

# Kuru Böcekler İçin Yeni Bir Yumuşatma Sistemi

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#### ÖZET

Bu çalışma kurumuş böcek örneklerini yumuşatmak için yeni bir yöntem sunar. Sistem ısıya dayanıklı cam malzemeden yapılmış 4 ana bölümden oluşur. Sistemin ana mantığı kuru böcekleri izole bir alanda sıcak buharla yumuşatmaktır. Sistemin en önemli başarıları, kuru böcek örneklerini çok kısa bir zamanda yumuşatması, mantarlaşmayı minimuma indirmesi ve ekstremite kayıplarını önlemesidir. Yumuşatma süresi böceğin boyutuna, kitin tabakasının yoğunluğuna ve böceğin ne kadar kuru kaldığına bağlı olarak değişir. Yapılan denemelerde böcek takımlarının bu sistemde yumuşatması 2 saatten fazla sürmez. Sistem 5 mm– 120 mm uzunluğundaki böcekler için kullanılabilir. Tasarlanan bu sistemle 2950 kuru ergin böcek yumuşatılmıştır.

## A New System for Relaxing the Dry Insects

#### ABSTRACT

The paper presents a new system for relaxing dry adult insect specimens. The system was designed with 4 main parts builted with high temperature resistant glass material. The main logic of the system was to relax dry adult insects in an isolated chamber with hot steam. The most important achievements of the system included relaxing the dry insect samples in a very short time, minimizing fermentation and preventing extremity losses. The relaxing duration varies based on the insect size, the density of the chitin layer and the degree of dryness. The experimental results show that it does not take more than 2 hours for the insect orders to relax in this system. The system can be used for insects of 5 mm to 120 mm in length. With the designed system, about 2950 dry adult insects were relaxed and prepared. Araştırma Makalesi

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### INTRODUCTION

According to the literature, slightly over 1 million insect species were described (Gullan and Cranston 2012). In other words, these species were reported in a taxonomic publication as "new" to science. Since certain insect species were noted as new more than once in the literature due to the failure of the recognition of a variation or the neglect of previous studies, it is not possible to ascertain the actual number of described species. Ongoing various studies on insect diversity are published globally. Certain, but not all, recent reanalysis studies reported lower estimates based on the calculations conducted by taxonomists and extrapolations from regional sampling methods rather than calculations based on ecological scaling. It would be safe and realistic to propose a figure of between 4 and 6 million species of insects to be described (Gullan and Cranston 2012).

Generally. insect materials are identified morphologically diagnostic and procedures are usually performed on adult specimens with classical methods when adult insect specimens are either fresh or relaxed. If the specimens are collected more than a few days before the processing, they may be hard, brittle, and it is almost impossible to pin them without breaking or damaging them. Certain extremities including antenna, mouthparts, wing, abdomen, and legs or all material that are used in the identification of insects. may be destroyed due to fragility. In the preparation process, the adult insect should remain fresh or moistened for a certain period. The process of relaxing the dried adult insects is very important since dry specimens could suffer from negative morphological properties and discoloration, which complicate the morphological diagnosis of the taxon. Especially during the relaxing of butterfly

(Lepidoptera) specimens, extremities loss is more than other insect orders. Relaxing the dry insect samples using several traditional methods takes a long time and mold is formed during the relaxing process. This is a serious challenge in taxonomic studies.

Any dry insect to pin should be relaxed, that is, remoistened sufficiently to prevent breaking when the pin is inserted or to allow the rearrangement or repositioning of the parts of the specimen. Insects, especially Lepidoptera with spread wings should be relaxed even when they have died only recently. Once the rigor mortis sets in, the muscles of Lepidoptera stiffen in a matter of minutes, preventing any desired adjustment. However, when treated in a relaxing chamber, the procedure becomes much easier. In typical cases eight hours in a relaxing chamber should provide sufficient relaxation; however, larger specimens may require 24 hours or more. Storing the specimens in a cyanide jar for a short period could relax them; however, the method is far from being reliable (Lane, 1965).

