The Proventriculus of Cicindelidae: Systematics and Functional Morphology

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Abstract

Internal structures of the proventriculus of adult tiger beetles were examined to determine their function and taxonomic and phylogenetic value. The overall appearance of the proventriculus suggested an intricate filter designed to permit the flow of nutrient fluids into the midgut while retaining particulate food; this corroborates earlier hypotheses of tiger beetle feeding behaviour. The intricate proventricular structure indicates that regurgitation of midgut liquids does not occur during regular feeding. S.E.M. and light microscope observations revealed eight longitudinal internal folds, four minor and four major plicae, lined with ctenidia. The minor plicae become apodemes posteriorly and displayed anteriorly directed ctenidia which were shown to vary among cicindelid subgenera. The ctenidia of the major plicae varied in length independently of the plical height at both the anterior and posterior regions of the organ. Slopes which were calculated from these measurements describe the inner proventricular structure between the anterior and posterior ends of the proventriculus and were shown to differ among cicindelid subgenera and species groups. Advanced taxa displayed flatter proventricular slopes. The proventricular slope data were compared to habitat types of seventeen species. Among classified species of the *maritima* group, proventricular slopes reflected traditional phylogenetic relationships rather than habitat distinctiveness. Significant differences of proventricular slopes were shown among species

groups having different ancestral habitats. A histological examination of the foregut of *Cicindela tranquebarica* Herbst third instar larva revealed that the proventriculus is absent at this stage. The overall structure of the proventriculus of the primitive tiger beetle *Omus audouini* Reiche displayed the close phylogenetic relationship of this genus with the genus *Cicindela* although some variations were noted. By comparison, in adults of the carabid *Carabus nemoralis* Müller, "socketed setae" and cuticular ctenidia covered the intima of this organ. Based on the comparative morphology of the proventriculus, members of *Omus* and *Cicindela* were concluded to demonstrate a phylogenetic relationship, with *Carabus nemoralis* more distantly related.

This document is based on original research conducted by the author and has not been previously submitted toward any degree. Concepts of other authors have been fully acknowledged within.

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Acknowledgements

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Introduction

The adult insect proventriculus was first described by researchers performing digestive tract studies of the Muscidae, (Giles,1906; Hewitt,1907), as well as bark beetles (Lindemann,1876). Although this organ varies significantly among the insects, it has been broadly defined by Borror et al. (1989) as ". . . the valve between the foregut and midgut."

Among the Coleoptera, the digestive tracts of numerous crop and forest pests of Polyphagan families have received a great deal of attention. Among the earliest accounts Lewis (1926) described the digestive tract of *Passalus* Fabricius (Passalidae) to explain the very specialized feeding of this insect and the significance of symbiotic fungi in the intestinal mucosa. Becton (1930) described the histology of the alimentary canal of the scarab *Phanaeus vindex* (Mach) including a brief description of the proventricular morphology. The proventriculus was included by Swingle (1930) in a histological description of the alimentary canal of *Popillia japonica* Newman (Scarabaeidae) in which he attempted to explain the ability of the beetle to digest a large variety of plants. Fletcher (1930), described the general profile of the proventriculus of *Phyllophaga gracilis* Burmeister (Scarabaeidae); however he did not discuss the structures of the organ's intima. The proventriculus and the histology of the alimentary canal of the asparagus beetle *Criocerus asparagi* Linneaus, (Chrysomelidae), was described by Davidson (1931). Eaton (1942) examined the anatomy and histology of the proventriculus of the scolytid *Ips radiatae*

Hopkins and discussed the possible taxonomic use of the proventriculus. He indicated that because there were so many genera and species for which proventricular structure was not known, the organ had not been used as a taxonomic character.

Concerning Adephagans, Balfour-Browne (1934, 1935) discussed the structure of the internal lobes of the proventriculus of numerous dytiscids and noted that a characteristic evolutionary pattern was displayed by the inner lobes of each genus examined and that these lobes were specialized for grinding in the lower groups and for filtering in higher groups. Smrz (1982) conducted a taxonomic and phylogenetic analysis of the internal structure of the proventriculus and intraelytral structure of approximately 400 species from 91 tribes of Adephagans and Archostematans. He concluded that the form of the proventriculus was generally distinguishable among tribes, and that genera within a tribe agreed with a tribe type proventriculus " . . . in spite of their geographic or ecological isolation from other ones of the tribe"; also that the proventriculus can be used for systematic studies because of ". . . its constancy and slight adaptability at the present time".

Among the Caraboidea, Bess (1935) described the structure of the alimentary canal of *Calosoma sycophanta* Linneaus. The gizzard was described in cross section as containing a peritoneal membrane of connective tissue, circular muscle, longitudinal muscle, epithelium and an intima with ridges of heavy cuticula or chitin from which large chitinous plumose spines arose and

projected caudad. The function of the spines was thought to prevent the food from being forced foreward during the grinding action of the gizzard. More recently Cheeseman and Pritchard (1984 b) described sclerotized protrusions on the intimal surface of the proventriculus, and explained the function of this organ as triturating in six species of particle injesting carabids. Evans and Forsythe (1985) conducted an extensive study of adult caraboid beetles in which they demonstrated that the overall head and mouthpart structure reflects the method of feeding rather than the specific type of food. Three main feeding methods were recognized, solid or fragmentary feeding, fluid feeding, and mixed and solid feeding. *Cicindela, Carabus, Cychrus* and *Scarites* were distinguished as fluid feeders that share among other structures, a proventriculus which acts solely as a filter and is characterized by poorly developed musculature and seta-covered inner lobes. The authors concluded that ". . . the fluid feeders include several systematic groups . . . which show similar adaptations to pre-oral digestion and the intake of fluids".

