

Study of Distinctive Diagnostic Characteristics of *Chrysomphalus dictyospermi* and Closely Related Species of *Chrysomphalus* Genus (Insecta: Sternorrhyncha: Coccoidea)

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Abstract. The paper presents the results of microscopic study of the basic diagnostic structures of the pygidium of the *Chrysomphalus dictyospermi*, which allow to distinguish this species from the closely related ones of the *Chrysomphalus* genus.

Keywords. *Scales, Chrysomphalus dictyospermi, pygidium, lobes, plates, perivulvar pores, paraphysis.*

Introduction

The object of the study was the brown scale insect *Chrysomphalus dictyospermi* (Morgan, 1889), which belonging to the group of insects (Insecta: Sternorrhyncha: Coccoidea). By the decision of the Council of the Eurasian Economic Union No. 158 of November 30, 2016, the Unified List of Quarantine Objects of the Eurasian Economic Union had been approved, in which subsequently dictyospermum scale *Chrysomphalus dictyospermi* was included. Earlier this object was included into the list of quarantine objects that are absent in the Republic of Kazakhstan. This List was put into effect on July 1, 2017 in the territory of all EEU countries. With an increase in the import of planting material for open and protected ground, the risk quarantine pests distribution increases. Detection of quarantine objects on plant products, laboratory research, precise species diagnosis of the object are the main stages of phytosanitary procedures.

Purpose of this paper — study of distinctive diagnostic characteristics of dictyospermum scale and closely related species of *Chrysomphalus* genus.

Aims of the paper:

- microscopic study of the female pygidium structure of the *Chrysomphalus dictyospermi* using modern microscopic technologies to identify the main diagnostic features;
- presentation of illustrative material indicating identified diagnostic structures;
- comparative analysis of the diagnostic structures of the *Chrysomphalus dictyospermi* and closely related species: *Chrysomphalus aonidum*, *Chrysomphalus bifasciculatus* and *Chrysomphalus pinnulifer*.

The obtained data will make it possible to reveal distinctive diagnostic features of the dictyospermum scale female from closely related non-quarantine scales species found on similar plant products from distribution countries.

General information about the pest

Chrysomphalus dictyospermi is a polyphage that causes damage to plants of about 80 families (ScaleNet, 2020). Dictyospermum scale feeds on the following forage crops: citrus crops (family: Rutaceae), rose (family: Rosaceae), palms (family: Arecaceae), oil-bearing plants (family: Oleaceae), asparagus (family: Asparagaceae), mulberry (family: Moraceae), legumes (family: Fabaceae), myrtle (family: Myrtaceae), grapes, fruit, subtropical and tropical plants, including potted plants (Cabi, 2020). This scale species is most harmful to citrus and subtropical crops, causing leaf fall, reduced yield and commercial quality of fruits.

Materials and methods

The diagnostic characteristics of the *Chrysomphalus dictyospermi* female were studied under a microscope (Carl Zeiss Microscopy GmbH). The material for the study was the slides of *Chrysomphalus* sp. from the collection fund of the FGBU “VNIKR” and scientific collections of plant material with brown shield colonies.

The data of CABI, ScaleNet and scientific publications on *Diaspididae* family were used for the comparative analysis of the diagnostic characteristics of *Chrysomphalus* sp.

Results

According of the study results, the main diagnostic micro-signs of pygidium of females of the genus *Chrysomphalus* are the following: the number of lobes, the peculiarities of the structure of plates, the presence of paraphyses and perivulvar pores.

The description of the main diagnostic features characteristic of the shields of the *Chrysomphalus* genus, as well as the necessary terms and definitions used in the diagnosis of the shields are presented in table 1.

