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Comparative morphology of scales in *Cionus* Clairville, 1798 (Coleoptera: Curculionidae) species

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Abstract
The external morphological characters of scales in *Cionus* (Clairville), 1798 (Curculionidae: Curculioninae) were examined by light and scanning electron microscope (SEM). Dried museum materials that are deposited in AEZMU Ent. (Zoology Museum of Ahi Evran University) were used in this study. Specimens were examined under the stereomicroscope (Olympus SZX-16) and measurements taken. Abdomens were removed to reach elytra by first softening the abdomen. Similarities and differences among the species were discussed. Although the scales look alike under the light microscope, they showed a rather different pattern with SEM. The fine structure of scales was shown to be useful for comparing species.

Keywords: Coleoptera, Curculionidae, *Cionus*, scales, morphology, SEM.

1. Introduction
Curculionidae is considered to be one of the most richest families in Coleoptera. The most of species in the family that except some species are phytophagous [1]. Larva and adults feed on plant organs such as: roots, stems, leaves and fruits. This group has a detrimental effect on crops and forest trees and can cause huge economic losses. Thus, this family has an economic importance [2, 3]. Although the phylogenetic classification of Curculionoidea is still in its infant stages, knowledge of the morphology of this large radiation of beetles also is relatively lacking. Few morphological reviews have been done at the family level, and even fewer have examined the subfamily level and below (beyond description of genital morphology) [4]. Morphological studies within the genus *Cionus* are limited and mostly restricted to the study of genital morphology, with a few exceptions (Sert, 2007; Freude *et al.*, 1983; Caldara, 2001) [5, 6, 7]. The tribe Cionini that included species of *Cionus* are recently used a sister-group of the Mecinini [6]. The members of *Cionus* Clairville, 1798 genus are ectophage. Larvae live in the open or hidden among several sheets. They are attack to *Verbascum*, *Scrofularia, Budleija* etc. and eat the leaves. The *Cionus* genus have forty species in Palearctic region [8].
In this paper, we present a detailed examination of the scales in *Cionus* Clairville, 1798 genus using both light and SEM. Separation can be best illustrated by utilizing the scanning electron microscope (SEM).

2. Material and methods
The six species of *Cionus* (Clairville), 1798 (Coleoptera: Curculionidae: Curculioninae) were used for the present study. They include *Cionus distinctus* Desbrochers, 1872; *Cionus hortulanus* (Fourcroy, 1785); *Cionus olens* (Fabricius, 1792); *Cionus olivieri* Rosenschöld, 1838; *Cionus thapsus* Fabricius, 1792 and *Cionus scrophulariae* (Linnea, 1758). The specimens were selected from the museum materials that had been collected from the central Anatolia in 2011. The material examined is deposited in the following collections: AEZMU Ent., Zoology Museum of Ahi Evran University, Kırşehir, Turkey. (*C. distinctus*) 22.VI.2013, Çiçekdağ-Kırşehir, M. Erbey leg., 1271m, 39056'86"N/34038'40"E; (*C. hortulanus*) 14.V.2012, Orta-Çankırı, M. Erbey leg., 1249m, 40075'56"N/33061'69"E; (*C. olens* and *C. olivieri*) 30.V.2013, Çandır-Yozgat, M. Erbey leg., 1109m, 39025'68"N/35054'74"E; (*C. thapsus* and *C. scrophulariae*) 31.V.2013, Gürün-Sivas, M. Erbey leg., 1083m, 38072'01"N/37017'27"E. The specimens were first retained in a pot for about 24 hours at 30 ^{0} for softening. Then semielytra of the specimens were removed from the abdomens and cleaned

through fine dissection. The samples were examined under light microscope. For examination under Scanning Electron Microscope, elytra were dried and mounted with double-sided carbon tape on SEM stubs, coated with gold in a Polaron SC 502 Sputter Coater, and examined with a JOEL JSM 6060 SEM operated at 10 kV. We then investigated the surface morphology of scales under x500 and x1500 magnifications. The classification that we used in this study is that of Alonso-Zarazaga and Lyal [9].

