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MOUTHPARTS OF ORIUS INSIDIOSUS (SAY), HEMIPTERA - HETEROPTERA : ANTHOCORIDAE, WITH EMPHASIS ON THE STIMULUS FOR BITING MAN

by

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INTRODUCTION

The family Anthocoridae, commonly known as the minute pirate bugs or minute flower bugs, contains about 250 world species, 50 of which occur in America north of Mexico. All known members of the family are predacious. Most species occupy a wide variety of unexposed niches as beneath loose or dead bark, in piles of decaying weeds or wood, in beetle galleries in shelf fungi, in the nests of birds and woodrats, and in caves. Many species are of significant economic value as a means of biological control for agricultural pests.

The genus _Orius_ is composed of very small insects (1.5-2.5mm in length) and is widely distributed in North America, where it is represented by four species. These are usually very numerous on flowers of various plants. These insects are known to be predacious, feeding on the eggs and larvae of other insects, and on a wide variety of other small arthropods. The State of Hawaii Department of Agriculture has recently introduced, on Oahu, the two common species: _O. tristicolor_ (White) for control of the microlepidopteran _Ithome concolorella_ (Chambers), the kiawe flower moth, and _O. insidiosus_ (Say) for the control of the corn earworm.

O. insidiosus is very abundant on a great variety of plants, but its distribution is limited to the eastern and midwestern United States, and southern Ontario and Quebec.

It is recognized by its small size (approximately 2mm long and 0.8mm wide), flattened and oval form, three veins in the wing membrane (with the middle vein almost invisible), shape of the ostiolar canal, and the asymmetrical genitalia in the male.

The morphology of the mouthparts became one of the first questions, naturally leading from the fact that this bug occasionally bites humans. It was hypothesized that they should be typically hemipteran--a labium surrounding a fascicle of central maxillary stylets, coupled as to form a dorsal food channel and a ventral salivary channel, and lateral mandibular stylets. A scanning electron microscope study was designed to elucidate the external mouthparts. The relationships of the stylets and channels, and their gross morphology, led to a composite diagramatic cross-sectional figure.

The next problem was to try to determine exactly why _O. insidiosus_ bites. Searching through the literature, it was discovered that hemipterans, in general, bite occasionally under three conditions: attraction to sweat, starvation, and the stimulus of artificial light or other unnatural environmental conditions. Assuming this to be true, experiments were designed to test the first and second hypotheses. Initial tests were devised to prove whether or not _O. insidiosus_ is attracted to human sweat. If the first hypothesis was confirmed, tests would be designed to fraction human sweat into its major components and determine exactly which component is attractive to the insect. To test the second hypothesis, that *O. insidiosus* bites humans when starved, initial tests were carried out. Thirty insects were starved for two days. These bugs were then placed in a test chamber (Fig. 2), affixed to a human forearm, and allowed to roam for 15 minutes. Numerous bites were observed. Having observed that *O. insidiosus* does indeed bite when starved, additional tests were done to determine if the frequency of biting is related to the number of days starvation. The third hypothesis, that hemipterans bite under the stimulus of artificial light or other unnatural conditions of the environment, was not directly tested. This was incorporated into the design of the other tests since they were conducted indoors, under artificial light, and under conditions not normally encountered by an insect in the field.

Throughout the remainder of this thesis, the tests are reported in the order in which they were performed. The sweat-tests, followed by the biting-tests, were conducted during the late summer and early fall of 1978, when *O. insidiosus* was still available in the field. Upon completion of these tests, the specimens were preserved in 70% ethanol. The S.E.M. study and the cross-section were done during the winter months when it became impractical to collect specimens.

REVIEW OF THE LITERATURE

The first anthocorid was described by Linnaeus (1758) as *Cimex minutes* in the family Cimicidae. Wolff (1811) described the genus *Orius* in the family Cimicidae and included *minutes*. *Orius insidiosus* (Say) was originally described as *Reduvius insidiosus* by Say (1832) in the family Reduviidae. Amyot and Serville (1843) erected the family Anthocoridae. Fieber (1860) created the genus *Triphleps* in the family Anthocoridae and changed *Reduvius insidiosus* to *Triphleps insidiosus*. Stål (1873) reduced the family Anthocoridae to the subfamily Anthocorinae in the family Cimicidae. Reuter (1884), following Fieber, erected the other three major subfamilies of the family Anthocoridae in what is still the most extensive work on anthocorids. Blatchley (1926) changed the genus *Triphleps* of the family Anthocoridae to *Orius* of the family Cimicidae, calling them synonyms and citing Wolff (1811) as having priority over Fieber (1860). He placed all currently recognized species of *Orius* into the family Anthocoridae, including *Orius insidiosus* (Say).

Because of the close relationships to anthocorids, the cimicids have been considered by some authors to be a subfamily equivalent either to anthocorids or to be at the same level as the anthocorid subfamilies. Anthocorids differ from cimicids in having a longitudinal suture on the meso- and meta-sterna, usually paratergites on the second and third abdominal segments, and usually well developed ocelli and wings. Reuter and others have stressed the habits of the anthocorid Lyctocoris campestris (F.), which sucks the blood of birds and mammals, including man. Carayon and Usinger (1965) described a new genus of the Lyctocorinae with brachypterous females that bear a striking resemblance to cimicids. To settle the taxonomic status of the anthocorids, it is necessary to decide whether the anthocorid subfamilies are more closely related to each other or to the cimicids. In the opinion of Usinger (1966) they are the former. This was also the judgment of China and Myers (1929), Carayon, and others. This seems to finally settle the question, after over 100 years of dispute.

White (1879) described Triphleps tristicolor, whose taxonomic status has been somewhat uncertain. Reuter (1884), Champion (1900), Poppius (1913), Barber (1914), Van Duzee (1916 & 1917), and Anderson (1962) considered it a valid species. Parshley (1919 & 1920), Britton (1926), Blatchley (1926), Torre-Bueno (1930), and Marshall (1930) considered it a synonym or only a color variety of O. insidiosus. This was finally resolved when Kelton (1963) proved the validity of tristicolor by comparing the male genital claspers in both species and finding them morphologically different.

The single most important book used in trying to locate the important pre-1925 papers is Parshley (1925).

There are many excellent dichotomous keys to the family level of adult hemipterans. The keys of China and Miller (1959), Borror, DeLong, and Triplehorn (1976), and Slater and Baranowski (1978) are the most recent and most complete. Lawson (1959), DeCoursey (1971), and Herring and Ashlock (1971) have constructed keys to the families of the nymphs.

There are only a few keys below the family level for Anthocoridae. DeCoursey (1971) only separates the family into two groups of subfamilies. Sands (1957) provides the only key to the immature stages and eggs of some British anthocorids. Pericart (1967) and Herring (1976) have keys to the generic level for European and North American Anthocoridae, respectively. These last two keys use the shape of the metapleural scent glands almost exclusively. J. Carayon (1952, 1957, and 1972) has done extensive work on the family at all levels of taxonomy, both external and internal, but has yet to publish a comprehensive key. Slater and Baranowski (1978) have used his 1972 paper exclusively in constructing their generic key.

Western Hemisphere species of <u>Orius</u> are difficult to identify since most of the characters used by the classic authors (Fieber, 1851 & 1861; Reuter, 1871 & 1884; Heymons, 1889; Poppius, 1909) are no longer adequate. Coloration was used almost exclusively. Ribaut (1923) was the first to investigate thoroughly the color variations and to establish useful characters for separation of the species. His major contribution was a critical study of the left genital clasper of the male. This structure has since been shown (Wagner, 1952; Kelton, 1963; Herring, 1966) to be the single most useful character for distinguishing the species. It appears to be so constant that any species can be recognized by it without recourse to other structures. Unfortunately, the females of many species are still very difficult to separate. Herring (1966) uses the shape of the head, pronotum, and pronotal callus to differentiate the female species. Since there is a gradation in these characters, this key is adequate only when separating distantly related species.

