči: štitasta vaš citrusa, Coccidae, prirodni neprijatelji, Srbija