Table 1 - Main terms and description of diagnostic features of *Chrysomphalus* genus scales (Danzig, 1993)

Diagnostic macro/microcharacteristics	Description
1. Scale cover	Round, more or less convex. Larval skins in the center of the scale cover, the 1st skin is raised in the crater form. The <i>Chrysomphalus dictyospermi</i> have size 2-2.2 mm.
2. Body of the female	The body of the female is wide-pear-shaped, weakly sclerotized.
3. Pygidium	Pygidium is the posterior part abdomen of the insect's, consisting of several sclerotized segments merged together. On the ventral surface of the pygidium there is a vaginal opening in the form of a slot, on the dorsal - anal, rounded shape. There are glands on both surfaces of the pygidium. Along the edge of the pygidium, in its rear part there are lobes and plates. Chrysomphalus genus have the edge of the pygidium is

Diagnostic macro/microcharacteristics	Description
	sclerotized and finely serrated in front of the plates.
4. Lobes	<p>Lobes are wide, strongly sclerotized protrusions located symmetrically along the edge of the pygidium. Lobes are denoted by the letter (L), for example, L₁ - the first pair of lobes.</p> <p>In the lobes of the genus <i>Chrysomphalus</i> pygidium with 3 pairs the similar size and shape; they are slightly asymmetric, the outer edge at L₁ and L₂ have small clippings, and L₃ finely serrated.</p>
5. Plates	<p>Plates are film-shaped, slightly sclerotized processes of various shapes, located in the indentations of pygidium between the lobes and very often along the edge of pygidium in front of the outmost lobes.</p> <p>The genus <i>Chrysomphalus</i>, the middle and lateral plates are large, branched, of the same length as the lobes, the front plate are longer than the lobes, of different shapes.</p>
6. Indentations	Indentations – sections of the edge of the pygidium between the lobes. In indentations are located the plates.
7. Paraphysis	<p>Paraphyses are chitinic thickenings located on the edge of pygidium, best expressed along longitudinal lines on the continuation of the lobes on both sides of the pygidium indentations.</p> <p>The genus <i>Chrysomphalus</i>, paraphyses are well developed, elongated, spindle-shaped.</p>
8. Dorsal ducts	<p>Dorsal ducts, are ducts located in oblique rows, in groups or randomly on the dorsal, less often on the ventral, surface of the pygidium and on the surface of other segments - the abdomen and thorax.</p> <p>The dorsal ducts of the genus <i>Chrysomphalus</i>, are long. They are numerous on pygidium and are of different width: several ducts located at the top of the pygidium are wider than the others.</p>

Diagnostic macro/microcharacteristics	Description
9. Ventral ducts	Ventral ducts located along the edge of the pygidium, sometimes located on other segments of the abdomen and (occasionally) thorax. the ventral ducts of the genus <i>Chrysomphalus</i> , are much smaller than the dorsal ducts
10. Perivulvar pores	The perivulvar pores, small ducts have disc-shaped and five-cell, located in groups around the vaginal area. Perivulvar pores present or absent the genus <i>Chrysomphalus</i>
11. Anal area (Anus)	The anal opening is small, moved away from the apex of pygidium

The main macro signs of the shields of the genus *Chrysomphalus* include the following: the shape and size of the scale cover, its color, number and location of larval skins included in scale cover, the color and shape of the female's body. Macro characteristics of brown shield females are shown in Table 2.

Table 2 - Description of macro signs of female *Chrysomphalus dictyospermi* (Dantsig, 1993)

Diagnostic macrocharacteristics	<i>Chrysomphalus dictyospermi</i>
Scale cover shape	A weak convex
Scale cover size, mm	1,5-2,0
Scale cover color	Brown with a copper tint
Location of larval skins, composing the scale cover	In the center of the scale cover
Live female body color	Yellow
Body shape	Pear-shaped



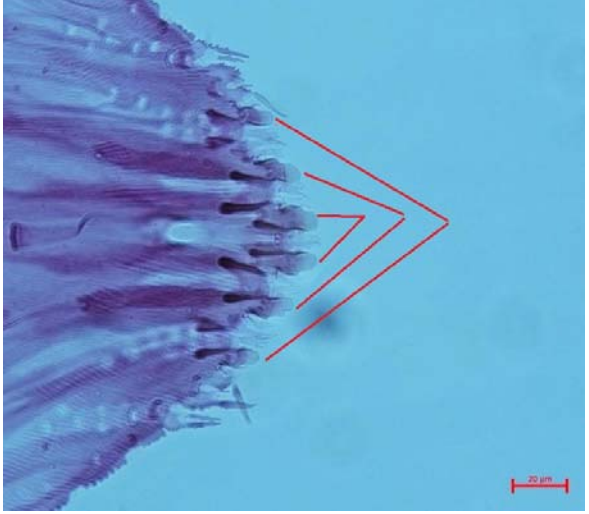

Fig. 1. A colony of *Chrysomphalus dictyospermi* (photo authors A.V. Shipulin, N.A. Gura)