Most of the published accounts of O. insidiosus have been on the abundance and importance of it as a predator of various pests of crop plants. This species is very abundant on a great variety of plants, but its distribution is limited to the eastern and midwestern United States and southern Ontario and Quebec. According to Marshall (1930) it has been recorded as preying on 53 species of insects and mites. This species is unique in that it is both a predator and a plant feeder. Among the most economically important prey are the bollworm (Quaintance and Brues, 1905), the cotton leafworm and cotton fleahopper (McGregor, 1942), and the tobacco budworm and cotton leafperforator (Lingren and Wolfenbarger, 1976) on cotton, the Fall armyworm, southern cornstalk borer, and Heliothis obsoleta Fab. (Barber, 1936), the corn earworm (Garman and Jewett, 1914), and the European corn borer (Dicke and Jarvis, 1962) on corn, and the soybean thrips (Robinson, Stannard, and Armbrust, 1972) on soybean. Other prey include a variety of thrips, aphids, whiteflies, leafhoppers, lace bug nymphs, the chinch bug, midges, and mites. Hyslop (1916) thought that it transmitted corn-ear rot (Diplodia sp., Fusarium sp.). It has also been indicated as doing some damage to plants either by feeding or oviposition (Riley, 1888; Marshall, 1930; Barber, 1936). All of the life-cycle (Garman and Jewett, 1914; Marshall, 1930; Barber, 1936) and population dynamics studies (Barber, 1936; DeLoach and Peters, 1972; Dicke and Jarvis, 1962; Shepard, Sterling, and Walker, 1972; Wiseman, McMillian, and Widstrom, 1976) of O. insidiosus have been conducted via its association with pests, on either cotton or corn.

Hemipterans biting man are well known in the literature. In the suborder Homoptera, various members of the families Aphididae, Cicadellidae, Cicadidae, Coccidae, and Membracidae (Myers, 1929; Usinger, 1934) are among the most common. Common biters in Heteroptera, excluding obligate human ectoparasites, occur in the families Anthocoridae, Belostomatidae, Cimicidae, Coreidae, Lygaeidae, Miridae, Nabidae, Naucoridae, Notonectidae, Pyrrhocoridae, Reduviidae, and Tingidae (Howard, 1900; Blanchard, 1902; Walton, 1908; Tucker, 1911; Bergevin, 1923 & 1925; Bequaert, 1926; Myers, 1929; Usinger, 1934 & 1966; Sailer, 1945; Bailey, 1951; Štys, 1973). Species of anthocorids that frequently bite man are: Orius insidiosus (Say) (Tucker, 1911; Malloch, 1916; Dicke and Jarvis, 1962; Robinson, Stannard, and Armbrust, 1972; Grzimek, 1975), Lyctocoris campestris (F.) (Butler, 1923; Jensen-Haarup, 1926; Royer, 1926; Imms, 1948; Woodward, 1951; Štys and Daniel, 1956 & 1957; Sands, 1957; Povolný, 1957; Štys, 1961 & 1973; Anderson, 1962; Vostál, 1962), Anthocoris antevolens White (Anderson, 1962), A. melanocerus Reut. (Anderson, 1962), and A. musculus (Say) (Torre-Bueno, 1930), all of which occur in North America. Other species include A. nemoralis F. (Jensen-Haarup, 1962) in Germany, A. pilosus Jak. (Marikovskii, 1965) in Russia, A. sylvestris L. (Morley, 1914), A. nemorum L. (Butler, 1923), and Dufouriellus ater (Dufour) (Dolling, 1977) in England, A. kingi Brumpt (Imms, 1948) in the Sudan, and Triphleps tantilus Mostch. and Septicius clarus Dist. (Misra, 1924) from India. S. clarus has also been reported as sucking juices from meat (Misra, 1924), while L. campestris has been listed as a parasite of the coypu, a small rodent, in England (Newson and Holmes, 1968).

There are no papers written describing the mouthparts of O. insidiosus or any other anthocorid, for that matter. The papers that are written about the mouthparts of Hemiptera can easily be divided into five groups. The first group, and also the earliest, containing papers by Kraeplin (1882), Geise (1883), Léon (1887, 1892, & 1897), Heymons (1896), and Smith (1892 & 1898), lays down the foundation of mouthpart morphology for later papers. The second group contains comparative anatomies between mouthparts of Hemiptera -- both Homoptera and Heteroptera (Wedde, 1885; Bugnion and Popoff, 1911; Muir and Kershaw, 1911; Weber, 1928). The third group contains papers by Marlatt (1895), Meek (1903), Nietsch (1908), and the classic paper by Snodgrass (1921). This group deals with homologies of the mouthparts of the cicada. The fourth group contains papers written about the mouthparts of a specific insect: Anasa tristis DeGeer in the case of Tower (1913 & 1914), and Cimex lectularis L. in the case of Usinger (1966). The last group includes the modern text books which give generalized views of the hemipteran mouthparts (Snodgrass, 1935; Imms, 1948; Borror, DeLong, and Triplehorn, 1976; Slater and Baranowski, 1978).

Cross-sections of insect mouthparts may be made by standard Paraplast imbedding techniques described in Steedman (1960). Helpful modifications are described in Palmgren (1954), Lower (1955), Beckel (1959 & 1960), Carlisle (1960), and Storch and Chadwick (1964).

MATERIALS AND METHODS

During the summer of 1978, specimens of Orius insidiosus (Say) were collected on White Sweet Clover, Melilotus alba Desr., between June 3rd and August 19th, feeding primarily on aphid nymphs. From August 13th to August 19th the Sweet Clover died out, the aphids disappeared, and the anthocorids migrated to Canada Goldenrod, Solidago canadensis L., where they were collected until November 2nd feeding primarily on thrips. The area of collection was a 6-acre field, partially surrounded by a swamp, in Cook County, Illinois, approximately one mile west of Wolf Lake. The specimens were collected with a suction-tube aspirator, after being knocked off the plant onto a beating sheet. They were then placed, in lots of 10, into 2.5cm diameter plastic cone-bottomed centrifuge tubes. The tubes contained a small cotton wad saturated with water and packed into the bottom to provide moisture for the bugs. A 2cm by 8cm piece of paper, folded into a "V", was also inserted, to provide a surface for the bugs to walk or rest on. The tubes were capped with cork stoppers. (Fig. 1) Approximately 200 specimens were collected per day and transported to the insectory at Loyola University.

The bugs were then tested for attraction to human sweat. The test chamber was constructed out of plastic, modified, "Hamster and Gerbil Castle" parts. (Fig. 1) A 15cm T-tube was connected to two 20cm long cylinders, each connected to a sealed chamber, approximately 20cm long by 10cm wide by 8cm high. The T-tube and cylinders were approximately 5cm in diameter. A funnel was placed between the chamber and the cylinder to retard the movement of the insects out of the chamber once they had entered. The funnel was sealed in place with non-hardening modeling clay. All connections and small holes in the test chamber were sealed with the clay, while all large holes were covered with very fine nylon monofilament screening. A 9cm disk of clean, dry filter paper was placed in the "control" chamber. A similar disk was saturated with 1ml of freshly collected human sweat and placed in the "test" chamber. Sweat was collected by scraping it from the body of the subject with a spatula and placing it in a glass vial. Twenty to fifty individual bugs were released into the chamber and allowed to roam for one hour. After this time, the five parts were separated and numbers of anthocorids in each section were counted and recorded. Animals were tested in lots of 50, on successive days, until there were insufficient numbers left to test i.e. less than 20. The animals were not fed but were supplied with ample water. After testing 1,205 individuals, the results were pooled and statistically analyzed.

Orius insidiosus was also tested for biting on humans. Special containers allowed the bugs access to the skin. (Fig. 2) The containers were constructed out of large, leather wrist-bands, fitted with a 7.5cm x 3cm plastic vial and connected by a standard screw clamp. The containers were strapped onto the skin of the forearm with the pressure of the vial against the skin. Since both males and females were previously determined to bite readily, ten bugs were randomly selected for all tests. (Fig. 3) The containers were removed after 15 minutes and the following data recorded: test date, subject, time of test, preparation of arm, test arm, number of bites, date the bugs were collected, and number of days the bugs were starved. The number of bites was determined by using a hand lens although most bites were clearly visible to the naked eye. (Fig. 4) Individuals collected on a certain day were tested until their numbers declined to less than ten, though not on successive days, whenever possible. No individuals were ever tested more than once on a specific day. One hundred twenty-seven tests were run using 1,270 anthocorids on ten human subjects. Results were tabulated and analyzed statistically.

Scanning electron micrographs of the head and mouthparts of <u>Orius insidiosus</u> were taken using the facilities at the Field Museum of Natural History in Chicago. The specimens were first cleaned ultrasonically. Mouthparts were then stretched out in front of the head and allowed to dry in this position for approximately 24 hours. Standard "T"-shaped mounting studs were prepared by attaching a strip of self-stick copper tape to the surface and dotting the corners with silver paint. The bugs were then mounted, using clear fingernail polish, to the copper strip and properly oriented.

Twenty-eight insects were mounted on ten studs, which were then transported to the Field Museum and gold coated. The specimens were then viewed on the museum's Cambridge Stereoscan S.E.M. Forty-five polaroid micrographs were taken of the mouthparts at various views and magnifications. These micrographs were then analyzed to determine homologies between anthocorid mouthparts and mouthparts of other hemipterans.

