SOME CYTOGENETIC OBSERVATIONS OF TWO *DORCADION* DALMAN, 1817 SPECIES (COLEOPTERA: CERAMBYCIDAE: LAMIINAE: DORCADIINI)

Atılay Yağmur Okutaner*, Hüseyin Özdikmen, Eşref Yüksel** and Yavuz Koçak*****

* Giresun University, Sebinkarahisar M.Y.O, Giresun / TURKEY. E-mail: atilayyagmur@gmail.com
** Gazi University, Faculty of Science, Department of Biology, 06500 Ankara / TURKEY. E-mails: (Ozdikmen) ozdikmen@gazi.edu.tr; (Yüksel) eyuksel@gazi.edu.tr
*** Ahi Evran University, Faculty of Science & Arts, Department of Biology, Kırsehir, TURKEY. E-mail: ykocak@gmail.com

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ABSTRACT: The paper gives some cytogenetic observations of *Dorcadion* (*Cribridorcadion*) *anatolicum* Pic, 1900 and *Dorcadion* (*Cribridorcadion*) *scabricolle* (Dlman, 1817) as the first time. Distribution in Turkey with a map and World, chorotype classification of the species, mitotic and meiotic metaphase plaques and karyogram are also given in the text.

KEY WORDS: Cytogenetic, meiotic metaphase, mitotic metaphase, karyogram, *Dorcadion anatolicum, Dorcadion scabricolle,* Lamiinae, Cerambycidae.

As known, approximately 1.500.000 species of animals have been described worldwide up to now. 80 % of them consist of insects. At the first views, carried out cytogenetic works have a minor number which is covered only about 1 % of all species. Thus, this considered to be too small.

Cytogenetic works had started in the XIXth century. The number of chromosomes at most of the insects is changed between n = 4 and n = 20 [extremely n = 1 (*Myrmecia croslandi* Taylor, 1991: Formicidae: Hymenoptera) and n = 217-223 (*Polyommatus atlantica* (Elwes, 1905))] (de Lesse, 1970; Crosland & Crozier, 1986; Gokhman & Kuznetsova, 2006).

At the present, unaccompanied external morphological taxonomy is not enough for an indisputable classification of some taxa. The remarks on the base of only external morphology are caused discussions and even mistakes on the systematic ranks for many taxa. For example, the status of Vesperidae. Svacha et al. (1997) that included larval morphologies and biologies of a few species, regarded the taxon as a subfamily of Cerambycidae. According to Brustel et al. (2002), Vesperidae is a separate family. Dutrillaux et al. (2007) that included caryotype definition of *Vesperus xatarti,* regarded the taxon as a subfamily of Cerambycidae too. Recently Löbl & Smetana (2010) gave the taxon as a subfamily of Cerambycidae in their catalog. The known differentiations of larval morphologies and obtained cytogenetic data do not encourage the last status. So status of the taxon is still under discussion.

Comparative caryology has more advantage than other methods in taxonomical studies of animals. In fact especially chromosomal characters are essentially morphological characteristics. On the other side, some characters of caryotype which are number of chromosomes, arms of chromosomes, nucleolar organisers, heterochromatic blocks etc., can be intraspecific values.

In particular, cytogenetic works on Cerambycidae have also been realized poorly worldwide until now. For example, Ehara (1956) gave chromosome numbers of 23 species that were distributed in Japan, belonging to the subfamilies Lepturinae, Cerambycinae and Lamiinae. Teppner (1966) determined chromosome numbers of 20 species that were distributed in Central Europe. Also Teppner (1968) mentioned chromosome numbers of 25 species that were distributed in Central Europe. Kudoh et al. (1972) gave chromosome numbers (both haploid and diploid numbers) of 5 species of the subfamily Lamiinae. Smith & Virkki (1978) revealed a large scaled work on cytogenetic of Coleoptera. They compiled all cytogenetic studies on Coleoptera up to 1978 and gave chromosome numbers of 157 species of Cerambycidae. Vidal (1984) stated chromosome numbers of 3 long-horned species. Vaio et al. (1985) observed meiotic plaques of 2 species of the genus *Trachyderes*. Lachowska et al. (1996) mentioned both haploid and diploid chromosome numbers of *Agapanthia violacea* of the subfamily Lamiinae. Holecova et al. (2002) gave chromosome numbers of 2 long-horned species. Rozek et al. (2004) stated chromosome numbers of 3 long-horned species. Dutrillaux et al. (2007) carried out a caryologic study on the species *Vesperus xatarti* of the subfamily Vesperinae. He stated chromosome number 54 + XX for female and 53 + XY1Y2 for male.