The tiger beetle proventriculus was first described by Gissler (1879) for *Amblycheila cylindriformis* Say as a powerful muscular complex consisting of eight flesh coloured fascicles each terminating in a tooth, the entire complex being covered with tooth-shaped excrescences or aciculi that are directed backward and outward, and the same apparatus was said to exist in *Omus* sp. and many other insects. Generally the cicindelid proventriculus has a system of soft, filtering hair-like structures such as that described for *Cicindela*

tranquebarica Herbst by Pomber (1972). Evans (1965) provided a detailed account of the feeding method of *Cicindela hybrida* Linneaus in which the mouthparts were explained as a pre-oral mill and that only fluids and very small particles enter the alimentary canal. Comparatively the pre-oral mill of the fluid feeding staphylinid *Philonthus decorus* Gravenhorst, was shown to rotate the food in the opposite direction to that of *Cicindela.*

Most tiger beetles are hygrophilous, living near or in humid to wet habitats. However unlike their nocturnal carabid relatives tiger beetles are diurnal specialists active mainly on exposed areas of bare soil during warm, sunny days and thus conservation of body fluids is especially critical for them. Tiger beetles have been perceived to be closely associated with their soil microhabitat since pioneer studies by Shelford (1908). Leffler (1979) associated the length of the second gonapophysis of female genitalia with the soil type of the habitat. Among others Wallis (1961) has described the various habitats among Canadian tiger beetles. Some species are strictly riparian, others are found away from water and a few occupy sand dunes. Adults and larvae were found to drink from the substrate by Palmer (1978) and Hadley et al. (1990) respectively. The concepts of fluid feeding mechanisms and the filtering function of the proventriculus as described by Evans and Forsythe (1985) for Caraboidea and the reduction of particle size of prey cuticle by the proventriculus as proposed by Cheeseman and Pritchard (1984 b) for carabids, appear to complement the overall life style of tiger beetles. It seems reasonable to

assume that in their desiccating environments the predaceous tiger beetles would be best served by maximizing consumption of the nutrient body fluids of their arthropod victims, and by minimizing the passage of undigestible, cuticular particles of prey to the midgut.

The evolution of a tiger beetle in a particular environment is likely to be reflected by its external appearance and this has been demonstrated by Acorn (1991) by the elytral patterns. However, the effect of the habitat on the proventriculus has not been explored. This study attempts to correlate structural differences of the proventriculus with specific habitat features, discussed above, among closely related taxa of tiger beetles. Also an attempt is made to assess the taxonomic and phylogenetic value of the proventriculus by determining its structural variation among monophyletic taxa of tiger beetles. Essential to these objectives is a thorough description of the proventriculus which may contribute to the understanding of the organ's function as it is currently perceived, and thus a detailed examination of the proventriculus of *Cicindela repanda* Dejean, a relatively unspecialized tiger beetle, was conducted and a hypothesis of its functioning proposed.

Materials and Methods

Collection of Adult Specimens

For histological studies of adult specimens, roadside collections were made with an insect net during the summer of 1992 along a route following the Trans Canada Highway west from Thunder Bay to the west coast of Vancouver Island. Additional specimens were collected from Missouri and Texas during May, 1993. Collected specimens were immediately removed from the net and placed in jars of Bouin's solution and 70% ethanol. Specimens were identified in the lab with the aid of a key to the species of *Cicindela* of North America north of Mexico by Willis (1968). These specimens were then assigned to their respective subgenera and species groups according to Rivalier (1954) and Boyd and Associates (1982). These specimens have been listed according to this classification from primitive to derived in Table 1. Species names and collection sites are listed in Appendix A.

Histological Preparation of Samples

Specimens were rinsed briefly under running water to remove excess Bouin's solution, and dissections were made with a Wild-Heerbrug dissecting microscope at 12X. The head and distal portion of the abdomen were cut off with scissors, and the sides were cut open between the tergum and sternum of the abdomen and through the episterna and epimera of the thorax. The entire

dorsum was then gently removed with forceps exposing the alimentary canal.

The crop and proventriculus could then be lifted out of the specimen intact. The

proventriculus was gently removed from the crop with forceps. Proventriculi

were initially washed in 30% tertiary butyl alcohol to remove any remaining

Bouin's solution before dehydrating through increasing concentrations (30%,

50%, 70%, 90% & 100%) of tertiary butyl alcohol for two hours. Next the

specimens were placed in a 1:1 solution of tertiary butyl alcohol and liquid

Paraplast X-tra paraffin in an oven at 56^0 C for 24 hours. The solution was

replaced with liquid paraffin and changed twice at two hour intervals, after which

specimens were oriented for cross sectioning under a dissection microscope at

25X. Moulds were left to solidify on a cooling tray, and each specimen was

blocked and sectioned at five microns with a microtome. Ribbons were floated in

distilled water on slides coated with Haupt's adhesive (Berlyn & Miksche, 1976).

Slide specimens were stained with Mallory's triple stain following the procedure

outlined by Pantin (1962). Modifications to this procedure included quickly

dipping the slides through the final alcohols to remove excess stain only and

deparaffinization was conducted with histoclear rather than xylene. Sections

were mounted directly from the last treatment of histoclear with Diatex. Museum

specimens did not produce ideally stained sections. Fresh specimens preserved

in Bouin's solution and 70% ethanol were used and showed optimal sectioning

and staining results. Photomicrographs were taken with a Zeiss Standard

photomicroscope equipped with a 35 mm camera and automatic exposure unit.

The time required to collect fresh adult specimens and process the histological samples of the proventriculus for the taxonomic analysis section largely dictated the sample number produced within the time allowed for the study. Thus it was decided that more taxa with fewer samples be taken into account rather than the reverse.

Measurements

An initial examination of the major plica of the proventriculus was conducted by the scanning electron microscope. Ctenidia of the same location in a sample individual were found to be nearly the same length. However measurements of the anterior ctenidia of the major plicae were found to vary among species. These scanning electron microscope measurements were compared with ctenidial measurements taken from the same location of the major plica in transverse section of a histologically treated proventriculus of the same species. Ctenidial lengths were found to be similar using both methods. However, cross sections were a more convenient method, particularly for locations where ctenidia were long, and were therefore used for all species in the study.

For each specimen one cross section from the anterior end of the organ and one cross section from the posterior end were chosen. The anterior end is identifiable by tall spade-shaped major plicae and tall minor plicae. The posterior end of the proventriculus is characterized by short round major plicae,

and short minor plicae with thickened sclerotized arrowhead points. Plical

height was measured at the anterior and posterior ends of the organ under 100X

oil emersion with a Zeiss compound scope. Measurements were made from the

inner circular muscle layer to the top of the cuticular layer of two plicae in both

the anterior and posterior regions of the proventriculus in each specimen in the

manner displayed by Fig. 1 (a), and Fig. 2 (a). One ctenidium was chosen for

measurement from each of the two plicae measured in the regions identified in

Fig. 1 (b), and 2 (b). Ctenidial lengths were measured from the base of the

sclerotized plate to the tip of the ctenidial fingers Fig. 3. Each plica then

provided two points, anterior plical height and ctenidial length, and posterior

plical height and ctenidial length. From this a slope could be calculated for each

plica that was measured, rise being the ctenidial length, and run, the plical

height. Each individual therefore provided two slopes. 31 individuals provided a

total of 62 slopes which are listed in Table 1. The plical and ctenidial

measurements of these specimens are found in Appendix B.