3. Results

Under the light microscope; the color and shape of the scales look like (Figs. 1a-e), except in *C. scrophulariae* (Fig. 1f). The scales distinctly yellow and nearly appear same shape (Figs. 1a-e), only in *C. olens*, interstria of the elytra have differently a series of erected scales (Fig. 1c). In *C. scrophulariae,* the shape of the scales oblong or longitudinal, the color brownish-black, and the scales more dense than the others (Fig. 1f). In all species, two big black spots in the sutur and several small spots in interstria look a like (Figs. 1a-f).

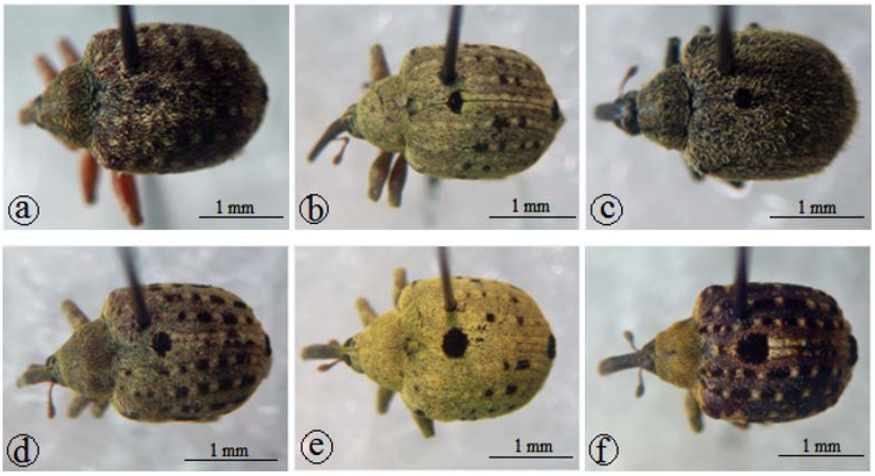

Fig 1: a- *Cionus distinctus, b-C. hortulanus, c- C. olens, d- C. olivieri, e- C. thapsus, f- C. Scrophulariae*

Under the scanning electron microscope; the details of the surface morphology of the scales seen clearly. The ultrastructure of scales that covered the body appear different from one to another. In *Cionus distinctus*, the scales are longitudinal, like elliptical, lateral margins serrate, the surface have three longitudinal process that are chitinized (Figs. 2a, 3a); in *C. hortulanus*, the scales longitudinal, parallel sided, apical part serrate, lateral side smooth, the surface has one longitudinal process, the hole of surface widen (Figs. 2b, 3b); in *C. olens*, the scales long and elliptical, apical part sharped, the surface of scales have two or three longitudinal process, the lateral side serrate near the apical part (Figs. 2c, 3c), the interstria have a row of scales that clearly erected (Fig. 4a); in *C. olivieri*, the elytra have short or long scales, but the most of scales short and wide, lateral side smooth or have one or two teeth, apical part distinctly serrate, the surface of scales have three longitudinal process (Figs. 2d, 3d), in *C. thapsus*, the scales long and smooth, the surface of scales have distinctly three process, lateral sides smooth (Figs. 2e, 3e), in *C. scrophulariae*, elytra have three different scales, the first type like hair, second type long and third type look like a plate that short and wide, they mixed in elytra