As seen below, diploid number of chromosomes of members of long-horned beetles is changed between 10 and 36. Sex-chromosome system of long-horned beetles is parachute type (Xy_p). Most of the diploid chromosomes number is 2n = 20 (18AA + Xy_p) (Smith & Virkki, 1978) (Graph 1).

Anyway, the genus *Dorcadion* Dalman, 1817 and thereby the species *Dorcadion* (*Cribridorcadion*) *anatolicum* Pic, 1900 and *Dorcadion* (*Cribridorcadion*) *scabricolle* have not been investigated cytogenetically until now. In the species, diploid numbers of chromosomes are determined 2n = 24 and 2n = 20 in mitotic metaphase respectively.

As known, the genus *Dorcadion* is a very rich and problematic group. Identification of the species of this genus on the base of external morphology, therefore, is difficult. Obtaining new taxonomic characters by cytogenetic works will be useful for both identification of species and classification of the group.

MATERIAL AND METHOD

The specimens were collected from Ankara and Konya provinces of Turkey in 2010 and were deposited in Gazi University, Ankara, Turkey.

The chromosomes are obtained according to Rozek (2004) with some alterations. The method is presented as follows:

The specimens were placed in killing-jar with ethyl acetate to anaesthetize. Abdomens of the specimens were cut and abdominal contents (especially testicle tissue in males, and middle-gut tissue in males and females) were transferred in petri dishes with distilled water. So the tissues were sustained on hold for 10-15 minutes in the hypotonic solution. They were transferred cryotubes with 0.05 % cholcicine solution and were maintained for 45-60 minutes in room temperature and then, fixed in 3:1 fresh ethanol-acetic acid solution for at least 1 hour. Small pieces from the treated tissues were taken and mounted on a clear lam. On tissue pieces were dropped 45 % acetic acid and were dissected with using dissection pins and bisturi. Then, tissue pieces were mounted and pressed directly between lam and lamel or lam and lam. These preparates were submerged into liquid nitrogen. Lam and lamel or lam and lam were uncoupled and left for drying. Later, the dry preparates were stained by 4 % Giemsa Phosphate Buffer (pH = 6.8) for 10 minutes and were washed with distilled water. After drying the preparates were examined under stereo microscope (Leica DMLB). The observed plaques were photographed zoom in (10X).(100X) (Graph 2).

RESULTS AND DISCUSSIONS

Subfamily LAMIINAE Latreille, 1802
Tribe DORCADIINI Latreille, 1825

Genus *DORCADION* Dalman, 1817

Type species: *Cerambyx glicyrrhizae* Pallas, 1773

The genus has Palaearctic chorotype (N Africa to China). It includes 5 subgenera as *Dorcadion* s. str.; *Cribridorcadion* Pic, 1901; *Maculatodorcadion* Breuning, 1942; *Carinatodorcadion* Breuning, 1943; *Acutodorcadion* Danilevsky et al., 2004. Recently, Löbl & Smetana (2010) also added *Megalodorcadion* Pesarini & Sabbadini, 1998 as 6th subgenus.

Almost all subgenera except the subgenus *Acutodorcadion* Danilevsky et al., 2004 were recorded from Turkey. The genus *Dorcadion* is represented by about 200 species in Turkey according to Özdikmen (2010). However, Löbl & Smetana (2010) gave a total of 150 species for Turkish *Dorcadion* (1 species for *Carinatodorcadion*, 140 species for *Cribridorcadion*, 4 species for *Maculatodorcadion* and 5 species for *Megalodorcadion*).

With this work, *D.* (*Cribridorcadion*) *anatolicum* Pic, 1900 that is endemic to Turkey and *D.* (*Cribridorcadion*) *scabricolle* (Dalman, 1817) that is the widest distributed species in Turkey, were studied taxonomically and cytogenetically.