A cross-section of the mouthparts was also obtained, using the following procedure:

1. Dehydrate through changes of 70%, 85%, 95%, and 100% ethanols for 20-30 minutes each.
2. 100% ethanol + 100% xylene in a 1:1 ratio for 20-30 minutes.
3. 100% xylene for 20-30 minutes.
4. Infiltrate through two changes of Paraplast, for 30 minutes each, in a vacuum oven at 15 P.S.I.
5. Imbed properly oriented insect, chill block, and section on a microtome (5-10 microns).
6. Fix sections to a clean glass slide, using albumin fixative, by floating sections on a small volume of 35% ethanol and drying overnight in an oven.
7. Dewax in two changes of 100% xylene for 5 minutes each.
8. Mount a coverslip with Cover-Bond.
9. Allow mounting media to dry overnight, in

a dust-free chamber, in a horizontal position.

It was not necessary to stain the mouthparts since they were dark colored and easily found. A composite diagramatic figure was constructed by examining numerous cross-sections. (Fig. 15) This figure was then analyzed to determine homologies between anthocorid mouthparts and comparative mouthparts of other hemipterans.

RESULTS

O. insidiosus was tested for attraction to human sweat. Table 1 is a tabulation of all tests done per day starvation. It does not show the results of individual tests. The numbers are numbers of individuals. Days 0-6 were treated by the "Binomial Distribution, Normal Approximation" (Zar, 1974) test due to large numbers of individuals tested (above 25). The following formula was used:

$$Z_c = \frac{\left| n\hat{p} - nc \right| - 0.5}{\sqrt{n\hat{p}\hat{q}}}$$

where "c"=0.5 (50% chance of going either way if no effect due to sweat), "n" equals the total number of animals exhibiting a preference (animals in the T-tube exhibited no preference), "$\hat{p}$" equals the number of animals attracted in one direction divided by n, and "$\hat{q}$" equals the number of animals attracted in the other direction divided by n or $1-\hat{p}$. In all cases, the critical value ($Z_{0.05(2)}$) equals 1.96; values of Z_c above 1.96 are significant. Days 7 and 8 were treated by the simple "Binomial Test" (Zar, 1974) due to small numbers (below 25).

All columns in the table were analyzed in two ways: (1.) Number of individuals actually reaching one chamber vs. the other chamber, and (2.) Number of individuals moving out of the T-tube in one direction vs. the other direction.

# Days Starvation	0	1	2	3	4	5	6	7	8	Sum
Control Chamber	65	121	63	20	48	32	21	5	2	377
Contol Tube	18	20	37	3	19	7	4	5	4	117
T-tube	25	30	52	8	37	9	14	7	7	189
Test Tube	18	36	43	6	27	9	3	8	6	156
Test Chamber	67	121	61	24	54	17	17	2	3	366
Total # Tested	193	328	256	61	185	74	59	27	22	1,205
% Moved	87	91	80	87	80	88	76	74	68	84

Table 1

The numbers of individuals actually reaching the chambers after 5 days starvation was significant at the 0.05 level but not at the 0.02 level. However, this is misleading because for 18 tests we expect 0.05 x 18=0.9 to be significant at the 0.05 level by chance alone. There was no other significant difference.

Since none of the tests was significantly different from a 50:50 ratio, O. insidiosus is not attracted to human sweat, either when fed or starved for up to eight days.

Per cent moved, those individuals moving out of the T-tube, was also tabulated to determine if there was increased movement proportional to time of starvation. This is obviously not the case. In fact, after 5 days starvation, they moved less, probably due to weakness.

O. insidiosus was also tested for biting behavior on human subjects. Table 2 shows the results of individual tests. The first test applied was the "Kruskal-Wallis Single Factor Analysis of Variance by Ranks" (Zar, 1974) to determine if there were any differences between biting occurrences after a specific number of days starvation. The following formula was used:

$$H=\frac{12}{N(N+1)}\sum_{i=1}^{k}\frac{R_i^2}{n_i}-3(N+1).$$

# Days Starvation	0	1	2	3	4	5	6	7	8	9	10	11	12
Number of Bites/Test	3	6	4	1	0	5	0	9	3	8	3	5	3
	4	1	3	2	1	2	1	9	2	5	5	5	
	4	0	6	5	5	10	3	8	5	4	4		
	1	2	5	3	4	4	5	4	4	3	3		
	1	4	5	3	4	2	4	9	7	6	3		
	2	4	3	3	4	4	3	4	4	8	3		
	3	5	3	1	5	5	5	3	2	0			
	0	3	3	2	4	6	2	5	4	0			
	2	8	5	3	3	6	2	5	4	1			
	0	5	7	3	4	5	4	6	6	5			
	4	3	0	3	0	7	5	3	5				
	0	0	2	3	0	11	4	7	4				
Sum Total	24	41	46	32	34	67	38	72	50	40	21	10	3
Average	2.0	3.42	3.83	2.66	2.83	5.58	3.17	6.0	4.17	5.0	3.5	5	3

Table 2

where "H" is the Kruskal-Wallis test statistic, "n_i" is the number of observations on day i, "N" is the total of all the n_i's for all days, and the "R_i's" are the sums of the ranks of the n_i observations for each day i. Since, in this case, N=127, H reduces to:

$$7.382 \times 10^{-4} \sum_{i=1}^{k} \frac{R_i^{2}}{n_i} - 384$$

The "H" calculated, 67.34, when compared to the critical value ($H_{0.05,12} = X^2_{0.05,12} = 11.340$) is significant. This indicates that there is at least one group that is significantly different from one other group.

The "Neuman-Keuls Test" (Zar, 1974) was then applied to the data to determine which differences were significant. Only the days with equal numbers of tests could be tested, days 0-8, all of which have twelve tests. The Standard Error, "SE", for each pair of days was calculated from the formula:

$$SE = \sqrt{\frac{n(np)(np + 1)}{12}}$$

where "n" equals the number of tests, and "p" equals the absolute value of the difference in ranks between days plus one (including the compared ranks). Since, in this case, n=12 in all cases, this reduces to:

$$SE=\sqrt{(12p)(12p+1)}$$

A "q" was then calculated for all possible differences in ranks by the formula:

$$q=\frac{R_B - R_A}{SE}$$

where "R_B" is the rank sum of day "B", "R_A" is the rank sum of day "A", and "SE" is the Standard Error for the two days. Each "q" determined was compared to its critical value ($q_{0.05,\infty,p}$). It was determined that biting occurrences on days 1, 2, 4, and 6 were not significantly different from each other. All other days are significantly lower or higher than each other without apparent pattern.

The next analysis employed was the "Chi-square Index of Dispersion Test" (Elliott, 1971) to determine if there was a group effect i.e. does the occurrence of one bite make a second bite more likely. The statistic for each day was determined as:

$$X^2=\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{\bar{x}}$$

where "x" is the number of bites per test on a certain day, "$\sum x$" is the total number of bites on that day, and "$\bar{x}$" is the mean number of bites on that day. The "X^2" for each day was compared to the critical value of $X^2_{0.05,11}=19.675$ to determine significance. None of the calculated numbers was significant. Therefore, all individuals act independently.

The last step was to fit a curve to the points. A "Simple Linear Correlation" (Zar, 1974) was attempted two ways: once with the averages of the numbers of bites per day starvation of all the days vs. days starvation, and once with the averages of the numbers of bites per day starvation of the days containing twelve tests (days 0-8) vs. days starvation. The Linear Correlation Coefficient, "r", was determined by the following formula.

$$r = \frac{N\sum xy - \sum x\sum y}{\sqrt{N\sum(x^2) - (\sum x)^2} \times \sqrt{N\sum(y^2) - (\sum y)^2}}$$

where "x" is the number of days starvation, "y" is the average number of bites per day x, and "N" is the number of trials (number of different x's). In both cases the "r" calculated was compared to critical values of $r_{0.05(2)}$ with the appropriate degrees of freedom, 11 and 7 respectively. The correlation indices calculated were approximately 16% and 38% respectively, neither of which is statistically significant. The relationship is not linear.

A "Multiple Regression" was then attempted. Loyola's computer was utilized using Bio-Medical Data program "BMD02R". Averages of the numbers of bites per day starvation of all the days were used. Again two attempts were made: $\sqrt{x}$ and x vs. y, and x^2 and x vs. y where "$\sqrt{x}$" and "x^2" are the transformed variables of x. The F-levels calculated were not statistically significant, at a 0.05 significance level, as compared to the F-levels read from the tables i.e. probability is greater than 0.05. Thus, a simple curve does not fit the points.