Collection and Dissection of *Cicindela tranquebarica* Herbst Larvae

Four third instar larvae were collected from the Thunder Bay area during

May 1993, and identified as *C. tranquebarica* with the key provided in Hamilton

(1925). Coloured golf tees were dropped into the holes of the larval burrows.

Larval activity was easily observed when golf tees were pushed upward and out of the burrow. At this point a spade was inserted into the soil cutting off the burrow. The soil was then turned and the larva recovered. Larvae were placed immediately into vials of Bouin's solution. The alimentary canals of the specimens could be successfully removed if dissections were performed within 24 hours. After this period the Bouin's solution hardened the tracheae and fat surrounding the alimentary canal and removal of the gut became difficult due to tearing. Thus 70% ethanol is recommended for longer term storage of larvae from which the alimentary canal is to be removed.

Larvae were dissected by cutting the sides of the body open from the posterior end to the mouthparts, and the gut was teased away from surrounding fat tissue with forceps under a dissection scope at 12X. Two gut specimens were subjected to the same histological preparation, staining regime and photography as were the adult proventriculi.

Drawings

Drawings were made using the Wild-Heerbrug dissection scope equipped with the M5 Zeichentubus attatchment.

Scanning Electron Microscopy

Dried pinned adults were boiled for fifteen minutes in water to soften tissues. The proventriculi were dissected out using the dissection scope under 12X, and boiled in 10% KOH solution to remove all tissue and dehydrated through an ethanol series for one hour in each of (30%, 50%, 70%, 90%, and 100%) concentrations of ethanol. They were then cut open longitudinally with a razor blade, spread open with minutin pins and left to air dry for half an hour. They were then placed on a stub with two sided tape and coated with gold by a Fullam sputter coater and examined with a Hitatchi 570 Scanning Electron Microscope. Photomicrographs were taken with the attatched camera unit.

Critical point drying was conducted for some of the specimens. However, when compared there was no difference between the specimens that were critically point dried and those that were air dried. Because the inner proventricular surface is entirely chitinized, it contains little or no water which would cause specimen distortion under vacuum. For good results air drying from a solvent with a low air-water interface such as chloroform 27 dynes/cm or propylene oxide 20 dynes/cm was suggested by Crang & Klomparens (1988). Thus specimens air dried from absolute alcohol with an air-water interface of 23.64 dynes/cm were acceptable.

Soil Microhabitat Evaluation

Each of the collection areas was classified by an index (Fig. 4) for proximity to water and water retention of the soil in the microhabitat. Large particle size such as gravel allows water to drain quickly, and was considered to be driest. The dry gravel habitat has an index value of 0.5. Small particles such as clay and silt exhibit a greater surface area and more readily absorb water (Carmean, 1993); these soils were considered wetter. Soils containing a high clay content were expressed by water retention index values of 0.1 and 0.2. Thus decreasing water retention values of the soil are expressed by an increasingly larger decimal value (Fig. 4). Proximity to water was divided into three categories, the index value increasing with the distance from a water source (Fig. 4). Although the index is discontinuous and very general, it provided a useful method of categorizing collection sites.

Statistical Methods

Ctenidial Length and Plical Height

Measurements of plical height and corresponding ctenidial length from both the anterior and posterior ends of the proventriculus (Appendix B) were subjected to a regression analysis, based on an hypothesis of independence of ctenidial length and plical height.

Slopes

Slopes (Table 1) were compared among subgenera, species groups and species within the *maritima* group using a two tailed Mann-Whitney U nonparametric analysis. This analysis indicated whether significant variations occurred in the slopes among the designated taxonomic categories. Since no cladogram exists for cicindelid taxa, slopes were arranged from primitive to derived according to the order of taxa by Rivalier (1954) and Boyd and Associates (1982).

Slope/Habitat Water Relationships

The mean value of the slopes was calculated and used to divide all slopes into steep and flat categories. The steep and flat categories were each tested with the Chi-square test of independence with, first, the categories representing the proximity to fresh water, and second, the categories representing soil drainage. The observed frequencies were displayed in two separate contingency tables to determine the significance of each separately. Separation of proximity to water and soil water retention was required to determine the significance of each to the habitat of the species in relation to the proventriculus. Thirty-one individual specimens were used each yielding two slopes and are displayed in Table 1.

Statistical analyses were conducted using Stat View II (Feldman et al.,1990). A 95% confidence level was used to determine significance of the Chi-square tests of independence and two tailed Mann Whitney U (Zar, 1984).

Generalized proventricular structure in *Cicindela*

Figure 1. a) Anterior cross section of the proventriculus: H, measurement of plical height.

b) Anterior cross section of the proventriculus: a, selected area for measurement of ctenidia.

Figure 2. a) Posterior cross section of the proventriculus: H, measurement of plical height.

b) Posterior cross section of the proventriculus: a, selected area for measurement of ctenidia.

Figure 3. Ctenidium of the proventricular intima: Cl, measurement of ctenidial length.