High humidity must be provided in a relaxing chamber for periods between several hours and up to about 3 days, based on the circumstances, without completely wetting the specimens. Mold growth should also be avoided, since it may destroy the specimens that are left for too long in relaxing chambers unless a chemical mold inhibitor is introduced. Insects killed with cyanide could usually be relaxed more easily; however, certain terminating agents, especially chloroform, ether, and carbon tetrachloride may stiffen muscles extremely, leading to brittle specimens that resist the humidity in the relaxing chamber. For instance, very hot water is injected into butterfly thoracic muscles using a fine hypodermic needle before spreading in Korea. However, certain specimens could not be relaxed sufficiently using any method (Schauff, 2001).

Insect relaxing is a procedure that has been conducted for many years. Some different studies performed in the past years are as follows.

Kılıç (1987) adopted a relaxing method for dry insect samples. In this method, a layer of moistened thin sand was placed in a large chamber and the sand was covered with a blotting paper. Then, dry adult insects were placed on the blotting paper for relaxing during 1-2 days. Doğanlar (2003) placed the dry butterfly specimens in a petri dish, the base of which was covered with a blotting paper, and moistened the surface of the blotting paper. Then, dry insect adults were placed on the blotting paper for relaxing during 24 hours. Doğruöz (2005), placed a water-saturated sponge and covered the sponge with blotting paper in a large chamber. In this System, the samples did not come into direct contact with the water and were allowed to relax for 1-2 days. In another relaxing process for bees, Plant and Dubitzky (2008) placed a Styrofoam in a large chamber and added hot water. As the Styrofoam floated on the water, the chamber lid was closed and dried adult bee samples were relaxed for 12-24 hours.

In this paper, it was aimed to relaxing dry insects quickly, undamaged, without fungus and sustainable.

## MATERIALS and METHODS

*Equipment*: High-temperature resistant glass chamber, high-temperature resistant glass pipe, high-temperature resistant glass lid, heater, cloth barrier, net barrier.

In a relaxing system, heat-resistant glass material was used. However, it can be used instead in other heat-resistant materials (eg. heat-resistant metal)

*Procedure*: Based on the process principle, the system includes 4 sections. The laboratory type of electric heater at the bottom, 15x15x15 heat-resistant glass cube on the top of the heater, 2 cm diameter pipe on the top of the glass cube that provides both the cover and the vapor passage. At the top there is a glass cube of 15x15x15, which is connected to the pipe and at the top where the vapor reaches.

The heater should be at 100 °C until relaxation is finished, because distilled water needs to boil until relaxing is finished and Distilled water is placed in the glass cube above the heater and the lid must be closed. The water evaporating from this glass cube passes through the steam pipe and reaches the upper part ensureing the formation of the steam at the bottom to go up quickly and directly. There is a cloth barrier with a network barrier in the top glass cube which is also used to prevent the steam coming from below from directly touching the insects. There is a network barrier where dry insects are placed 5 cm above the cloth barrier. Approximately 20 minutes after the relaxing system is started, the vapor transfer through the vapor pipe to the upper chamber begins.

## RESULTS

In this study, most of the insect specimens were collected with Robinson Type Light Trap and an insect net in different provinces of Turkey. For insect specimens preparation, they should be fresh; however, it could dty since it could take a very long time in certain cases. This situation makes the preparation process impossible without losing extremities. During the PhD research of the first author, most adult Geometridae specimens were collected and stored in the Gaziantep University Entomology Laboratory (GUGT). For the preparation process, a designed relaxing system was used (Figure 1). Several methods were reported to relax tissues and chitin structures during the preparation of dry insects. However, in many methods reported by different researchers, the relaxing process could take up to 2 days, which causes insect fermentation or extremity loss. The present study aims to shorten the relaxing process and to prevent both mold and extremity loss. In this study, dried insect adult sizes varied between 5-120 mm. The number of relaxed insects , average relaxing time, testes insect orders are presented in Table 1.



Figure 1: Relaxing system A: Overview (photo), B: Overview (illustrated), C: Top view (photo), D: Top view (illustrated).

Şekil 1: Yumuşatma sistemi A: Genel bakış (fotoğraf), B: Genel bakış (çizim), C: Üstten görünüş (fotoğraf), D: Üstten görünüş (çizim).