Dorcadion (*Cribridorcadion*) *anatolicum* Pic, 1900

Material examined: Konya prov.: Taşkent, Gevne Valley, 23.III.2010, 1 specimen (Fig. 1)

Records in Turkey: Konya prov. (Pic, 1900; Breuning, 1962); Konya prov.: Akşehir (Demelt, 1963); Konya prov.: Akşehir, Sultan Mts., Ilgın and Kızılören, Antalya prov.: Irmasan pass, Adana prov.-Kahramanmaraş prov.: between Tufanbeyli and Göksun (Braun, 1978); Konya prov.: Dedegöl Mts. (Adlbauer, 1988); Isparta prov.: Eğirdir, Konya prov. (Önalp, 1990); Konya prov.: Taşkent (Çukuryurt pass) (Özdikmen & Hasbenli, 2004); Kahramanmaraş prov.: Göksun (Özdikmen & Okutaner, 2006); Konya prov.: Taşkent (Faşikan plateau), Hadim-Beyreli road (Turgut & Özdikmen, 2010) (Map 1).

Range: Turkey.

Chorotype: Anatolian.

Note: Male genitalia cannot give due to deformation of genitalia during the study.

Dorcadion (*Cribridorcadion*) *scabricolle* (Dalman, 1817)

Original combination: *Lamia scabricolle* Dalman, 1817

According to Löbl & Smetana (2010) and Özdikmen (2010), this species has 9 subspecies in the world. It is distributed widely in Turkey and is represented by five subspecies in Turkey. *D. scabricolle caramanicum* Daniel & Daniel, 1903 (Southern subspecies) occurs in Cilician Taurus (South Turkey), *D. scabricolle paphlagonicum* Breuning, 1962 (Northern subspecies) occurs in Kastamonu province of North Turkey, *D. scabricolle balikesirense* Breuning, 1962 and *D. scabricolle uludaghicum* Breuning, 1970 (Western subspecies) occur in Balıkesir and Bursa provinces of North-West Turkey and the nominative *D. scabricolle scabricolle* Dalman, 1817 occurs in Transcaucasia and Armenia to Anatolia. The other known subspecies of this species are *D. scabricolle nakhiczevanum* Danilevsky, 1999 and *D. scabricolle paiz* Danilevsky, 1999 occur only in Caucasus. According to Braun (1978), *D. sevangense* Reitter, 1889 that described from Transcaucasia as *D. scabricolle* v. *sevangensis* is a distinct species. He mentioned that it separated clearly from *D. scabricolle*. According to Danilevsky (2011), - *sevangense* Reitter, 1889 is a subspecies of *D. scabricolle*.

Besides, Danilevsky (2011) mentioned that "*Dorcadion scabricolle elisabethpolicum Suvorov, 1915 was described from near Elisavetpol (now Giandzha in Azerbajdzhan) on the base of small size of available specimens. According to my series from Khanlar (7km S Giandzha) the local population really consists of relatively small specimens. So, the name can be regarded as valid. In fact each population of D.scabricolle is more or less peculiar*". So this taxon is presented as a subspecies of this species.

Consequently, this species is the widest *Dorcadion* species in Turkey. The taxonomic status of subspecific level of the species needs to be clarified with future works. The present materials belong to the subspecies, *D. scabricolle paphlagonicum* Breuning, 1962.

Dorcadion scabricolle paphlagonicum Breuning, 1962

Material examined: Ankara prov.: Ayaş, Ankara–Ayaş road, 20.III.2010, 3 specimens; Ankara prov.: Kazan, 22.III.2010, 1 specimen; Ankara prov.: Kızılcahamam, Işık Mt., 26.IV.2010, 4 specimens (Fig. 2).