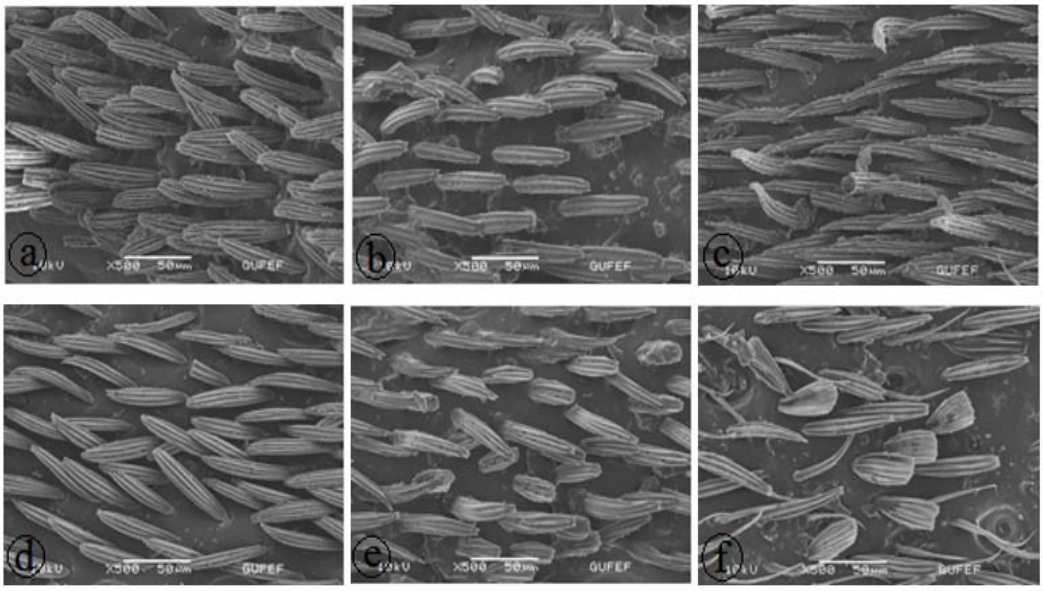

Fig 2: SEM photos of scales in species of *Cionus* genus at x500; a- *C. distinctus*, b- *C. hortulanus*, c- *C. olens*, d- *C. olivieri*, e- *C. thapsus*, f- *C. Scrophulariae*

(Figs. 2f, 3f), but short and wide scales exist some group in the interstria (Fig. 4c), in all species, the black spots existed a different scales that like plate, they are different from the other scales in surface of elytra (Fig. 4b). They are concave, nearly similar shape and have a little differences (Figs. 4d-f).

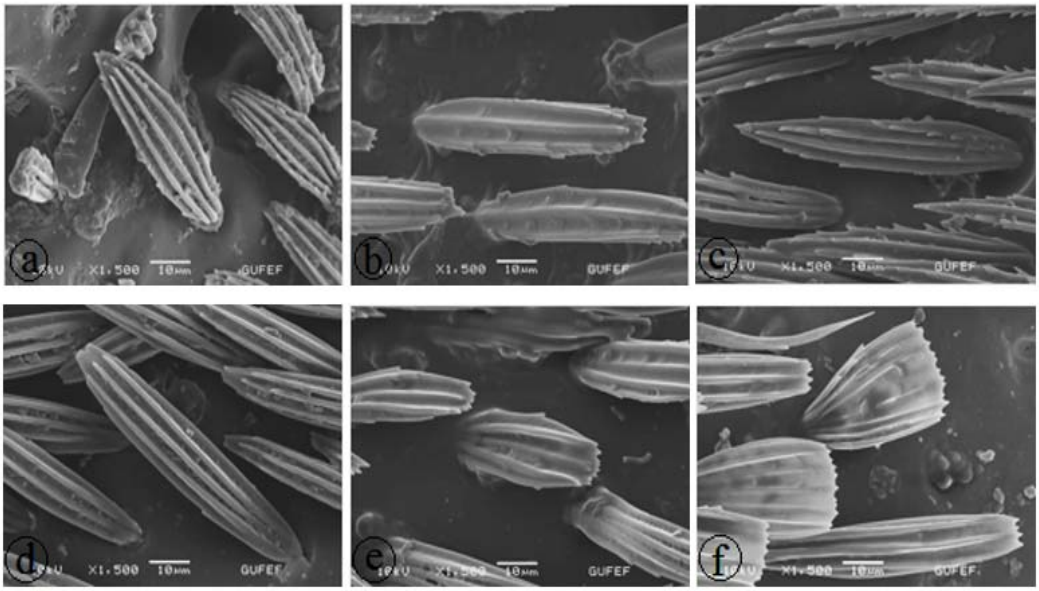

Fig 3: SEM photos of scales in species of *Cionus* genus at x1500; a- *C. distinctus*, b- *C. hortulanus*, c- *C. olens*, d- *C. olivieri*, e- *C. thapsus*, f- *C. scrophulariae*