Since regression analyses show no effect of starvation on biting, results are inconclusive. Some other factor or factors, which may or may not be testable, must influence the propensity to bite. More tests are required before any general statements can be made.

A scanning electron microscope study of the head and mouthparts was also conducted. (Fig. 5-14) Since the sclerities of the head are solidly fused together, the general head regions are all that can be described. The occiput lies behind the ocelli and compound eyes, and forms the posterior portion of the head, surrounding the foramen magnum. It is marked off from the vertex by a shallow transverse groove. The vertex (v) comprises the dorsal region in front of the occiput and bears the ocelli (oc) (Fig. 5). This area is not deliminated from the frons (fr), which lies above and between the bases of the antennae (ant). The anterior margin of the frons is united with the base of the tylus. Below the vertex and occupying most of the lateral area of the head, are the very large and conspicuous compound eyes (e). The gena, due to the extremely large size of the eyes, is absent below. It is present behind the eyes and grades into the vertex above, occiput behind, and gula below. Anterior to the eyes, it occupies an area below the frons, behind the jugum (j) and maxillary lobe (mx-l), and above the gula (gu). The genae contain short anterio-ventral protuberances called the antenniferous tubercles which form the bases of the four-segmented antennae. The complete ventral portion of the head capsule forms the gula. The tylus, the most anterio-dorsal sclerite of the head, is only partially delimited by lateral sutures, which extend a short distance into the dorsal aspect of the head and then disappear, thus not defining the area posteriorly. On either side of the tylus are paraclypeal lobes or juga, which are bounded by faint sutures (Fig. 11). Surrounding the base of the rostrum are the very distinct and rugose maxillary lobes (Fig. 6-11).

The mouthparts come off of the most anterio-ventral portion of the head. The labrum (lbr) appears as a free sclerite at the extreme anterior margin of the head (Fig. 5). It is bilobate at its apex and marked by a definite labral suture. Ordinarily it is deflexed beneath and covers the base of the rostrum. The labium (lbm) arises from just within the anterior margin of the head beneath the labrum. It is surrounded laterally and ventrally by the maxillary lobes (mx-l) (Figs. 6-11). A small sclerite, in the membrane (mem) at its base, may be a vestige of a fourth segment (lbm-v), but for all practical purposes the labium may be regarded as 3-segmented (Fig. 7-8). On the anterior or (at rest) ventral surface is a longitudinal groove which contains the fascicle of stylets (sty) (Figs. 5-6). The apex of the labium consists of two lobes bearing minute sensilla (sen) (Fig. 12). The fascicle is composed of four stylets: an inner pair of maxillary stylets (mx) and an outer pair of mandibular stylets (md). The mandibular stylets are minutely serrate apically (Fig. 13). The maxillary stylets are asymmetric apically but similar (Fig. 14). The outer edge of one side is smooth, while the opposite edge is slightly concave and with small serrations in the concavity. The smooth edge of one stylet articulates with the concave edge of the other and vice versa. The stylets diverge at the base of the rostrum where the maxillary stylets are met by the cibarium and salivary ducts. The mandibular stylets also separate from the maxillary stylets at this point. The bases of all four stylets widen posteriorly, forming attachments with the protractor and retractor muscles which operate them. (Fig. 16)

Figure 15 is a diagram of the cross-section of the fascicle of <u>O. insidiosus</u>. The maxillary stylets form the core of the fascicle. They are interlocked and fluted forming two tubes. The larger, somewhat square-shaped food channel (fc) lies dorsal to the smaller, round salivary channel (sc). The outer surface of each stylet contains three ridges for locking the mandibular stylets to them.

The mandibular stylets are much more slender and lie lateral to the maxillary stylets. Their outside surfaces are smooth, while their inside surfaces are notched to "dovetail" with the lateral ridges in the maxillary stylets. The sutures marking the interlocking mechanism for the maxillary stylets were not observed due to their extremely small size and close proximity.

DISCUSSION

Hemipterans, in general, occasionally bite man under three conditions: attraction to sweat, the stimulus of artificial light or other unusual conditions of the environment, and starvation. The test for attraction to human sweat vs. days starvation was originally designed only to test the insects and their attraction to sweat. After all the tests were completed, it was discovered that the best way to treat the data statistically was with the two variables. This seemed acceptable since the assumption, that the anthocorids were fed on day zero, was the same as that incorporated into the biting tests. Now, there is some question as to the validity of this assumption. There is only scanty data on the feeding habits of O. insidiosus in general and virtually no data on its feeding habits in relation to thrips or aphids. The food requirements of this bug, as a function of time, are not understood. Put simply, no one knows how long after an anthocorid has fed, that it will again become hungry. Another problem with the pre-test assumption is that all individuals were thought to be in the same state of hunger on day zero. It was probable that some individuals, when caught, had not fed for some period of time while others, caught at the same time, had just fed or were feeding when captured. As a matter of fact, some specimens, when collected, had the prey still impaled on the apex of their stylets.

The problem with the pre-test assumption was also born out by a subsequent test. Ten anthocorids were starved for three days. These were then given the opportunity to feed on aphids and thrips. When placed in the biting-test chamber, they, for the most part, remained motionless on the sides of the container. This is contrary to what was expected and contrary to their usual "seeking" activity, moving about rapidly and probing everything with their labial apices. This test is interesting but in no way conclusive since so few individuals were tested. It can therefore be stated that the numbers of days starvation, in both the sweat and biting tests, represent number of days of known starvation rather than numbers of days of actual starvation.

This problem in no way invalidates the results of the sweat tests since these tests were qualitative rather than quantitative. They were designed only to test if anthocorids are attracted to sweat and not to what degree they are attracted. Taking everything into consideration, the results stay the same: *O. insidiosus* is not attracted to sweat.

The biting tests, on the other hand, were quantitative. They were designed to try to determine if there is an increase in biting proportional to number of days starvation. Known vs. actual days of starvation may have caused the results to be inconclusive. More tests, keeping known days starvation equal to actual days of starvation, need to be done before any general statements about biting frequency proportional to starvation can be made.

The rest of the study bears out the fact that the mouthparts of O. insidiosus are typically hemipteran. (Fig. 16) From the small thin size of the protractor muscles and the large broad size of the retractor muscles, it seems logical to assume that the piercing mechanism is also typically hemipteran. The stylets are not moved by simultaneous contraction of their muscles. The mandibular stylets are the chief piercing structures. When the insect begins an insertion of its fascicle, one mandibular protractor muscle (md-pro) contracts causing its mandibular stylet to be thrust out a short distance in advance of the other, to puncture the food source. Then the opposite mandibular stylet is protracted until its apex meets that of the first. Finally, the maxillary protractor muscles (mx-pro) contract simultaneously causing the maxillary stylets to be lowered together until their apices lie between those of the mandibular stylets. At a single thrust, a stylet is extruded no farther than the maximum distance the short muscle can protract it with one contraction. This distance, at best, is insignificant compared to the depth to which the fascicle can finally be sunken into the tissue. Repeated thrusts are therefore necessary. A repetition of the insertion process necessitates that the protracted stylets be, in some way, secured in their new position in order to resist the backward pull of the retractor muscles (md-ret and mx-ret) which restore the protractors to their functional lengths. In O. insidiosus, the fascicle is anchored in the food tissue by the recurved barbs at the apices of the mandibular stylets. (Fig. 13)

When the stylets are not in use they do not normally protrude from the tip of the labium. The stylets are not long enough to be protracted from the labium except for the very short thrust given them by the protractor muscles. The exposure and insertion of a greater length of the fascicle is made possible by a retraction or folding of the labium that does not involve the stylets.

The cross-section of the fascicle was also compared to cross-sections of other hemipterans. The central interlocking maxillary stylets and the lateral mandibular stylets, with the mechanism to lock the latter to the former, are typically hemipteran. The dorsal position of the food channel and ventral salivary channel are also typical, with respect to location. The "squarish" shape of the food channel is noteworthy, since it seems to vary from the normally round condition found in most other hemipterans. There must be some selective advantage in the shape.

One possible explanation has to do with the relative sizes of the food channel compared to the salivary channel.

