

## MATERIALS AND METHODS OF STUDY



## Material studied

All specimens of Oryctes gnu Kohn. used in the course of this study were borrowed from collections of the Department of Agriculture, but specimens of Oryctes rhinoceros (Linn.) are private collections. The specimens were collected from various coconut-growing provinces in Thailand, such as Choburi, Rayong, Chiangmai, Lampoon, Chachoengsao, etc. The larvae and pupae are kept in 70 percent ethyl alcohol after fixed in KAAD for 48 hours.

## Methods

The method of preparing beetle for study is as follows. All specimens were tentatively identified and arranged according to species. The external morphology and genitalic structures of both sexes of two species were critically examined and illustrated. In dissecting the genitalia and mouthparts, the beetles were removed from the pins and placed in a relaxing jar for one week where the moisture softened the beetles. A few crystals of lacto-phenol was added to the water in the jar to protect from mold.

The genital capsule was removed by holding the abdomen ventral side up in the left hand. The venter can be

pressed apart with thumb and index finger, using sharp pointed forceps to pull the capsule loose at its point of attachment. The genitalic capsule was then placed in ten percent solution of potassium hydroxide in a small beaker for 12 to 24 hours or until unwanted tissues were dissolved. Then it was cleaned in water. The clear genitalic structures were placed in 70 percent ethyl alcohol for study. The structures were studied under a binocular dissecting microscope. The dissected and extended male or female genitalia were stored in glycerine in microvials attached to the specimens from which they were removed.

The mouthparts, especially, the maxillae and mandibles, were removed from the beetle and kept in 70 percent ethyl alcohol otherwise they would easily be lost. The maxillae and mandibles were cleared in a ten percent solution of potassium hydroxide for 15 to 30 minutes. They were then washed in water. The dissected maxillae and mandibles were kept in 70 percent ethyl alcohol in a small vial and kept together with the beetle from which they were removed.

To study the larvae and pupae, a deep petri-dish was used and about one third filled with 70 percent ethyl alcohol. The head of the larva was removed intact from the

thorax, and the maxillae, mandibles, and labium were removed from the head capsule and cleared in a ten percent solution of potassium hydroxide for 30 minutes. They were then washed with water. After studying, all the structures were kept in 70 percent ethyl alcohol in a small vial and kept together with the larva.

In the study of genitalia, the papers by Sharp and Muir (1912), Muir (1918), Metcalfe (1932), Snodgrass (1935), Pu (1938), Michener (1944), Wood (1952) and Wattanapongsiri (1959) were consulted.

#### Measurements

Measurements of the length and width of larva, pupa, and adult's body, horn, were made with an ocular micrometer in a Bausch and Lomb binocular microscope.

Body length of the larva were measured dorsally from the head capsule to the posterior end of the last abdominal segment; width was measured at the widest point on any abdominal segment, usually on the seventh, or eighth abdominal sternite. The length of the head capsule was measured medially from the anterior end of the clypeus to the posterior end of the capsule; width was measured at the widest point across the capsule.

Body length of the pupa was measured dorsally from the anterior end of the pronotum to the posterior end of the last abdominal segment; width was measured across the metanotum.

Body length of the adult was measured from the anterior end of the pronotum to the posterior end of the pygidium; width, at the base of the elytra across the humeri at their widest point. The length of the horn was measured from the base to the apex; width, at the base across its widest point.

#### Drawings

All figures were made with the aid of an ocular grid in a Bausch and Lomb binocular microscope, using 1 X 1 centimeter graph-paper, except figures 1, 2, 3, 4, 27, 28, 29, which were made with the squared-scale plate beneath the specimens and then completed free hand for the details in the body.