Records in Turkey: Malatya prov. (Heyden,1888); Konya prov.: Akşehir (Sultan Mt.) (Bodemeyer, 1900); Antalya prov.: Toros Mts., Niğde prov.: Çamardı as *D. s.* v. *caramanicum* (Bodemeyer, 1900); Niğde prov.: Çamardı as *D. scabricolle* v. *caramanicum* (Aurivillius, 1922); Cilician Taurus as *D. s. caramanicum* Daniel, 1903 / Niğde prov.: Bolkar Dağları-Maden (=Çamardı) as *D. s.* m. *bulghardaghense* Breuning, 1946 / Kastamonu prov. as *D. s. paphlagonicum* Breuning, 1962, *D. s.* m. *subbasalireductum* Breuning, 1962, *D. s.* m. *humeralibivittatum* Breuning, 1962 / Balıkesir prov. *D. s. balikesirense* Breuning, 1962 (Breuning, 1962); Konya prov.: Akşehir (Demelt, 1963); Sivas prov.: Zara, Çorum prov.: Boğazkale (Perissinotto & Luchini, 1966); Erzurum prov. (Breuning et Villiers, 1967); Bursa prov.: Uludağ as *D. s.* m. *corpulentum*(Breuning et Villiers, 1967); Bursa prov.: Uludağ as *D. scabricolle balikesirense* (Breuning et Villiers, 1967); Van prov.: Özalp *D. s.* m. *corpulentum* (Fuchs et Breuning, 1971); Yozgat prov.: Central (Fuchs et Breuning, 1971); Van prov.: Kuzgunkıran as *D. s. paphlagonicum* (Fuchs et Breuning, 1971); Malatya prov.: Arguvan (Gfeller, 1972); Konya prov.: Akşehir (Tuatay et al., 1972); Konya prov.: Akşehir (Cankurtaran), Kayseri prov.: Bakırdağı, Ankara prov.: Central / Kızılcahamam (Central / Güvem), Çorum prov.: Yazılıkaya, Yozgat prov., İçel prov.: Sertavul pass, Kahramanmaraş prov.: Göksun / Elbistan, Adana prov.: Saimbeyli / Tufanbeyli, Bilecik prov.: Söğüt (Braun, 1978); Balıkesir prov., Bursa prov., Uşak prov., Isparta prov., Ankara prov., Kastamonu prov., İçel prov., Kayseri prov., Kahramanmaraş prov., Erzurum prov., Van prov., Ağrı prov., Ardahan prov. (from map in Braun, 1978); Erzurum prov. and near (Özbek, 1978); Konya prov.: Sertavul pass, Gümüşhane prov.: Köse (Sama, 1982); Turkey (Danilevsky & Miroshnikov, 1985; Özdikmen, 2006); Erzurum prov.: İspir, Ankara prov.: Güvem (Adlbauer, 1988); Konya prov.: Akşehir / Sultandağ, Ankara prov.: Central / Gölbaşı / Çal Mt. / Hüseyin Gazi Mt., Erzurum prov., Van prov., Kars prov.: Central / Kağızman, Niğde prov.: Bulgar-Maden (Çamardı), Afyon prov.: Emirdağ (Önalp, 1990); Yozgat prov.: Yozgat pine grove, Erzincan prov.: Çayırlı (Başköy), Sivas prov.: Tahtıkement village, Gümüşhane prov.: Kelkit (Akdağ), Yozgat prov.: Yozgat National Park (Özdikmen & Hasbenli, 2004); Konya prov.: Akşehir (Özdikmen et al., 2005); Ankara prov.: Çal Mountain (Özdikmen & Demir, 2006); Kahramanmaraş prov.: Göksun (Central / Küçüksu plateau / Korkmaz) (Özdikmen & Okutaner, 2006); Ankara prov.: Ayaş, Kızılcahamam (Işık Dağı) (Özdikmen et al., 2009); Konya prov.: Bozkır (Kuruçay-Ahırlı) (Turgut & Özdikmen, 2010); Kahramanmaraş prov.: Afşin (Emirilyas village, Mağaraözü district) (Özdikmen et al., 2010) (Map 2).

Range: Caucasus (Armenia, Azerbaijan, Georgia), Turkey, Iran.

Chorotype: SW-Asiatic (Anatolo-Caucasian + Irano-Caucasian + Irano-Anatolian).

Cytogenetics: First of all, we must to state that observation density of chromosomes is low due to a low of mitotic and meiotic activations in the examined material. Long-horned beetles have also holometabolous development such as other members of the order Coleoptera. The larval, pupal and imaginal stages of holometabolous insects in terms of observed mitotic and meiotic activities are displayed diversity. This case was evaluated by Teppner (1968) with regard to spermatogenesis. He stated that meiosis is started in pre-pupal stage; spermatogenesis is accelerated in last instar larva and is continued in adult. He also mentioned these findings are varied among the subfamilies. In respect to this, spermatogenesis that occurring in last instar larvae, decelerates in adult stages in the subfamilies Lepturinae and Aseminae, while it is continued with the same density in adult stages in Cerambycinae and Lamiinae. Moreover, duration of meiosis differs from stage to stage.