There seems to be a gradation in hemipterans. The so-called "seed predators" have an enlarged salivary channel with a slightly smaller food channel. This condition can be explained by the fact that more salivary secretions are necessary to dissolve the substances inside the seeds. The smaller food channel in these "seed predators" may be related to an increase in cibarial pump pressure necessary to draw the food up the fascicle to the functional mouth. The plant sap-feeders exhibit a condition in which both channels are the same size. This may be explained by less need for salivary secretions and less need for high pressure to draw the liquid food up to the mouth. Ectoparasites such as the common bed bug occupy a position at the other end of the spectrum. These insects have a greatly reduced salivary channel and a relatively large food channel. In the bed bug, the latter is in excess of six times the diameter of the former. This has obvious evolutionary advantages. The salivary channel has been reduced by selection because it functions only in injecting anticoagulents into the already liquid blood of its host. The food channel may be enlarged for two reasons. The first is that not much pressure is required to draw the blood up to the mouth, and capillary action may also play an important part. The second reason is that much more blood can be taken up in a shorter period of time. This is an obvious advantage to the bugs, who are always in danger of being interrupted during feeding. The large food channel gives them the ability to engorge with blood quickly and escape.

Anthocorids, being primarily predators, but sometimes either plant feeders or facultative ectoparasites, occupy a position somewhere between the plant feeders and the obligate ectoparasites. It seems logical that the size of their maxillary channels should be somewhat intermediate. This is born out by the cross-section. (Fig. 15) This intermediate morphology gives the insect the ability and genetic potential of going any of three directions, with regards to food, depending upon which source presents itself.

The "squarish" shape of the food channel may also be a case of evolution in progress. The closest relatives of anthocorids are the family of bed bugs, which are all ectoparasites of mammals. Since the first cases in the early 1900's, more and more accounts have been written about anthocorids biting humans and a few other mammals. There have also been a few accounts, within the past twenty-five years, where they have been proven to be facultative ectoparasites. The "squarish" shape of the food channel may be the result of evolutionary pressures acting to enlarge it, due to the new niche anthocorids might be exploiting.

<u>O. insidiosus</u> may become a pest of humans in the future because of the destruction of its natural habitat in urban areas. In rural areas <u>Crius</u> is found on food crops, such as corn or soybeans, or industrial crops, such as tobacco or cotton. In the city it is found primarily on White Sweet Clover and Canada Gondenrod, plants which thrive in "waste"

areas. This species has been collected in vacant lots, alleys, industrial complexes, along highways and railroad tracks, and in marsh reclamations in southern Chicago, always on these plants. If populations of Orius increase to the point where their natural prey cannot continue to support them, they will look elsewhere for food. This makes man a potential food source, especially since Orius is attracted to artificial light. Even if a natural food source is available, there is also the possibility that numerous individuals may become trapped in a building, get hungry, and bite humans in a feeding probe.

Today, this may even be a problem. Humans are, most likely, being bitten all the time, but because of their ignorance of entomology and the extremely small size of the insect, these occurances are rarely reported.

Much more research, in both comparative morphology and behavior, is needed before any of the aforementioned speculations can be substantiated. It is hoped that this thesis will stimulate such research in order to better understand these economically and medically important insects.

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FIGURES

Table 3 is a list of the abbreviations used in the figures. Most of the abbreviations and terms follow those used by Borror, DeLong, and Triplehorn (1976). The only exceptions are: ant-f, lbm-v, and mx-l, which are my own abbreviations of terms used by Usinger (1966), and c, md-pro, md-ret, mx-pro, and mx-ret, also my own abbreviations of terms used by Snodgrass (1935).

Table 3

Abbreviations Used in Figures

ant	antenna
ant-f	antennal foramen
c	cibarium
e	compound eye
fc	food channel
fr	frons
gu	gula
j	jugum
lbm	labium
lbm-v	fourth labial segment vestige
lbr	labrum
md	mandibular stylet
md-pro	mandibular protractor
md-ret	mandibular retractor
mem	membrane
mx	maxillary stylet
mx-l	maxillary lobe
mx-pro	maxillary protractor
mx-ret	maxillary retractor
n_1	pronotum
oc	ocellus
sc	salivary channel
sen	sensilla
sty	stylets
ty	tylus
v	vertex

Plate 1

Fig. 1 Author with Cone-bottomed Centrifuge Tube and "Sweat-test" Chamber.

Fig. 2 "Biting-test" Chamber on Subject's Arm.

Fig. 3 *O. insidiosus* in Typical Biting Position on Human Skin, 25x.

Fig. 4 Arrows Indicating Bite-marks of *O. insidiosus* on Inner Forearm of Human Subject.