0.5mm

Figure 1.   a   b

0.5mm

Figure 2.   a   b

Cl

10 um
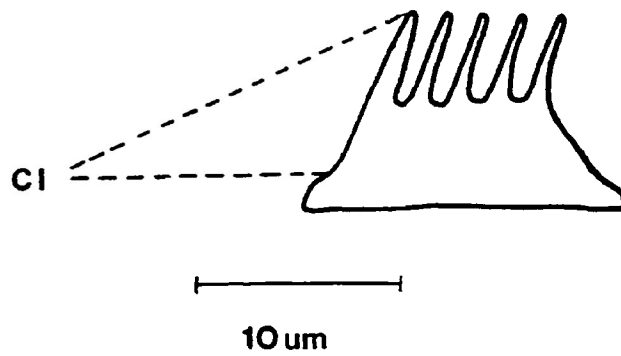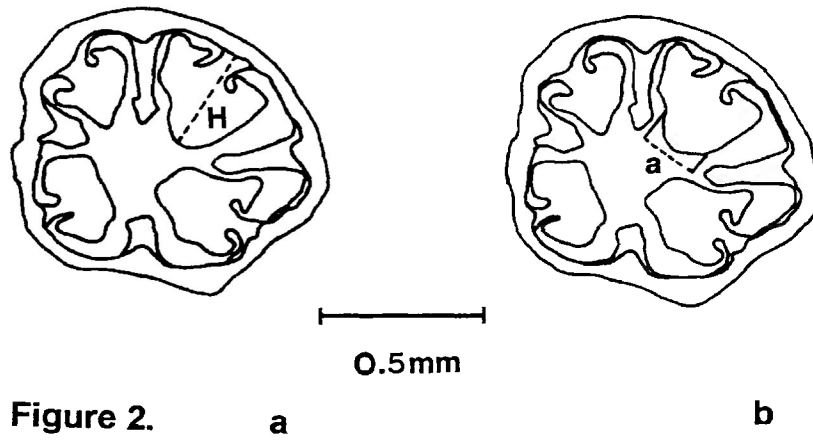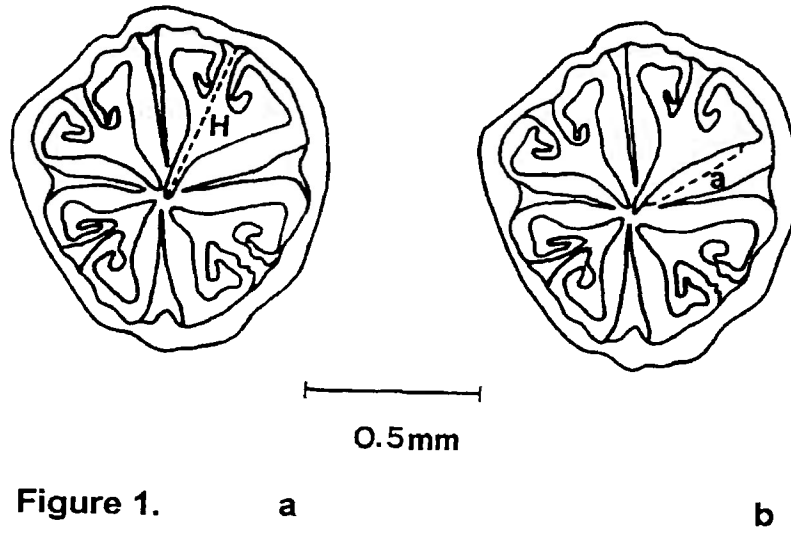
Figure 3.

Figure 4. Microhabitat water availability index.

Three Categories Representing
Increasing Distance From Fresh Water Source

	< 20 m = 1	20 - 200 m = 2	> 200 m = 3
Clay = 0.1	1.1	2.1	3.1
Clay/Sand = 0.2	1.2	2.2	3.2
Sand = 0.3	1.3	2.3	3.3
Sand/Gravel = 0.4	1.4	2.4	3.4
Gravel = 0.5	1.5	2.5	3.5

Increasing Soil Particle Size

Decreasing Soil Water Retention
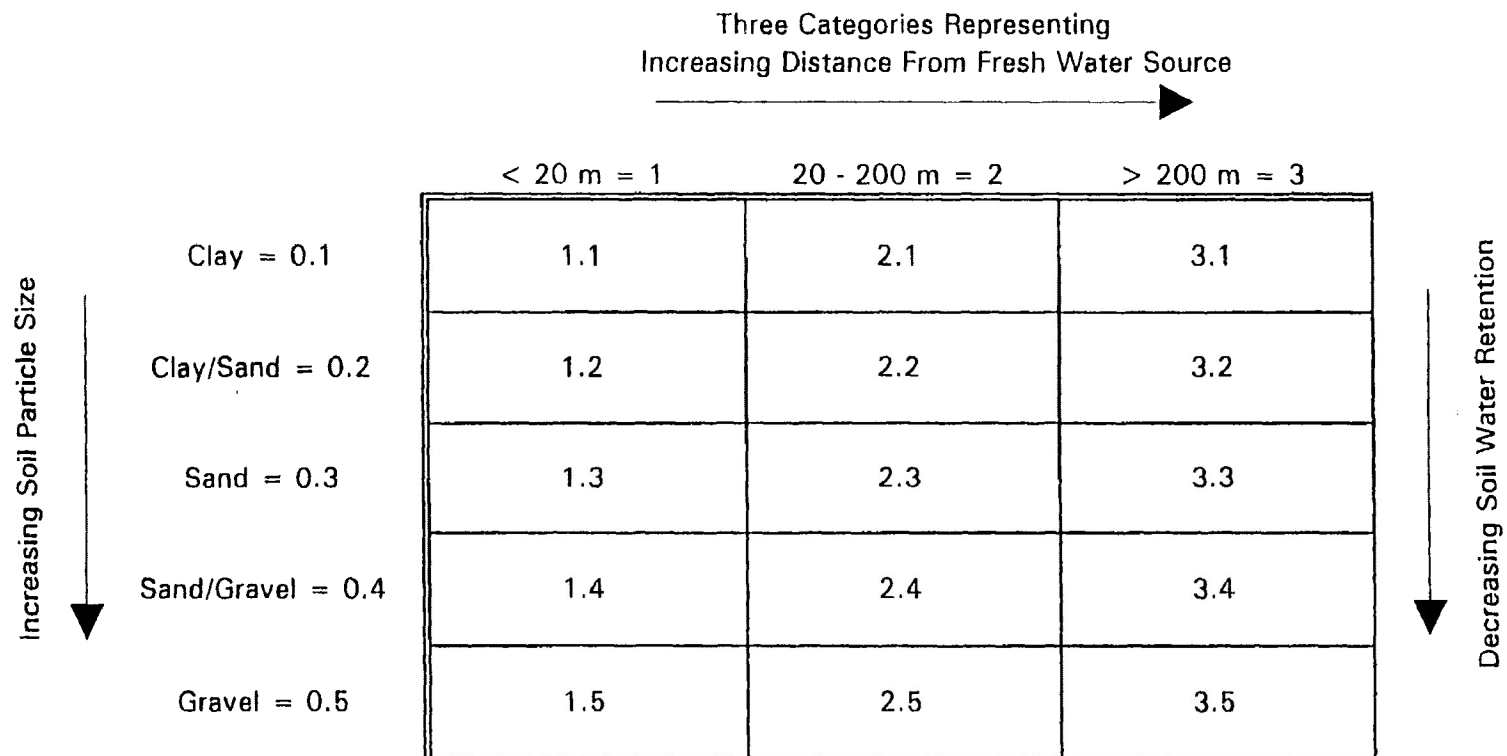
Figure 4.