Fig. 2. Body of female of *Chrysomphalus dictyospermi* (photo authors A.V. Shipulin, N.A. Gura)

Next, Table 3, Figure 3 shows an illustrative material from a microscopic examination of the pygidium of a brown shield female indicating the main diagnostic structures detected on the microscope slides.

Table 3 The main diagnostic micro-signs of the brown shield *Chrysomphalus dictyospermi* detected on the micropreparation slides

Dagnostic microcharacteristics	Illustrations
A) 3 pairs of elongated lobes on pygidium	
B) Features of the structure of plates in pygidium indentations: the first 2 plates of the front group with a solid (non-bifurcated) club-shaped or lanceolate apex (indicated in the red circle)	

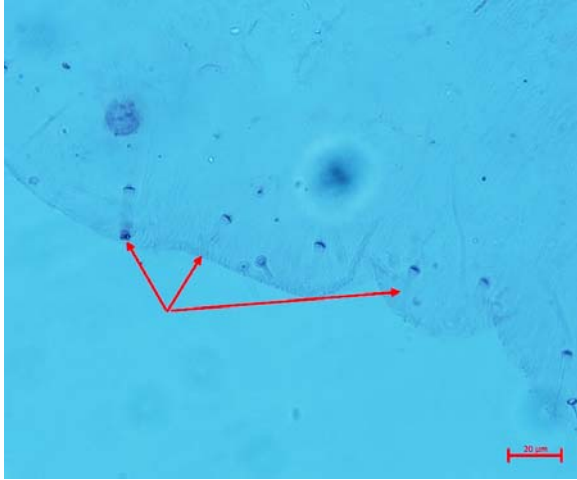
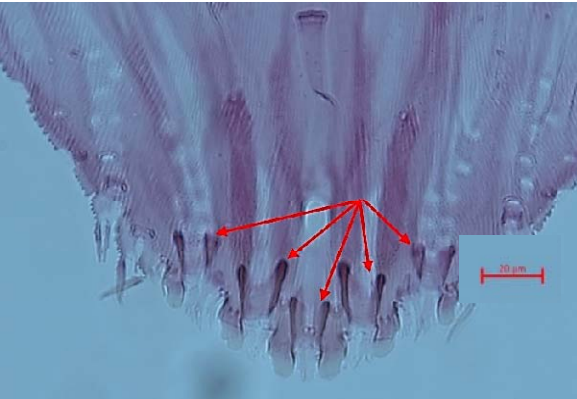





Dagnostic microcharacteristics	Illustrations
C) Microducts along the edge of the abdomen segment (I-III)	 <p>A light micrograph showing the edge of an abdomen segment. Several small, dark, circular structures (microducts) are visible along the edge. Red arrows point to these structures. A scale bar in the bottom right corner indicates 20 μm.</p>
D) Paraphysis present. 5 pairs	 <p>A light micrograph showing a close-up of the paraphyses, which are small, hair-like structures. Five pairs of paraphyses are visible. Red arrows point to these structures. A scale bar in the bottom right corner indicates 20 μm.</p>
E) Perivulvar pores present. (7-8) 3-4.	 <p>A light micrograph showing a close-up of the perivulvar pores, which are small, circular structures. Three to four pairs of perivulvar pores are visible. Red arrows point to these structures. A scale bar in the bottom right corner indicates 20 μm.</p>

Fig. 3. The main diagnostic micro-signs of the female *Chrysomphalus dictyospermi* (photo A-E - authors A.V. Shipulin, N.A. Gura)

Tables 4-5 show comparative analysis and illustrative material of diagnostic macro- and micro-signs of *Chrysomphalus dictyospermi* and closely related species.