Fig. 1

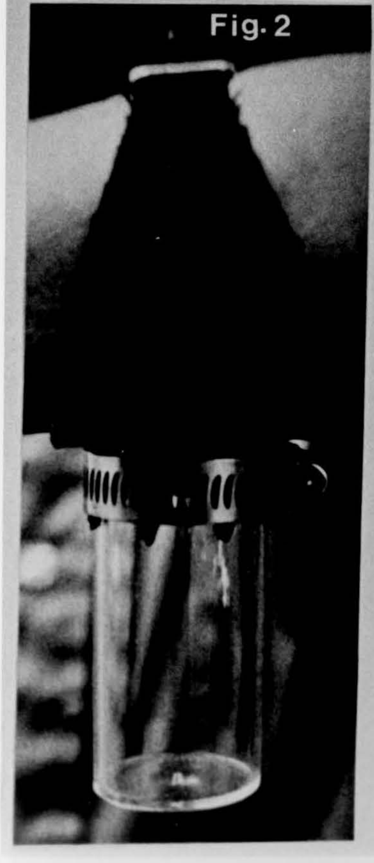


Fig. 2

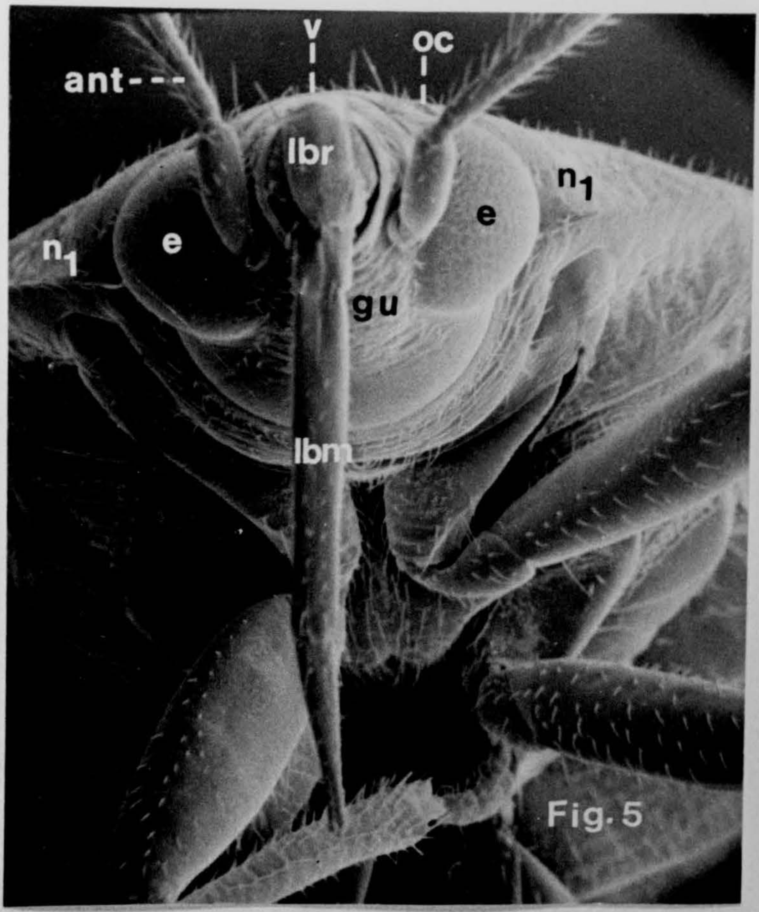
Fig. 3

Fig. 4

Plate 2

Fig. 5 O. *insidiosus*, Anterior View, 154x

Fig. 6 Mouthparts, Ventral View, 200x



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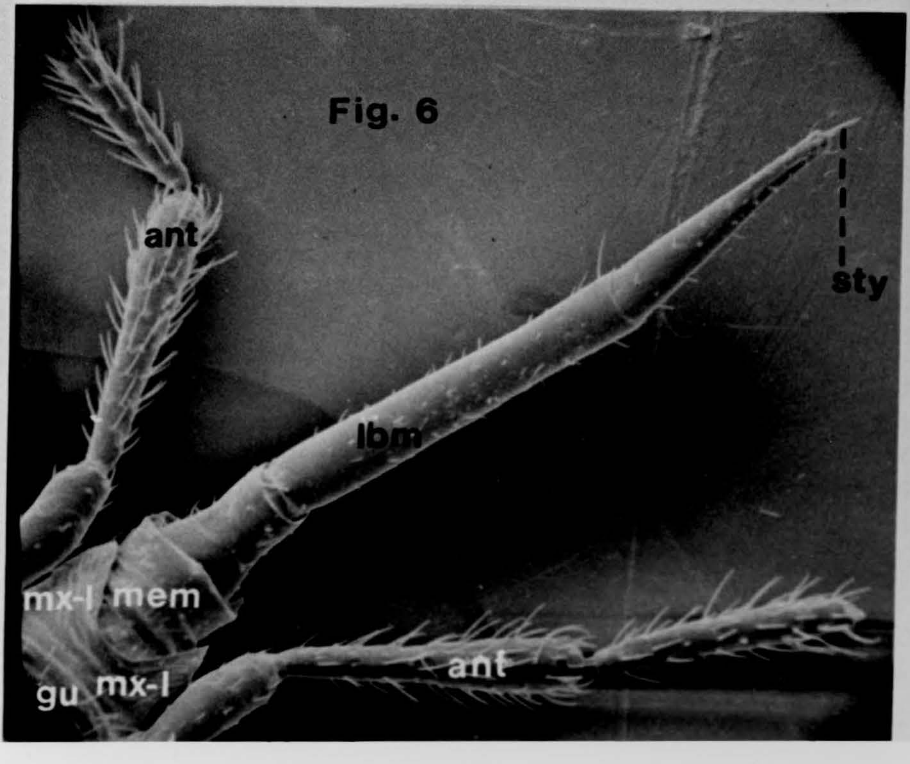
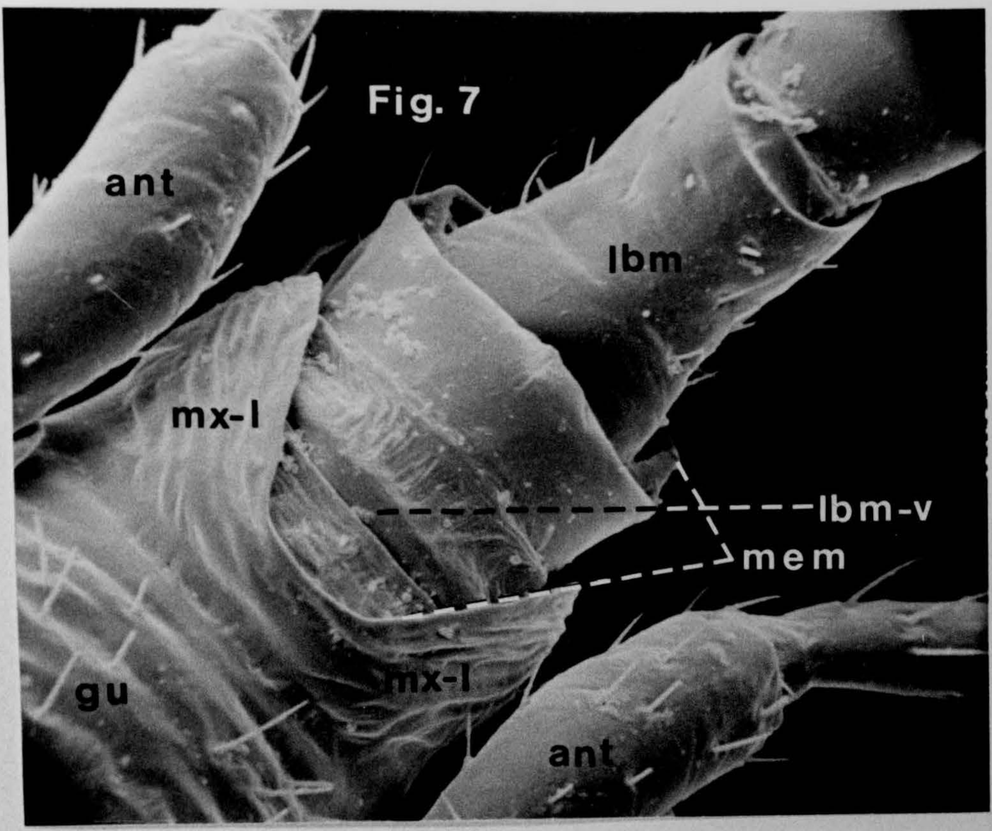


Fig. 6

Plate 3

Figs. 7 & 8 Variation in Membranous Area Containing the Vestige of a Fourth Labial Segment, Ventral Views: 538x, 550x.

Fig. 7



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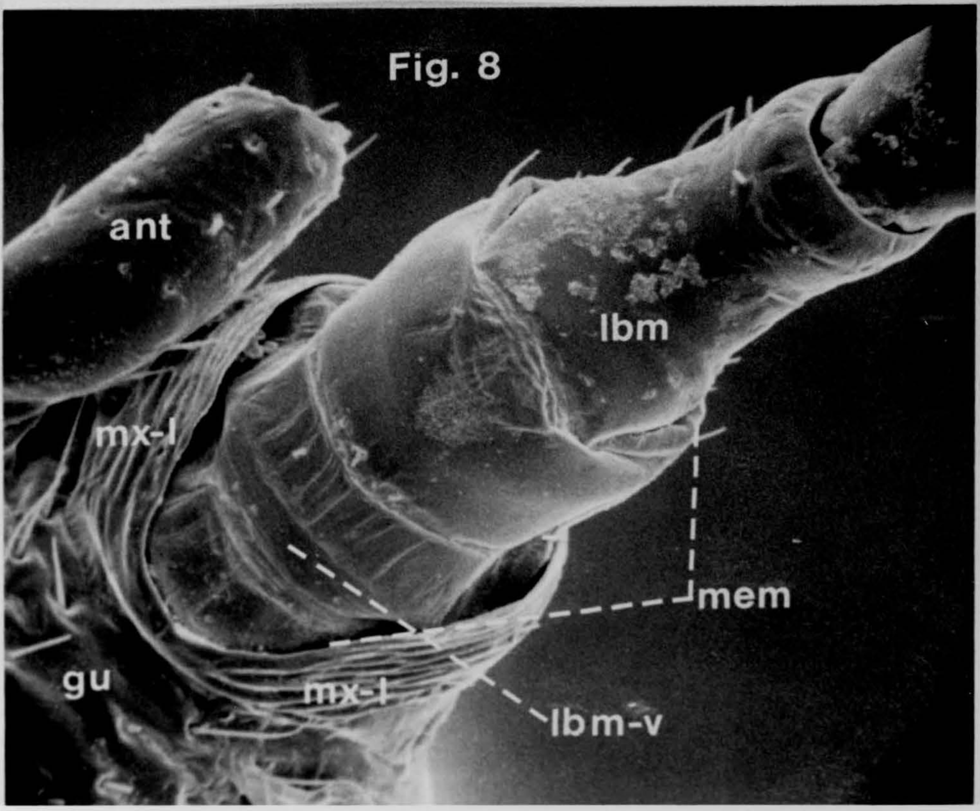

Plate 4

Fig. 9 Mouthparts, Showing Stylets Protruding from Apex of Labium, Lateral View, 176x.

Fig. 10 Base of Mouthparts, Lateral View, 352x.