In the present work, cytogenetic researches carried out on the adult due to the larval and pupal identification are very difficult.

Observed chromosomes of long-horned beetles are small. Centromere regions and length of arms of the chromosomes are not clear. The chromosomes, therefore, evaluated only on account of the number.

With regard to the present study, haploid number of chromosomes for the species *D. anatolicum* was determined as $n = 11 + Xy_p$ in meiotic metaphase from testicle tissues (Fig. 4), and diploid number of chromosomes was determined as $2n = 24$ in mitotic metaphase from testicle tissues (Fig. 5).

Also, diploid number of chromosomes was determined as $2n = 20$ in mitotic metaphase from testicle tissues (Fig. 6).

As a result of this study, number of chromosomes is varied in the genus *Dorcadion*. Probably, determination of chromosomal number variance can aid to solve some systematic problems of this group.

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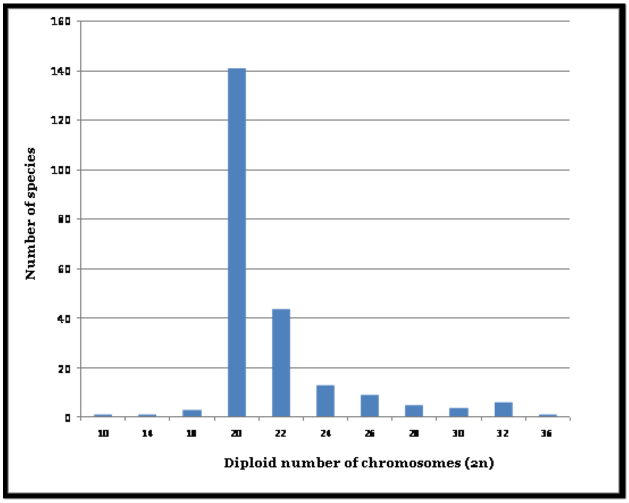


Graph 1. Diploid number of chromosomes – Number of species in Cerambycidae.

Graph 2. The main steps of the used method for cytogenetic researches.

Figure 1. Habitus of *D. anatolicum* (dorsal view).

Figure 2. Habitus of *D. scabricolle* (dorsal view).

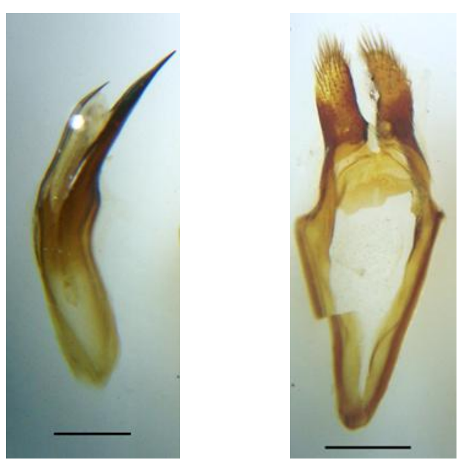


Figure 3. Male genitalia of *D. scabricolle*. A. Aedeagus, B. Paramers.

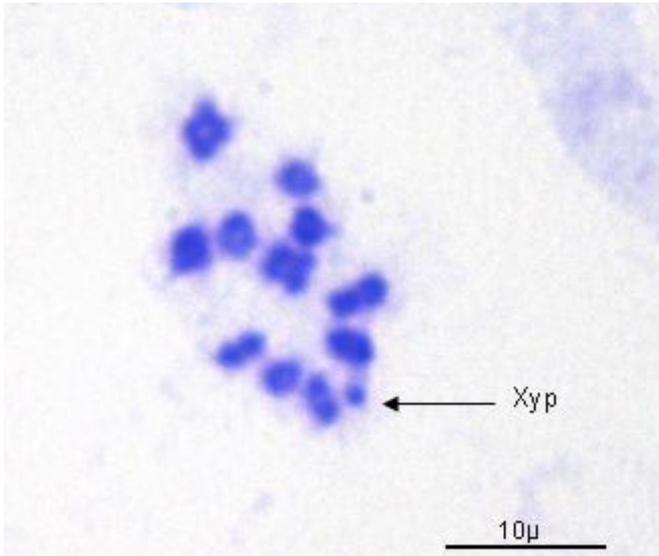


Figure 4. Meiotic metaphase plaque from testicle tissue of *D. anatolicum* (n = 11 + Xy_p).