Results and Discussion

Morphology of the proventriculus: *Cicindela*

Location

The proventriculus of *Cicindela* is a narrow chitinized channel which connects the foregut to the delicate, multidiverticulated midgut. In the studied species (Appendix A), this organ lies within the first three abdominal segments and protrudes into the hind end of the sac-like crop (Fig 5), in a manner similar to that discussed by Judd (1948) for *Cicindela sexguttata* Fabricius. In this discussion I have used *C. repanda* to illustrate the overall structure of the cicindelid proventriculus.

External Structure

In lateral profile the proventriculus resembles a fleshy funnel with four posterior apodemes (Fig 6), as noted by Smrz (1982) for other members of Cicindelinae. The apodemes are heavily sclerotized and continuous with the thin cuticular lining of the foregut. Their dark colour makes the proventriculus easily recognizable although it is a very small organ largely concealed by surrounding tissues. The apodemes provide support for the posterior region of the organ which is very narrow and positioned away from the crop which provides support for the anterior end of the organ. The organ appears to be

enveloped by a membranous coating which has been described as connective

tissue (Bess 1935). This coating is continuous with the crop and extends to the

midgut.

Internal Structure

The proventriculi of cicindelid species listed in Appendix A have an

internal structure that consists of eight longitudinal folds. These folds project

farthest into the anterior and middle portions of the proventricular lumen and

become less prominent in the posterior end in which the diameter of the lumen

decreases. Four of the folds are large and spade-shaped in cross section (Fig

7). These folds alternate with four narrow less prominent folds. This cross

sectional pattern has been reported for *Cicindela maritima* Dejean by Brunetti

(1931) and *Cicindela sexguttata* Fabricius by Judd (1948). In this discussion I

refer to the large spade-shaped folds as major plicae and the narrow folds as

minor plicae after Miller (1972).

C. repanda illustrates the eight-plicae configuration in the anterior half of

the organ. An outer layer of circular muscle surrounds the proventriculus, and

an interior layer of longitudinal muscle protrudes into the bases of the minor

plicae, and fills the major plicae. A cuticular lining of the lumen (Fig 8), has

many small bristle-like structures known as ctenidia (Smrz 1982).

In the posterior half of the proventriculus, the major plicae are rounder

and the minor plicae are thicker and shorter each with a conspicuously

sclerotized apex (Fig 9). There is less obstruction of the lumen by plicae, and an increase in the length of the ctenidia which reach almost to the center of the lumen. At this point the proventriculus is no longer invaginated into the crop and the overall construction takes on a solid appearance.

Near the posterior end of the proventriculus, the major plicae are triangular, and the minor plicae are reduced to sclerotized triangular bumps (Fig 10). Long ctenidia extending from the major plicae fill the lumen. At the end of the proventriculus there is an abrupt decrease in the diameter of the lumen to approximately half the anterior width, and there is a notable increase in thickness of the outer circular muscle layer (Fig 11). The lumen in this area begins to resemble the lumen of the midgut, with the disappearance of the chitinous intimal layer and associated ctenidia.

The insect foregut shares its ectodermal origin with the exoskeleton and similarily is chitinized throughout (Snodgrass 1935). Some structures of the foregut intima and exoskeleton are alike in detail and considered here to be homologous. Smrz (1982) indicates that the primitive type of intimal proventricular structure is the "setal ctenidium" that can be found on the walls of the crop as well as on the surface of the body. "Setal ctenidia" depicted by Smrz (1982) are the same as those within the cicindelid proventriculus, and are similar to the form of microsculpture known as diffraction grating discussed by Hinton (1970). For morphological and taxonomic accounts of microsculpture, in particular for ground beetles see Ball and Erwin (1969), Lindroth (1974), Ball

and Hilchie (1983), and Acorn and Ball (1991). Schultz and Rankin (1985) investigated microsculpture of the elytral surface of *Cicindela*. Freitag and Lee (1972) and Schincariol and Freitag (1986) have described structures of the cicindelid elytra and flagellum of the male genitalia respectively which also resemble microsculpture.

The ctenidia which sparsely cover the apodemes of cicindelids, are directed anteriorly (Fig 12), and those which cover the plicae of the anterior three quarters of the proventriculus are directed posteriorly (Fig 13). Anteriorly directed ctenidia cover the apodemes from their posterior apex (Fig 12) to the point where they disappear into the fold of the minor plicae (Fig 14). Figure 15 shows the ridges created by opposing ctenidia at the anterior end of the apodeme. These ridges rise up along both sides of each apodeme and converge anteriorly.

The changing proventricular structure from the anterior to the posterior ends is shown in Figure 16. The anterior end of the organ shows prominent and intricately folded major plicae bearing ctenidia. The minor plicae are tall narrow folds that flank the major plicae anteriorly to the midsection. Figure 16 shows the point where the minor plicae turn into heavily sclerotized apodemes which project posteriorly towards the midgut.

Figure 5. Alimentary canal of a tiger beetle: pv, proventriculus.

Figure 6. External view of a tiger beetle proventriculus.

PV

1 mm

Figure 5.