Table 4 Comparative analysis of macrocharacteristics of closely related *Chrysomphalus* sp.

(https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/introduction/topic.php?id=3377&epi=155)


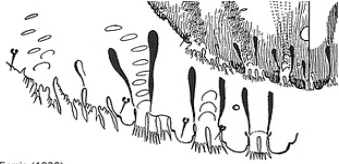

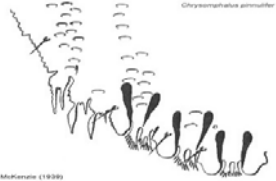
Closely related species/diagnostic macrocharacteristics	<i>Chrysomphalus dictyospermi</i>	<i>Chrysomphalus aonidum</i>	<i>Chrysomphalus bifasciculatus</i>	<i>Chrysomphalus pinnulifer</i>
Scale cover shape	Round scutellum	Round scutellum	Round scutellum	Round scutellum
Scale cover size, mm	1,5-2,0	1,5-2,5	1,5-2,5	1,5-2,0
Scale cover color	brown, often with a copper tint	dark brown with reddish-brown central edging	from dark gray to black, larval skins of reddish tint	The central part of the shield is dark-brown and pale brown along the edge of the scale cover
Location of larval skins, composing the scale cover	In the center of the scale cover 	In the center of the scale cover 	In the center of the scale cover 	In the center of the scale cover 
Live female body color	Yellow	Yellow	Yellow	Yellow

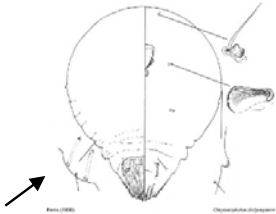
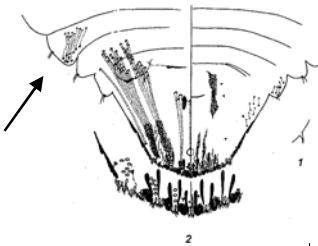
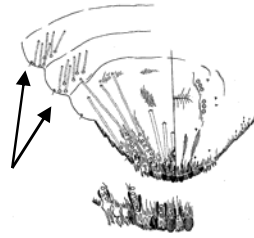
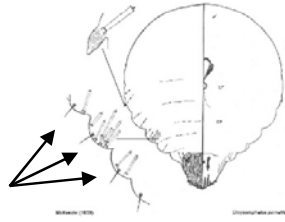
Among the main microcharacteristics of female pygidium of *Chrysomphalus* genus, the following have been analyzed: the number of lobes and their shape, the presence of paraphyses and their structure, the presence or absence of perivulvar pores. The results of comparative analysis of diagnostic microcharacteristics of four species of *Chrysomphalus* sp. scales are presented in Table 5.

Table 5 Comparative analysis of microcharacteristics of closely related *Chrysomphalus* sp.

(https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/introduction/topic.php?id=3377&epi=155)

Closely related species/diagnostic microcharacteristics	<i>Chrysomphalus dictyospermi</i>	<i>Chrysomphalus aonidum</i>	<i>Chrysomphalus bifasciculatus</i>	<i>Chrysomphalus pinnulifer</i>
Number of lobules	3 pairs	3 pairs	3 pairs	3 pairs
Features of plates structure	In the 1st and 2nd indentations, on pygidium is located on the 2 of wide plates, in the 3rd indentations - 3 plates. Each of the first two plates lying in front of the 3rd lobes has one long club-shaped or lance-shaped branch (Danzig.1993, Pesotskaya, 1959)	The front plates are strongly serrated with a comb apex (Danzig, 1993)	In the 1st and 2nd indentations of pygidium there are two, in the third indentations of pygidium-three wide serrated scallops. Further along the edge of the pygidium are three branched scallops (Tereznikova, 1986)	The first two plates of the front group with a smooth club-shaped apex (Danzig, 1993)