Fig. 9

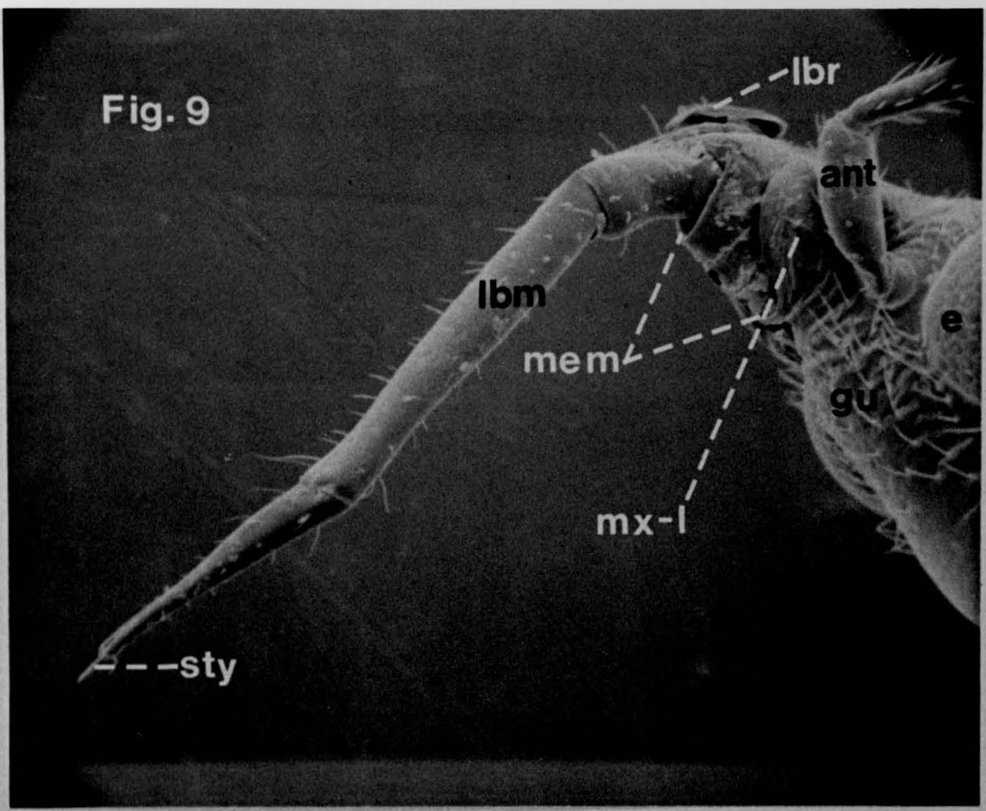

Fig. 10

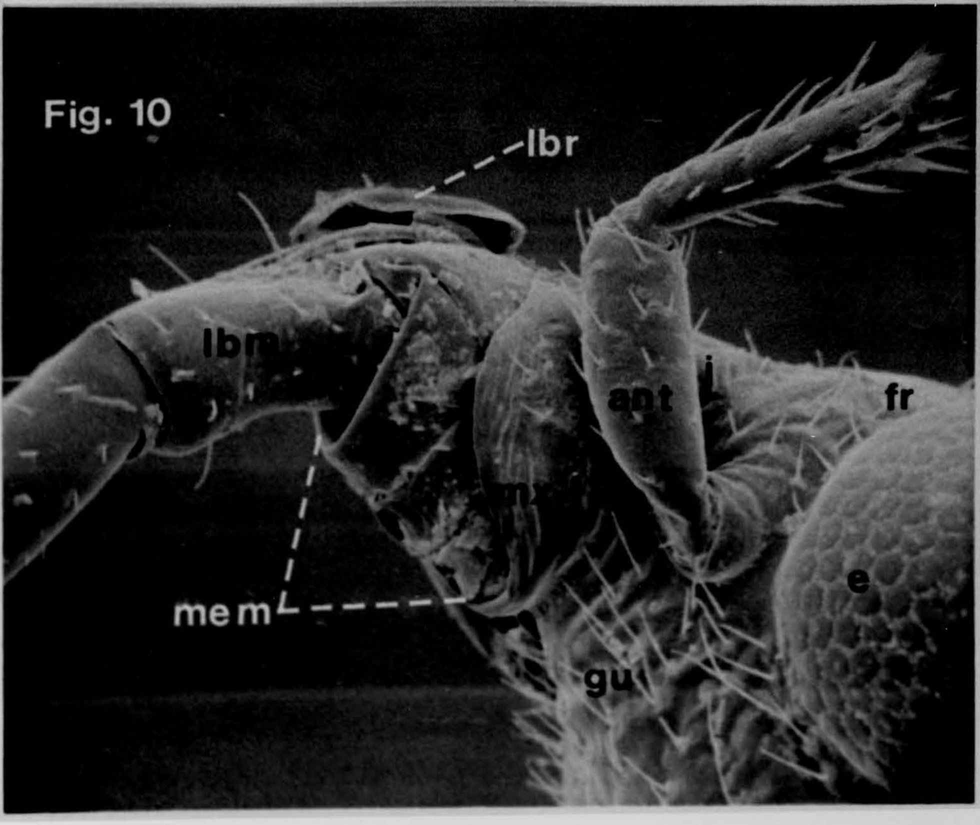

Plate 5

Fig. 11 Base of Stylets, Showing Stylets Exiting Head Proper below Labrum and Entering Labial Groove, Lateral View, 550x.

Fig. 12 Labial Apex, Showing Sensilla and Protruding Fascicle, Ventro-lateral View, 2420x.

Fig. 11

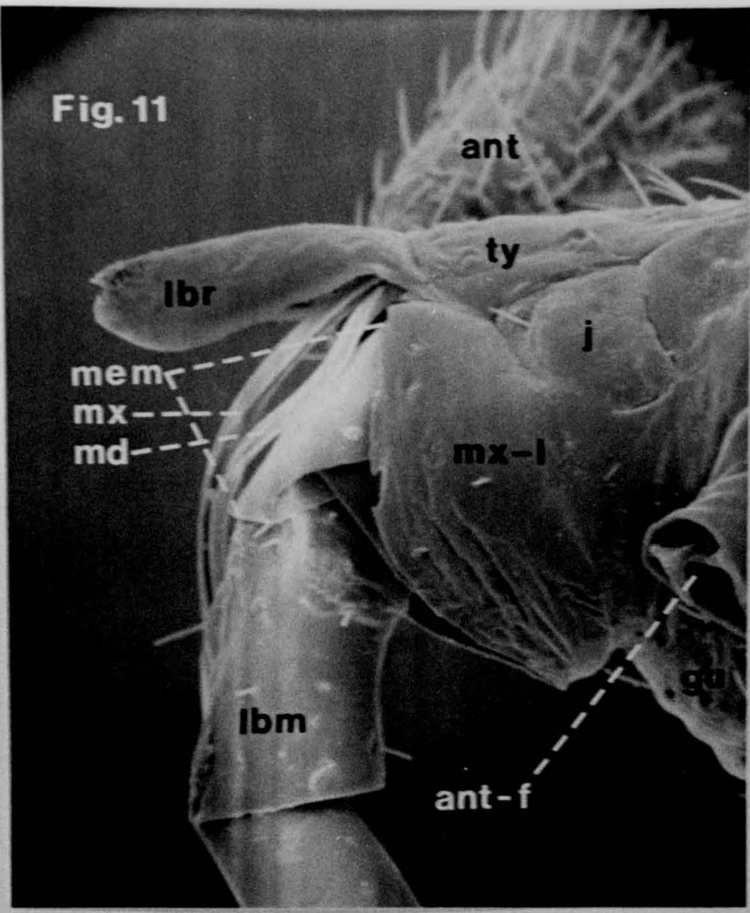

Fig. 12

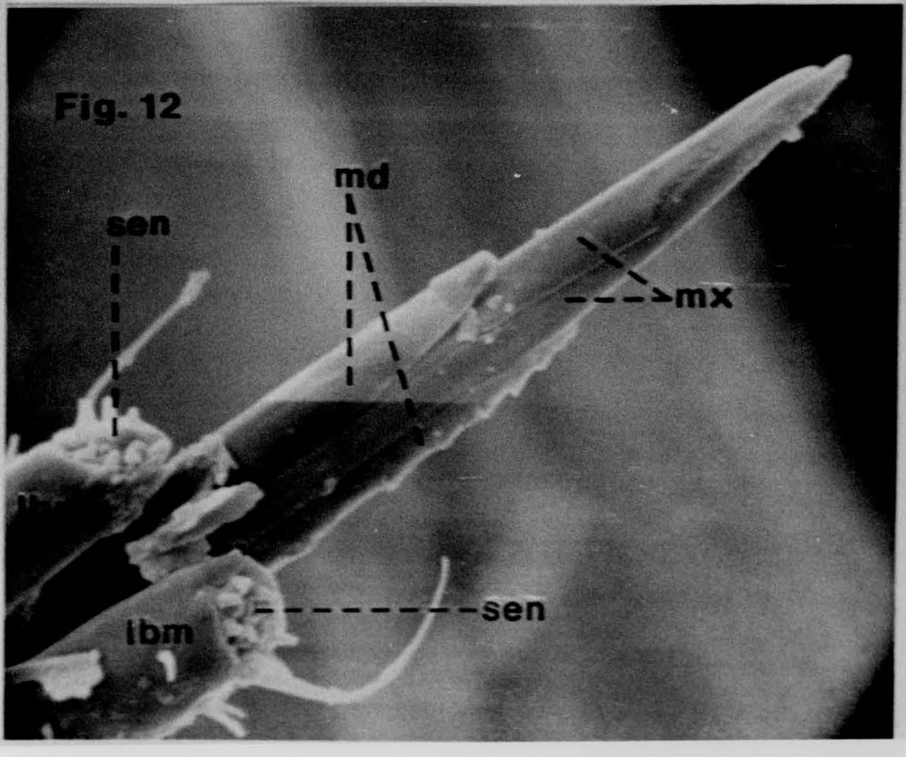

Plate 6

Fig. 13 Fascicle, Showing Apex of Mandibular Stylets, Ventro-lateral View, 5750x.

Fig. 14 Fascicle, Showing Apex of Maxillary Stylets, Ventro-leteral View, 5060x.