1 mm
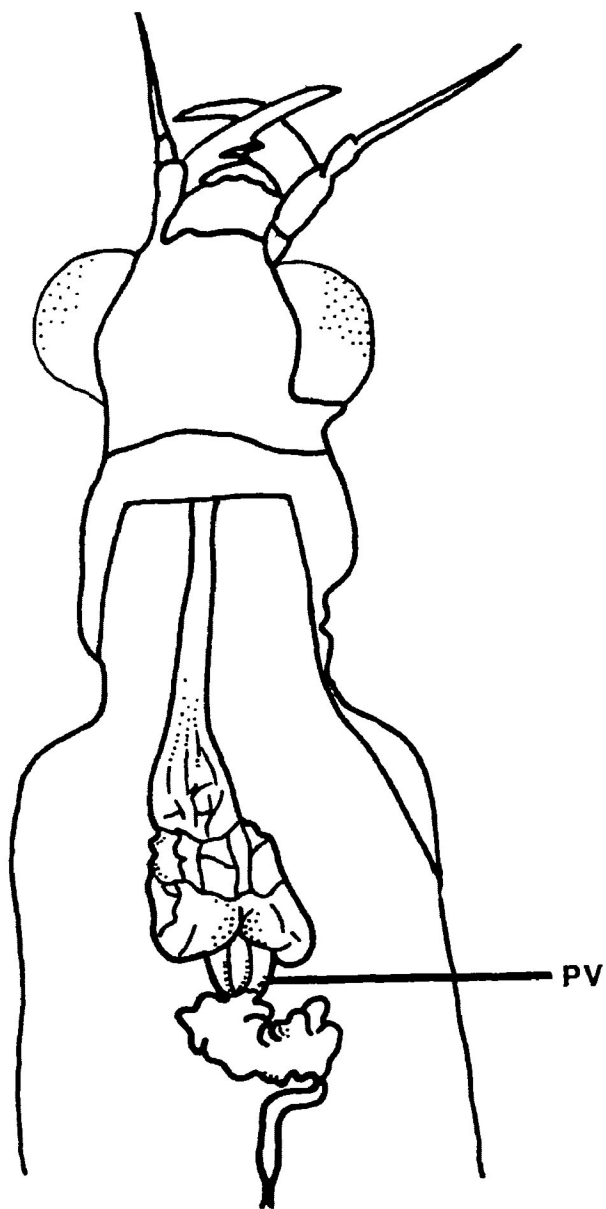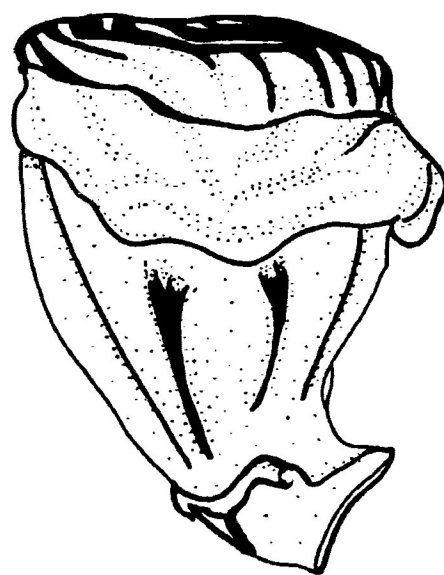
Figure 6.

The proventriculus of C. repanda

Figure 7. Cross section of the proventricular anterior end: MP, major
plica; mP, minor plica; CM, circular muscle; LM, longitudinal muscle;
I, intima.

Figure 8. Major plica of the proventricular anterior end: I, intima; C, ctenidium.

Figure 9. Cross section of the proventricular posterior end: MP, major plica;
mP, minor plica; C, ctenidium.

Figure 10. Cross section of the proventricular posterior end, (Further back than
9): MP, major plica; mP, minor plica; C, ctenidium.

Figure 11. Extreme posterior end of the proventriculus: CM, circular muscle; L,
lumen.

7

LM
I
MP
mP
CM
100 um

8

I
C
50 um

9

MP
C
mP
100 um

10

C
100 um

11

L
CM
100 um
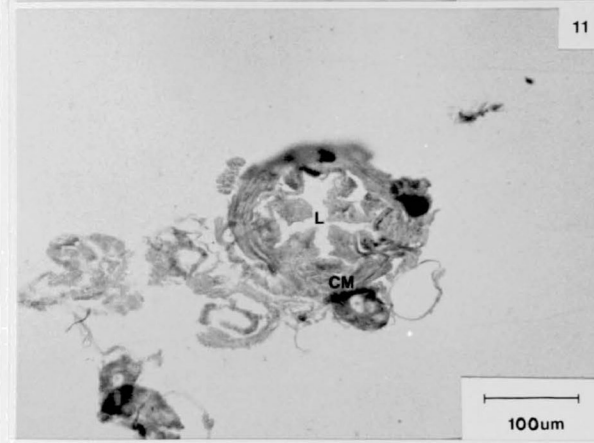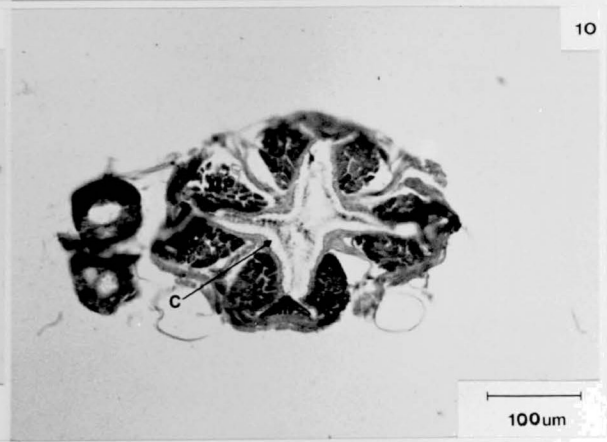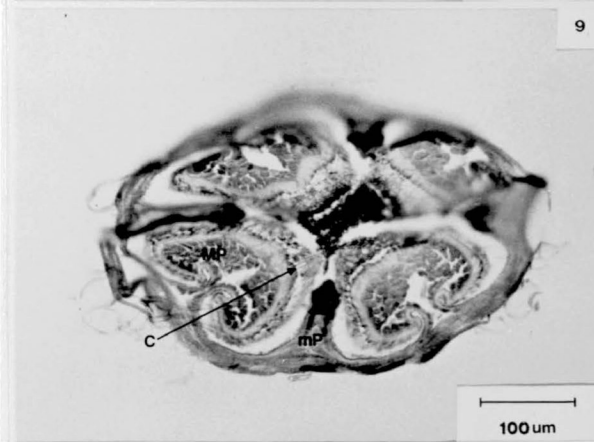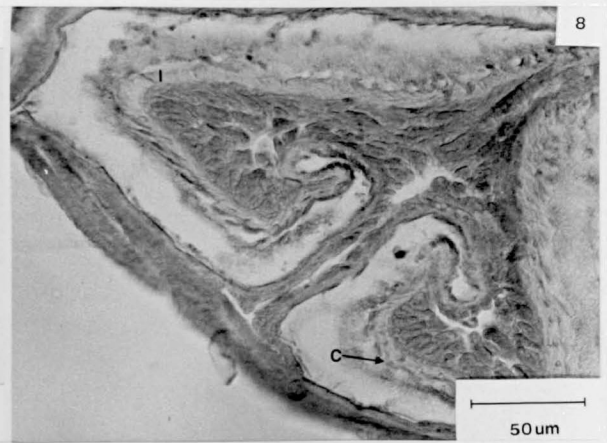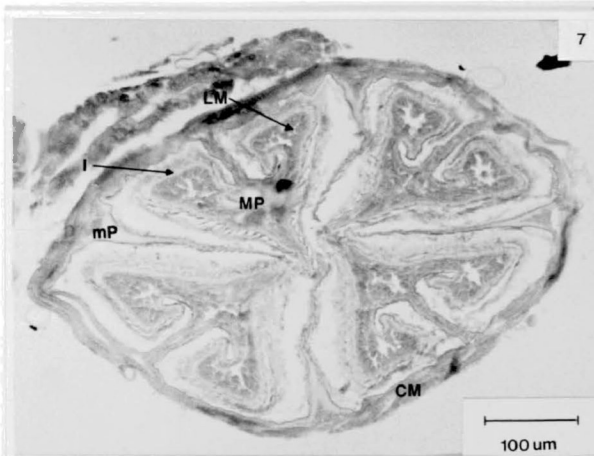
Figure 12. Posterior end of proventricular apodeme of *Cicindela marginata* Fabricius displaying the anteriorly directed ctenidia of the inner surface: C, ctenidium; A, apodeme.

