# SOME CYTOGENETIC OBSERVATIONS OF *MORIMUS ORIENTALIS* REITTER, 1894 (COLEOPTERA: CERAMBYCIDAE: LAMIINAE: LAMIINI)

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ABSTRACT: The paper gives some cytogenetic observations of *Morimus orientalis* Reitter, 1894 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, *Morimus orientalis*, 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 XIX$^{th}$ 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 *Morimus* Brullé, 1832 and thereby the species *Morimus orientalis* Reitter, 1894 have not been investigated cytogenetically until now. The first and single cytogenetic study on Turkish long-horned species is Okutaner et al. (2011). Diploid number of chromosomes of *Morimus orientalis* Reitter, 1894 is determined 2n = 24 in mitotic metaphase.

As known, the genus *Morimus* is a problematic group. Some members of *Morimus* resemble each other external morphologically. Identification of these species on the base of external morphology, therefore, is very 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 specimen was collected from Bolu province of Turkey in 2010 and was 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 LAMIINI Latreille, 1802

### Genus *MORIMUS* Brullé, 1832

Type species: *Lamia lugubris* Fabricius, 1792 = *Cerambyx asper* Sulzer, 1776.

The genus *Morimus* has a problematic group as *M. asper* and related taxa. Some old species that are *M. funereus, M. verecundus*, however, are regarded as a subspecies of *M. asper,* and *M. ganglbaueri* is regarded as a synonym of *M. asper* by some authors (e.g. Sama, 2002; Sama & Löbl in Löbl & Smetana, 2010). However, Sama's proposal does not seem useful to take into consideration both morphological differences and distribution areas of the taxa. Recently, Sama & Löbl in Löbl & Smetana (2010) gave the distribution areas of these taxa as follows:

- The range of *M. asper asper*: South Europe (Spain, France, Italy, Switzerland, Yugoslavia, Croatia, Albania, Greece).
- The range of *M. asper funereus*: Italy, Hungary, Czechia, Austria, Slovakia, Slovenia, Yuguslavia, Croatia, Albania, Bosnia-Herzegovina, Macedonia, Romania, Bulgaria, Greece, Moldavia, Ukraine.
- The range of *M. asper verecundus*: Ukraine, European Russia, Caucasus (Azerbaijan, Armenia, Georgia), Turkey, Iran, Turkmenistan.

In respect to this, at least *M. verecundus* (Faldermann, 1836) is a separate species. This proposal has also been supported by Danilevsky (2011). On the other side, according to Danilevsky (2010), *M. asper ganglbaueri* Reitter, 1894 that occurs Bosnia-Herzegovina, Croatia and Yugoslavia, is a good subspecies. He stated that *"for the distinguishing characters and distribution see Mikšić (1971), Mikšić & Korpič (1985)"*. Consequently, we accepted that *M. funereus* and *M. ganglbaueri* are the subspecies of *M. asper* and *M. verecundus* is a separate species.

So, this Palaearctic + Oriental genus, *Morimus* Brullé, 1832, is represented by 12 species (with 3 subspecies) in the whole world. With regard to this, *M. asper* is the most widely distributed member of the genus.

The members of the genus are distributed from Europe (Spain to Caucasus) to Central Asia (Turkmenistan) and China in Palaearctic region, and India, Myanmar, S China, Thailand, Vietnam and Malaysia in Oriental region.

A total of four species occur in Palaearctic region. Three of them are represented only in Palaearctic region as *M. asper* (Sulzer, 1776), *M. orientalis* Reitter, 1894 and *M. verecundus* (Faldermann, 1836). The species, *M. assamensis* Breuning, 1936 occurs in both Palaearctic and Oriental regions. None of them is endemic.