Closely related species/diagnostic microcharacteristics	<i>Chrysomphalus dictyospermi</i>	<i>Chrysomphalus aonidum</i>	<i>Chrysomphalus bifasciculatus</i>	<i>Chrysomphalus pinnulifer</i>
Presence and characteristics of paraphyses	<p>Paraphysis are present, 5 pairs, the vertices of the 2nd pair do not reach the anal opening</p>  <p>Ferris (1938) https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/species/taxon.php?id=113063,</p>	<p>Paraphysis are present, with each paraphyse longer than the median lobes</p>  <p>Ferris (1938) https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/species/taxon.php?id=113061</p>	<p>Paraphysis are present, with each paraphyse longer than the median lobes</p>  <p>Ferris (1938) https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/species/taxon.php?id=113062</p>	<p>Paraphyses are present, with each paraphyse longer than the median lobes</p>  <p>Mohamedali (1938) https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/species/taxon.php?id=113064</p>

<p>Features of the location of marginal groups of ducts on the prepygidium segments</p>	<p>There are no ducts, there are separate small glands along the very edge of the body</p> 	<p>Along the edge of the II segment of the abdomen there is a group of 10-15 small ducts</p> 	<p>On the II and III segments of the abdomen there are groups of 3-10 ducts</p> 	<p>Prepygidial segments with 5 ducts on each side</p> 
<p>Presence or absence of perivulvar pores</p>	<p>7-8 (3-4) (Danzig,1993) (3-4) 1-3 (Pesotskaya, 1959)</p>	<p>0-2 (7-8) 3-4 (Danzig,1993)</p>	<p>(3-4) 2-4 (Tereznikova, 1986)</p>	<p>No data</p>

Conclusions

1 Based on the results of a study of the structure of the dictyospermum scale female pygidium, an illustrative material of the main diagnostic features identified on a microslide is presented.

2 Diagnostic macro- and microfeatures of four closely related species of *Chrysomphalus* genus are analyzed and given in the form of comparative tables, which allows us to reliably distinguish a quarantined object from a non-quarantine species of scales during laboratory entomological study, which is an important part of phytosanitary procedures. This paper can be used by quarantine and plant protection specialists involved in the diagnosis of detected organisms.

References

1. Dantsig E.M. 1993. Coccinea suborder. Phoenicoccidae and Diaspididae families // Fauna of Russia and neighboring countries. Homoptera insects. Vol. 10. SPb: Nauka.
2. Pesotskaia E.A., Iakovleva N.S. 1959. Identifier of citrus fruit pests and diseases. M.: USSR Ministry of Agriculture.
3. Konstantinova G.M., Kozarzhevskaya E.F. Shchitovka. 1990. Pests of fruit and ornamental plants. M.: VO "Agropromizdat,". P.160.
4. Tereznikova E.M. Shchitovka. 1986. In book: Fauna of Ukraine. T. 20. Koktsida. Issue. 20. Kiev: Naukova Duma, P. 132.
5. Chou, I. 1947. A Study on the Genus *Chrysomphalus* of China. (Hemip. Homop. Coccidae). *Entomologia Sinica*, 2, (in Chinese). 9–24 pp.
6. Smith-Pardo A. H., Evans G. A., Dooley J. W. 2012. A review of the genus *Chrysomphalus* Ashmead (Hemiptera: Coccoidea: Diaspididae) with descriptions of a new species and a new, related genus // *Zootaxa*. T. 3570. №. 1: 1-24 pp.
7. Ferris, G. F. 1938. Atlas of the Scale Insects of North America. Series 2. Stanford University Press, Palo Alto, California.
8. Ferris, G.F. 1941. Atlas of the Scale Insects of North America. Series 3. Stanford University Press, Palo Alto, California.
9. Williams, D.J. & Watson G. W. 1988. The Scale Insects of the Tropical South Pacific Region. Part 1: The Armoured Scales (Diaspididae). CAB International Institute of Entomology, London, 290 pp.
10. [Electronic resource]. Access mode: ScaleNet: <http://scalenet.info/catalogue/>.
11. [Electronic resource]. Access mode: CABI. <https://www.cabi.org>.
12. [Electronic resource]. Access mode: https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/introduction/topic.php?id=3377&epi=155