Proventriculus of *C. repanda*

Figure 13. Intima of the proventricular lumen showing posteriorly directed ctenidia located on the major plica: C, ctenidium.

Figure 14. Anteriorly directed ctenidia of the apodeme shown creating a ridge where they meet the posteriorly directed ctenidia of the minor plica. C, ctenidium; A, apodeme.

Figure 15. Ridge created by anteriorly directed apodeme ctenidia, and posteriorly directed minor plical ctenidia, (orientation same as Figure 14).

Figure 16. Longitudinal aspect of the proventriculus (internal): MP, major plica; mP, minor plica; C, cteidium; A, apodeme.

12 C

30 um

13 C

0.5mm

14 C

43 um

15

6 um

16 MP

C

231 um
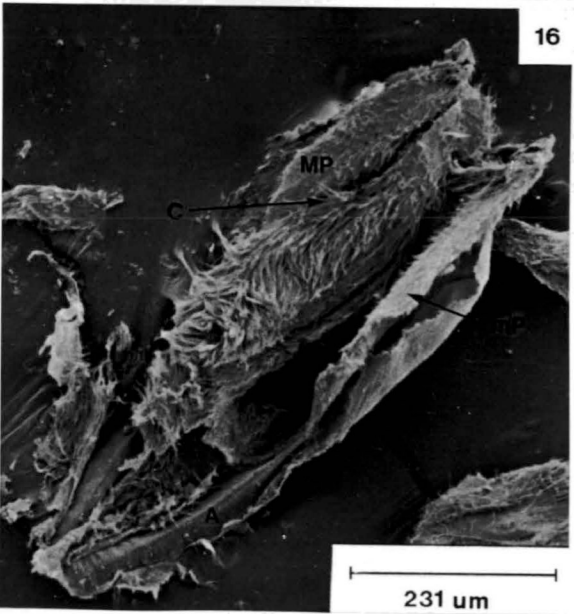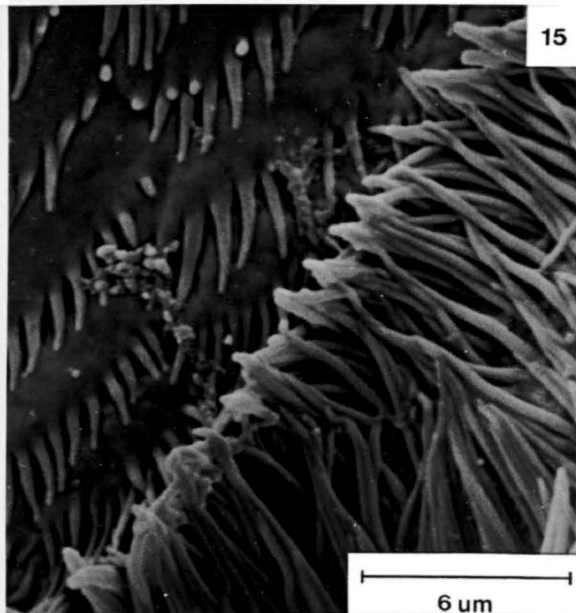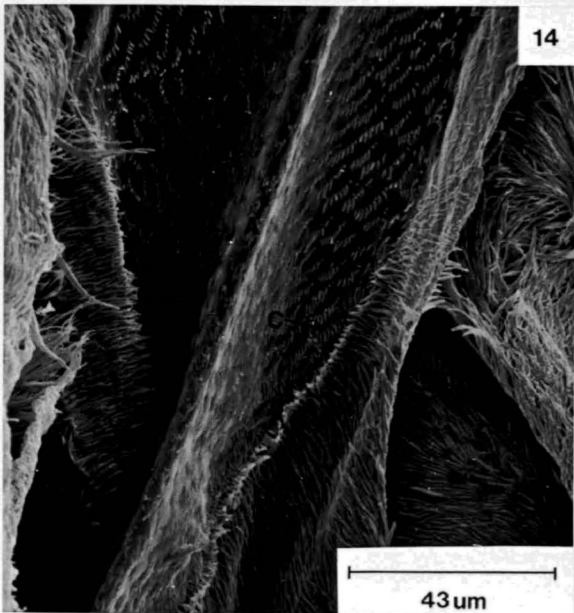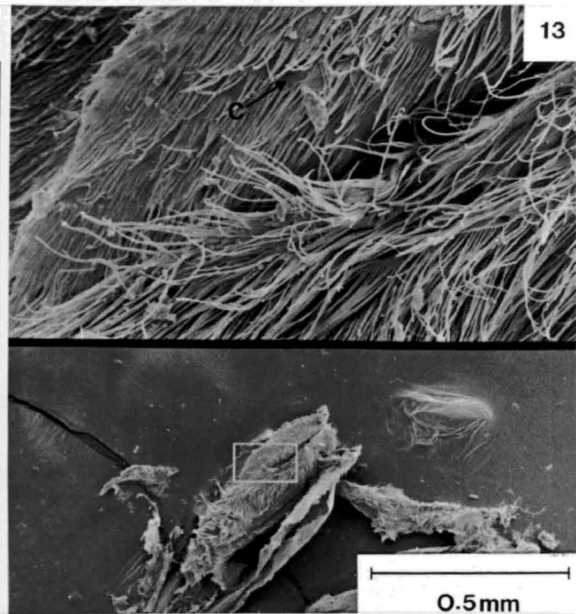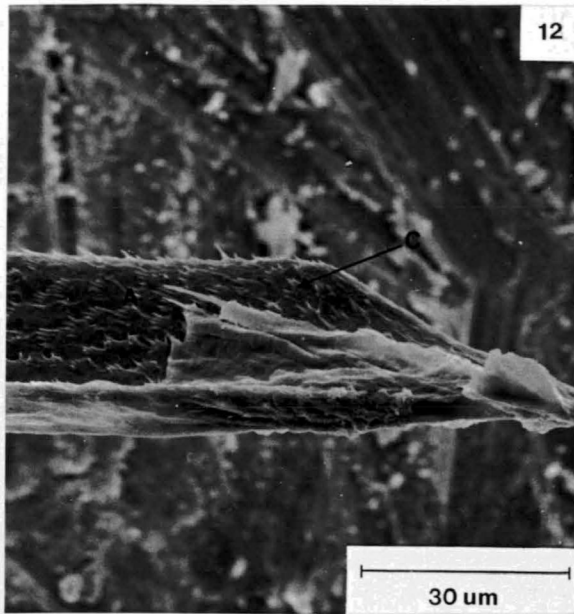
Functional Morphology