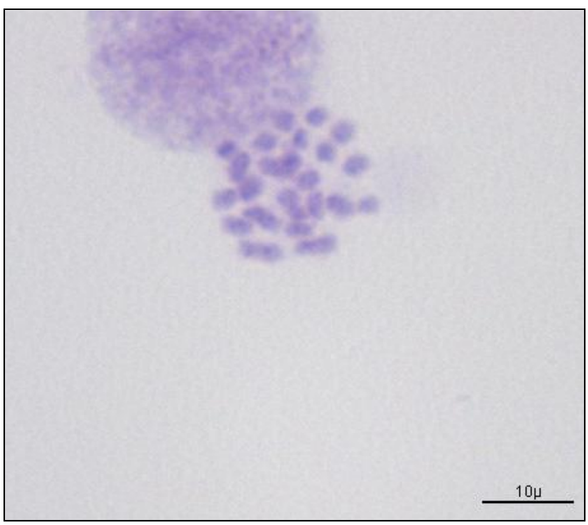


Figure 5. Mitotic metaphase plaque from testicle tissue of *D. anatolicum* (2n = 24).

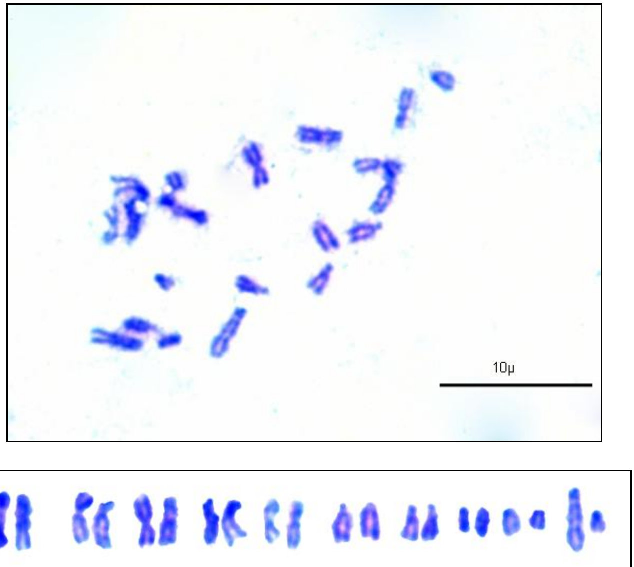
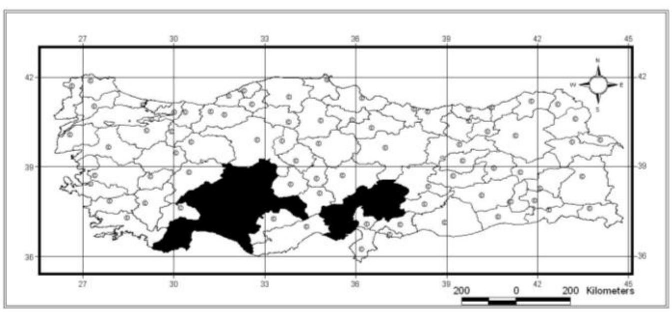
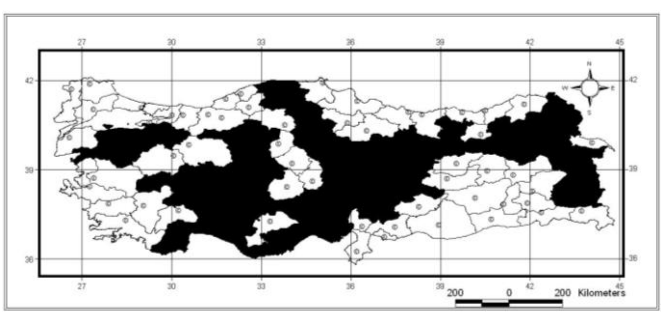


Figure 6. Mitotic metaphase plaque from testicle tissue and karyogram of *D. scabricolle* (2n = 20).



Map 1. Distribution in Turkey of *D. anatolicum* (in respect to provinces).



Map 2. Distribution in Turkey of *D. scabricolle* (in respect to provinces).