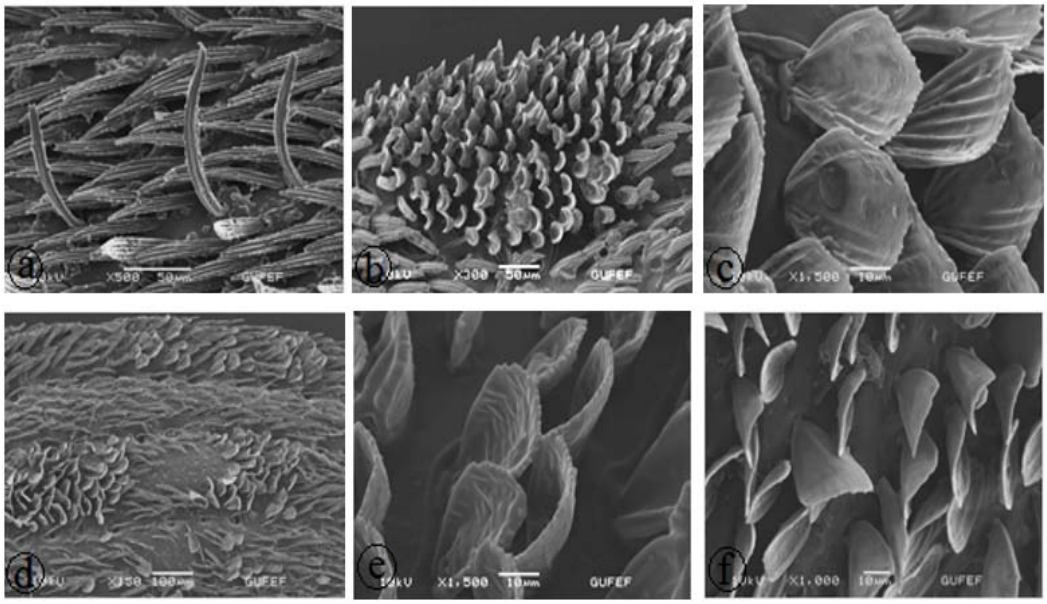

Fig 4: SEM photos of scales in species of *Cionus* genus; a- *C. olens*, b- *C. hortulanus*, c- C. *scrophulariae*, d- C. *scrophulariae*, e- *C. thapsus*, f- *C. Olivieri*

4. Discussion

In this study, the scales of six species of *Cionus* (Clairville), 1798 (Coleoptera: Curculionidae) were examined under light and scanning electron microscope in order to determine the differences and similarities among them. Several external characters as hairs, setae, scales, punctures and color were used for taxonomy [10, 11, 12, 13, 14, 15, 16, 17, 18]. In Curculionidae, many researchers have been using the scales of the insect for taxonomic studies, but they showed the simple drawing of shape and arrangement[9, 10, 15]. However, Sanchez-Ruiz and Alonso-Zarazaga[19] investigated the surface morphology of scales in all species of *Aspidiotes* Schoenherr, 1847 (Curculionidae: Entiminae) by the scanning electron microscope and used the morphology of scales in separation of species. Erbey and Candan[20] investigated the scales of *Tychius* Germar, 1817 (Curculionidae: Curculioninae) species with light and scanning electron microscope, and firstly determined that the surface morphology of scales are different in all species according to SEM micrograph. Also Erbey and Candan[21] investigated two species of *Brachypera* Capiomont, 1868 (Curculionidae: Hyperinae) and six species of *Hypera* Germar, 1817 (Curculionidae: Hyperinae) genera and showed surface morphology of scales by scanning electron microscope (SEM). In this group, we determined that the scales have distinct differences, such as, lateral sides are serrate (*C. distinctus*) (Fig. 3a), paralel sided and have two deep and wide grooves (*C. hortulanus*) (Fig. 3b), like elliptical, and have three longitudinal process and sharp point (*C. olens*) (Fig. 3c), have a short and wide scales (*C. olivieri*) (Fig. 4d), *C. scrophulariae* have three type scales (Figs. 2f and 3f), they are

different from the other scales. However, the details of these spots on elytral suture are not sufficiently seen under the light microscope (Figs. 1a-f), but the scales that exist to the spots like oval plate, and rather different from the other scales based on scanning electron microscope (Figs. 4b-f). Although the scales of spots are resemble, they have a little different details (Figs. 4c-f). Surface morphology of scales is an important character and it is useful for taxonomic studies along with other external morphological characters. The fine structures of scales may help in the differentiation of similar species and used for keys in insects, for example, Sanchez-Ruiz and Alonso-Zarazaga[19] used to the surface morphology of the scales for key in revision of *Aspidiotes* Schoenherr, 1847 (Curculionidae: Entiminae). Consequently, we think that electron microscope micrography can be used in many groups where the scales resemble each other in terms of shape and color.

5. Acknowledgement
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