METHODS AND MATERIALS

The specimens from Bang Phra were collected by means of an insect light trap which was constructed at the Walter Reed Army Institute of Research, Washington, D.C. This trap was designed to collect live mosquitoes and other arthropods. Instead of the usual killing jar, the insects are sucked into a wire screen cage and held there by the suction of the fan. The design was based on a trap originally made by Du Toit (1944) for the study of Culicoides in Africa. Use of this trap in the United States has yielded catches of over 100,000 Culicoides per night (Scanlon 1960)

At Bang Phra the trap was hung in or near one of the three large stables where horses were kept.

The insects collected in the light trap were killed with chloroform and the mosquitoes removed for virus studies. The remaining catch, including the Culicoides, was sent to Bangkok for further examination. The Culicoides were removed and placed in 70% ethyl alcohol, pending further study. From the early part of the Bang Phra study in 1961 until the present study was undertaken, most of the Culicoides collections were sent to Dr. Willis W. Wirth, United States National Museum, Washington D.C. for use in a revision of the Ceratopogonidae of Southeast Asia which he contemplates. These specimens have not been examined as yet, since the projected study has not yet begun.

In the case of the specimens from Bang Phra determined in Bangkok, the following steps were taken. Approximately one thousand midges were chosen from each day's dry light trap collection.

The midges from each day of the month for which a catch was available were mixed together. Approximately one-tenth of this mixed monthly sample was then placed in 70% alcohol, and identified. Some Culicoides in this sample could be identified readily with the dissecting microscope only; while in other cases it was necessary to make slides. Slides were made of all members of the Trithecoides species, since their wing patterns are very similar and it was necessary to use other structures, such as the spermathecae, for identification. In addition, some of the remaining midges in the dry portion of the monthly sample were examined with the stereoscopic microscope and species which appeared different or interesting were removed for slide examination. This was especially true after it was noted that relatively few species made up the bulk of the collections from Bang Phra.

Slides were made using the general technique advocated by Wirth. The following steps were followed:

1. Dissolve phenol (carbolic acid) crystals in absolute ethyl alcohol. Only a very small volume of alcohol is required if the bottle is shaken frequently.

2. Mix two parts of canada balsam in xylene with one part of the phenol-alcohol solution. This mixture is caustic to the skin and should be handled with care.

3. Remove Culicoides from alcohol and place in a small volume of the phenol solution overnight, or longer if necessary to obtain proper clearing. Midges are ready for mounting when they are soft and transparent. To hasten clearing the thorax and abdomen should be pierced with very fine needles (minuten pins) while in the phenol solution.

4. Transfer Culicoides to a vial containing a small quantity of the phenol-balsam mixture for one half hour. Transfer may be made most conveniently with a medicine dropper.

5. Transfer midges to a slide with a small brush (No.0) with a small drop of the phenol-balsam mixture. Dissect with fine needles. Remove the head, one wing, and the tip of the abdomen in the female. In the case of females with one spermatheca the entire abdomen is removed, to preserve the long ducts. The same precedure is followed for the males, with the tip of the abdomen containing the genitalia dissected.

6. Place four small pieces of broken cover slip in the medium to prevent crushing of the specimen. Let the preparation air herden for a few moments, then add cover slip carefully with the aid of a forceps. With wax pencil add locality, slide number, date to the slide. Place in oven at (50°-70°C) for several days. Add canada balsam in xylene as the medium drys to prevent shrinkage of the specimens.

Using this method even dry specimens can be relaxed and cleared. Also, the refractory index of the phenol-balsam permits the examination of minute details of the antennal and palpal sensoria, female spermathecae, and internal structures of the male genitalia.

Available keys and original and subsequent descriptions were used for identification. However, as noted above, there is relatively

little published material on the Culicoides of Thailand, and no single reference with keys to the Thailand species. The specimens were therefore first divided into groups based on obvious similarities of wing pattern. A series of photographs prepared at the Walter Reed Army Institute of Research from specimens supplied by Dr.W.W. Wirth was used as a guide in this initial separation. The following groups were used:

1. Trithecoides group : Wing pale with radial cells well developed, especially the long second radial cell. Wing markings consist of two anterior pale areas. One over the r-m crossvein, second over the apex of the second radial cell. Large distinct pale areas occasionally present on basal part of wing, and across extreme wing tip. Other pale areas usually very indistinct.

2. Peregrinus group : Wing with very dark area on first and second radial cells, and projecting into the large white spot distal to the radial cells. Two white spots in cell Cu, at base and tip of cell.

3. Sumatrae group : Wing with dark area on first and second radial cell slightly darker than the rest of the wing. Third white costal spot separated from costal margin. Only one white spot in cell Cu.

4. Orientalis group : Wing with large white band at extreme apex. Almost all of cell Cu occupied by large white spot. Dark spot at middle of cell R_5

5. Ornatus group : Macrotrichia over almost entire surface of wing. White band at tip of cell R_5. White spot at tip of and posterior to second radial cell. Larger white spots near base of cells M_1 and M_2

6. Shortti group : Wing with numerous distinct pale spots. Dark area over second radial cell.

7. Gymnopterus group : Wing long, with very distinct veins. Radial cells very long.

8. Guttifer group : Wing with distinct circular spots. Macrotrichia distributed over almost entire wing.

Within these groups it was usually necessary to use other structures to distinguish the individual species. These included the spermathecae, antennae, palps, mandibles, tibial combs and other structures of the males and females. Some of these structures were examined for color, outline, etc., while others were measured. A number of terms were used which require definition before the descriptions can be utilized. These were as follows:

1. Wing length - Measured from the basal* arculus* to the wing tip.
2. Wing venation- Follows the Tillyard variation of the Comstock-Needham system (Tokunaga, 1937).
3. Costal ratio- Length of costa divided by wing length.
4. Relative lengths of the flagellar segments of the antenna.
5. Antennal Ratio- The value obtained by dividing the combined lengths of the last five flagellar segments of the antenna by the combined lengths of the preceeding eight segments.

6. The number and location of the antennal segments bearing small, distal sensory areas, each ringed by a tuft of small setulae.
7. Palpal ratio- Length of the third palpal segment divided by its greatest breadth.
8. The number and development of the mandible teeth.
9. The number of spines in the comb at the apex of the hind tibia*.
10. The spermathecae- Their number, relative size and measurement in mm. Length was measured to include the sclerotized portion of the duct. No note was taken of rudimentary third spermathecae when these were present, nor of the sclerotized ring of the female internal reporductive organs.

Measurements of more than one specimen are followed by a notation of the number examined in the following manner :. 07 mm (n=6), where 'n' is the number measured. In some cases the minimum and maximum values are also indicated, in the following manner : 15 (13-17, n = 4).

Representatives of all species identified were sent to Dr.Willis W.Wirth, United States National Museum, for confirmation of the identifications. Wing photographs and outline drawings of some of the species were prepared as an aid in identification.

The same general techniques were used for the Culicoides obtained from other areas of Thailand. Most of these were also separated

---

discussed in Torre - Bueno. (1937)

from mosquito light trap collections. A small number were obtained by collectors conducting mosquito biting catches in several parts of the country.