A total of nine species occur in Oriental region. Eight of them are represented only in Oriental region as *M. granulipennis* Breuning, 1939, *M. inaequalis* Waterhouse, 1881, *M. indicus* Breuning, 1936, *M. lethalis* Thomson, 1857, *M. misellus* Breuning, 1938, *M. ovalis* Breuning, 1943, *M. plagiatus* Waterhouse, 1881 and *M. sexmaculipennis* Breuning, 1961. Seven of them except *M. lethalis* are endemic to different countries: *M. granulipennis* and *M. ovalis* to Myanmar;

*M. inaequalis, M. indicus, M. misellus* and *M. plagiatus* to India, and *M. sexmaculipennis* to Malaysia. Again, *M. assamensis* Breuning, 1936 occurs in both Palaearctic and Oriental regions.

In Turkey, the genus is represented by 3 species as *M. asper* (Sulzer, 1776), *M. orientalis* Reitter, 1894 and *M. verecundus* (Faldermann, 1836) (Özdikmen, 2007 and 2008).

### *Morimus orientalis* Reitter, 1894

**Material examined:** Bolu prov.: Central, 25.IV.2010, 1 specimen.
**Records in Turkey:** Sakarya prov.: Sapanca (Gökdağ) (Bodemeyer, 1900); İstanbul prov.: Alem Mt., Sakarya prov.: Sapanca (Gökdağ) (Bodemeyer, 1906); Turkey (Winkler, 1924-1932; Lodos, 1998; Sama, 2002; Tozlu et al., 2003); İstanbul prov.: Polonezköy, Alem Mt. (Demelt & Alkan, 1962; Demelt, 1963; İren & Ahmed, 1973); Erzurum prov. and env. (Özbek, 1978) (Map 1).
**Range:** Europe (Bulgaria, European Turkey), Turkey.
**Chorotype:** Turano-Mediterranean (Balkano-Anatolian).
**Remark:** This species is recorded for the first time from Bolu province in Turkey.
**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 *M. orientalis* was determined as $n = 11 + Xy_p$ in meiotic metaphase from testicle tissues (Fig. 1), and diploid number of chromosomes was determined as $2n = 24$ in mitotic metaphase from testicle tissues (Fig. 2). This results are the same with that of *D. anatolicum* (Okutaner et al., 2011).

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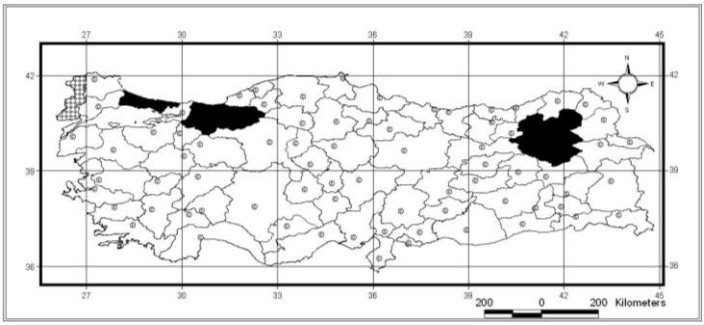
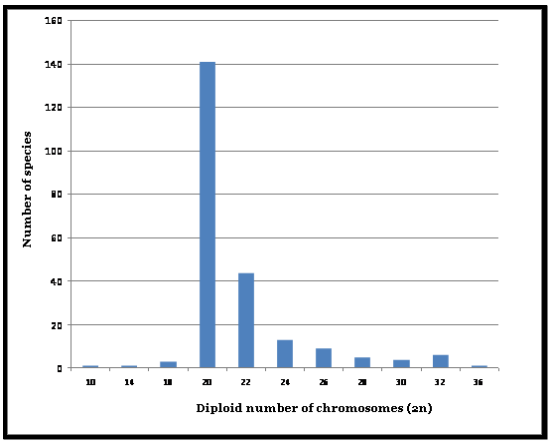



Map 1. Distribution in Turkey of *M. orientalis* (in respect to provinces).