The relaxing times of insects were measured as follows: measurements started with the beginning of the vapor transfer and the specimens were placed on the net barrier and finished with the preparation process. An average of 2300 adult butterfly specimens could be relaxed with the steam produced in the lower chamber, which was initially half-filled with distilled water and also the relaxing system was tested with different insect orders to determine the average relaxing time.

A review of the times presented in Table 1

demonstrates that there was a direct correlation between the thickness of the chitin layers and the relaxing time. It is known that the Coleoptera order species had the thickest chitin layer among all insect orders and due to the thick chitin layer, the relaxing process took a maximum of 2 hours. For Lepidoptera, Diptera, and Neuroptera orders, the average relaxing

time was approximately 25-35 minutes due to the thin chitin layers. The relaxing times varied based on the insect size, the drying time, and the proportion of the chitin; however, the relaxing period never exceeded 120 minutes and no specimen loss was experienced because of the system during the relaxing process.

Table 1. Average relaxing times for insect orders.

Tablo 1: Böcek takı	mlarının ortalama yumuşama süreleri.	
Insect Order	Relaxed Specimens Count (~n)	Average Relaxing Time (minutes)
Böcek takımları	Yumuşatılan Böcekler	Ortalama Yumuşatma süreleri (dakika)
Lepidoptera	2300	(25 – 35 )
Diptera	100	
Neuroptera	100	
Heteroptera	50	
Hymenoptera	100	(45 - 60)
Odonata	100	
Orthoptera	100	
Coleoptera	100	(100 - 120)

Relaxing of dry specimens by using the system and preparation process for the tested insect orders are explained in Figure 2. Using the system designed in the present study, adult insects were relaxed then prepared and stored in insect drawers.



Preserved as dry specimens

Figure 2: The relaxing and preparing process. Şekil 2 : Yumuşatma ve preparasyon işlemi.

The appearance of adult insect specimens (Figure 3) was not different when compared to that of the fresh specimens. The most important challenge during the preparation of butterfly specimens is the loss of wing scales and indirect loss of wing patterns. It was clear that these problems were not experienced in our system. Furthermore, during the preparation of dried adult specimens of other insect orders, negative conditions such as breaking, rupturing and tearing of insect head, antenna, abdomen, leg or wing were

minimized.

#### DISCUSSION

It is not possible to prepare all insect samples collected from nature without being transferred to the laboratory or it takes a long time to prepare and convert the specimens into museum-ready material in a short time after transfer to the laboratory. During this period, several insects are known to dry quickly and their body parts become fragile. This requires the



Figure 3: Adult specimens of different insect orders that were prepared by using a relaxing system developed in the present study.

Şekil 3: Bu çalışmada geliştirilen yumuşatma sistemi kullanılarak hazırlanan farklı böcek takımlarına ait ergin örnekler.

relaxation of insect adults to prepare them for study. In the literature, reported relaxing methods take a minimum of 12 and a maximum of 48 hours, in which various chemicals are used to prevent mold growth (Kılıç, 1987). The application of these methods is difficult because the specimens should be monitored continuously for the conditions, ie. molding, overrelaxation, which leads to serious problems with the material.

The priority in entomological studies is to conduct preparations in a laboratory environment without losing the colour, texture and morphological properties of dry adult specimens collected from nature. In the proposed relaxing system, all specimens in various insect orders were relaxed in the present study. Since the relaxing process was continuous and the dry specimens were kept in a hot area during relaxing, no mold growth was observed and the relaxing process took only a short time. No specimen loss was observed due to mold formation or systemic factors. Furthermore, the system only requires distilled water and no chemicals are used.

The basic rationale of the system was relaxing the specimens in an isolated environment with hot steam and the system was designed for use with all insect orders. The system could be used for Coleoptera with a thick chitin layer and Lepidoptera, which is one of the most delicate insect orders. Thus, the system could be considered as the most comprehensive and practical system where a higher number of adult insect preparations could be produced in a very short time without material loss.

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### Statement of Conflict of Interest

Authors have declared no conflict of interest.

## Author's Contributions

The contribution of the authors is equal

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