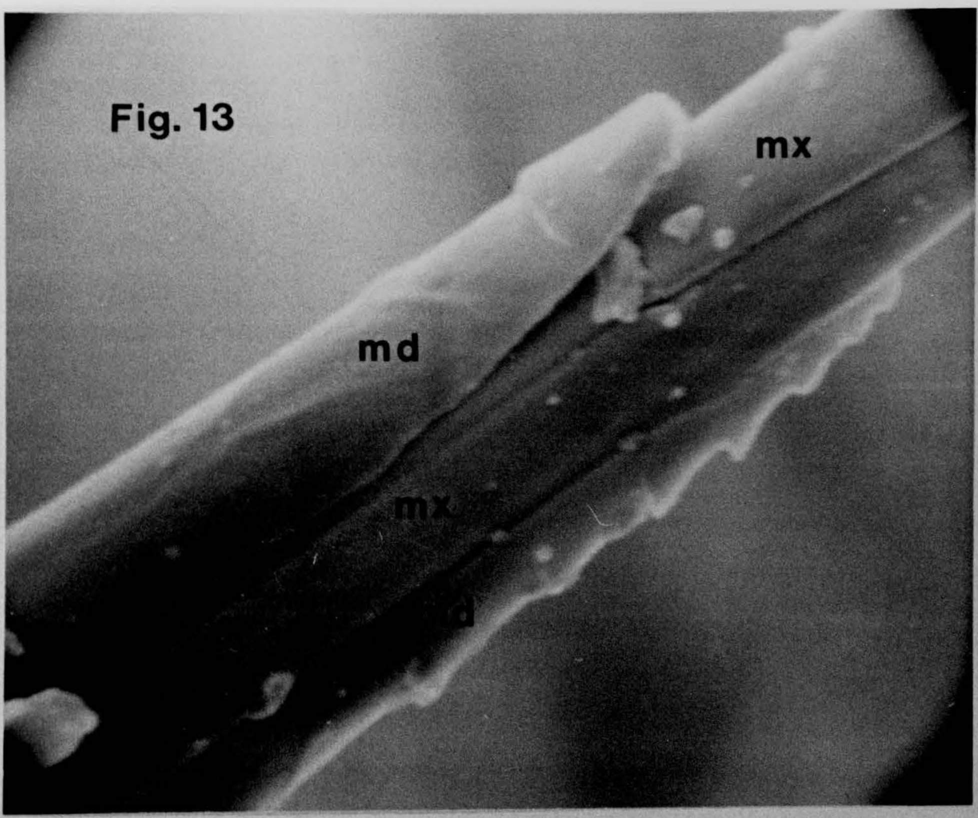

Fig. 13

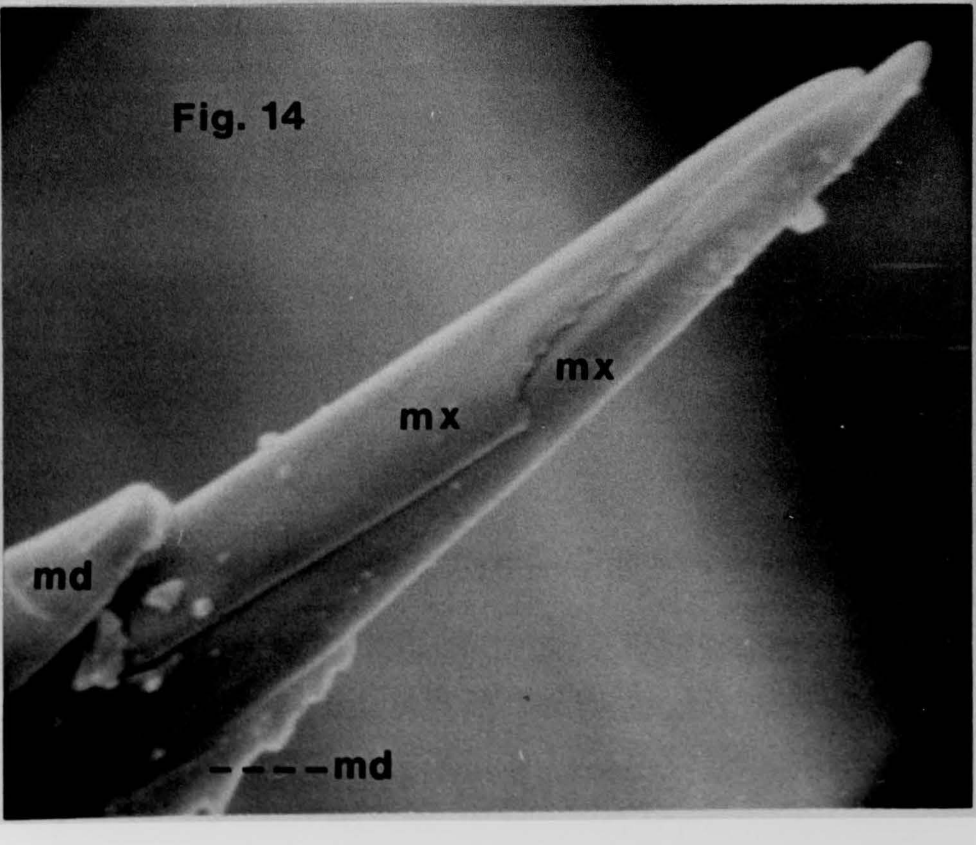

Fig. 14

Plate 7

Fig. 15 Fascicle in Cross-section, Showing Relationships of Stylets to Food and Salivary Channels, Approximately 10,000x.

Fig. 16 Diagramatic Representation of the Mouthparts, Showing Cibarium, Stylet Bases, and Stylet Musculature.

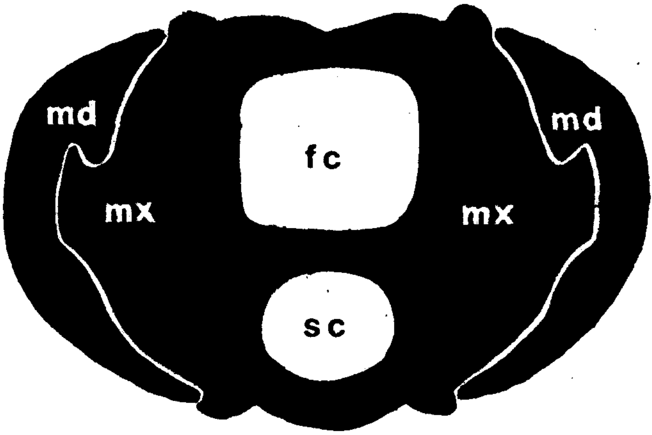

Fig. 15

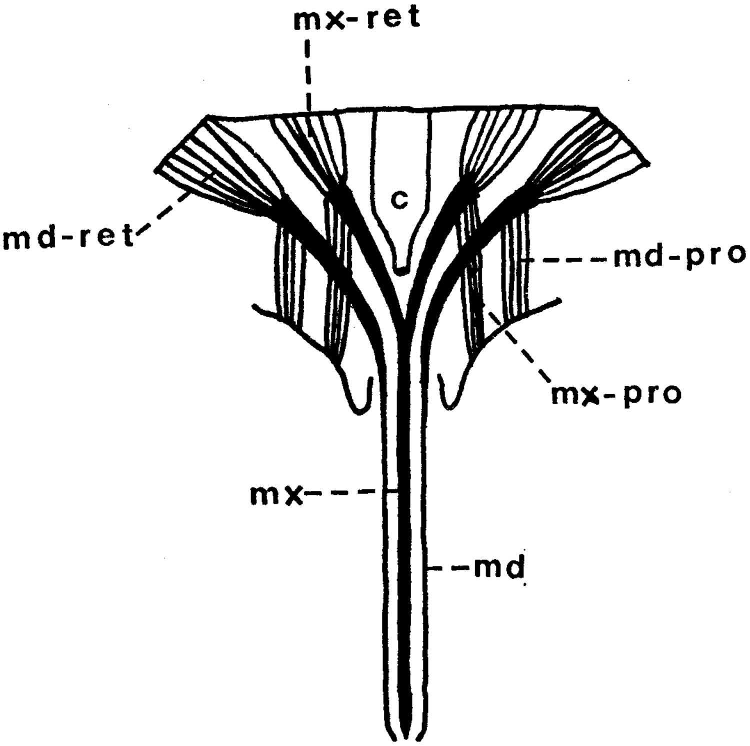

Fig. 16

APPROVAL SHEET

The thesis submitted by Mark N. Wisniewski has been read and approved by the following committee:

Dr. Robert W. Hamilton, Director
Associate Professor, Biology, Loyola

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

4-23-1979
Date

Robert W. Hamilton
Director's Signature