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


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

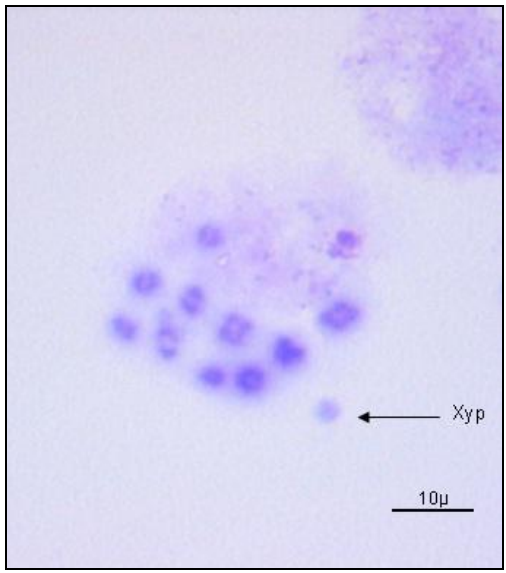



Figure 1. Meiotic metaphase plaque from testicle tissue of *M. orientalis* (n = 11 + $Xy_p$).

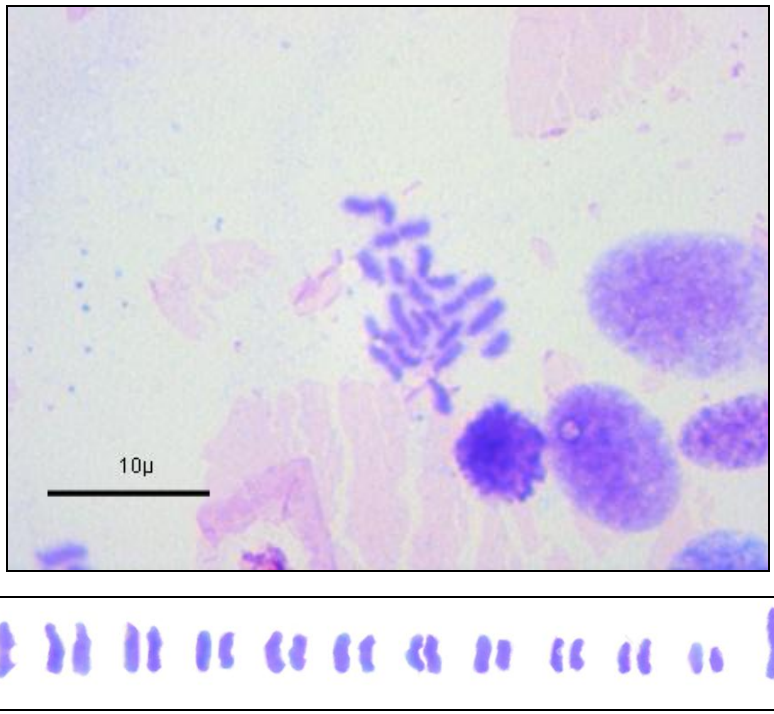
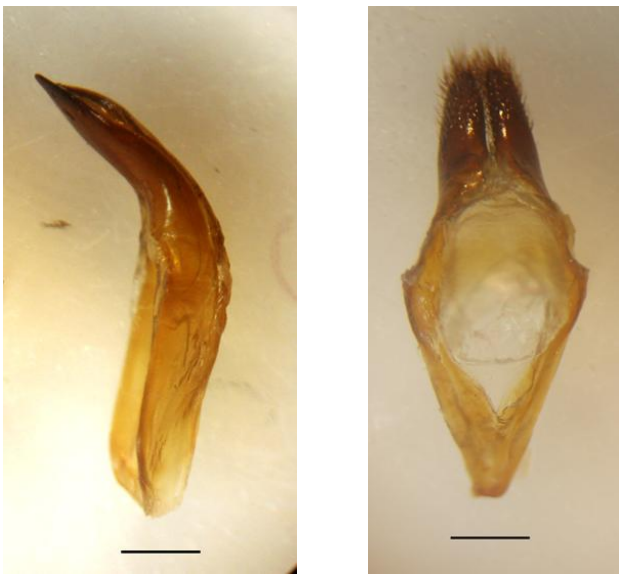



Figure 2. Mitotic metaphase plaque from testicle tissue and karyogram of *M. orientalis* (2n = 20).



**A** **B**

Figure 3. Male genitalia of *M. orientalis*. A. Aedeagus, B. Paramers.