Upon killing its prey, the tiger beetle removes the tough exoskeleton and rakes in the soft innards that are processed into a bolus in the pre-oral cavity. Because tiger beetles have downward pointing setae on the hypopharyngeal membrane and epipharyngeal flap before the cibarial chamber (Balduf 1925; Evans 1965) the entrance of large particles to the digestive tract is prohibited. Food material then travels to the crop, where it takes the form of soft grey pellets of unrecognizable matter (personal observation of numerous specimens).

Evans (1965) concluded that the proventriculus of *Cicindela hybrida* Linneaus contained a poorly developed system of filtering setae which was characteristic for a beetle with a fluid or semi-fluid diet. Beetles were fed with mealworms injected with india ink and with uninjected mealworms on subsequent days. Clumps of india ink were recovered from the food bolus of a beetle feeding on the uninjected mealworm. Thus he concluded the liklihood of regurgitation from the alimentary canal during feeding in *C. hybrida.*

Hengeveld (1980) in a literature review of food specialization in ground beetles included cicindelids among carabid species that digest extra-intestinally or pre-orally. In these "food specialists" midgut digestive fluids are presumed to move to the mouth as an adaptation to pre-oral feeding. However he observed that the proposed movement of this fluid through the food-filled proventriculus and crop to the mouth is not clear.

Forsythe (1982) described the proventriculi of *Cychrus caraboides*

Linneaus and *Carabus problematicus* Herbst as being similar. Both species

were described as having proventriculi that were ". . . adapted for dealing with

fluid food intake", and filter out large particles while liquid from the crop passes

through the proventricular channels. Evans and Forsythe (1985) conluded that

proventricular structure reflects different feeding methods and in fluid feeders

such as *Cicindela* the organ displays poorly developed musculature and is seta

lined acting solely as a filter.

Cheeseman and Pritchard (1984 a) described the feeding processes in an

adult carabid beetle, *Scaphinotus marginatus* Fischer and concluded that its

crop is a storage area for midgut digestive fluids from which they are

regurgitated onto food items and reinjested. "After injestion, food is stored and

digested in the crop". The proventriculus was described as a valve displaying a

thick ring of circular muscle at its anterior end that can close the passage

between the foregut and midgut, thus enabling crop regurgitation of fluids.

Interior plumose setae were described as an effective barrier to fluid flow

through the organ, and midgut fluids move forward to the crop by coordinated

contractions of the proventricular longitudinal muscles creating a paddling effect.

Jaspar-Versali et al. (1992) studied the structure and function of the

proventriculus of adult carabids with a semi-fluid diet. The arrangement of

musculature, cuticle thickness and organization and profile of teeth and setae

were correlated with the nature of the injested food. The proventriculus was

concluded as having two main functions, regulation of food to the midgut, and

filtration; contraction of the circular muscle creates an overlap of the plumose

setae that trap particulate matter and allow the liquid fraction of the food to reach

the midgut quickly, and the filtering mechanism prevents the loss of particulate

food during the anterior movement of midgut digestive fluids. The authors

concluded that in the fluid feeding Carabidae, Cicindelidae and Cychridae, the

proventricular structure indicates a parallel evolution towards a filtering system.

While the function of the proventricular structures of caraboids continues

to be controversial, it is apparent that detailed knowledge of the organ's

morphology is fundamental to the understanding of its functioning in a given

taxon. The following hypothesis attempts to describe the action and role of the

proventriculus in food manipulation in tiger beetles based mainly on the organ's

structure as examined in this study.

Muscle contractions combined with the plical folds probably lead to liquid

being squeezed from the food mass when it enters the anterior end of the organ.

Beyond the midpoint, where there is a reduction in the surface area of the plical

spade-shape and increase in ctenidial length (Fig 9), liquids may flow backwards

to the midgut and the remaining portion of the food may become trapped.

Dilation of the posterior sphincter-like muscle may draw digestive juices forward

from the midgut. Figure 17 depicts the proposed routes of liquid movement

through the proventriculus. Anteriorly directed ctenidia of the apodemes appear

to form pathways for the anterior flow of midgut digestive fluids. The ridges of

posteriorly directed ctenidia which flank the apodemes on both sides are

positioned to aid in directing digestive fluid movement along the apodemes into the area of filtration (Fig 9). Where apodemes become tall narrow minor plicae (Fig 16), midgut fluids are probably caught among the long ctenidia at the posterior end of the major plica where they digest trapped food material. The ctenidial ridges may also function in obstructing particles which have passed through the area of filtration (Fig 9), thus trapping them in an area where they are subjected to the activity of midgut digestive fluids.

The structure of the proventriculus seems to indicate that the anterior movement of midgut fluids through the proventriculus anteriorly to the mouth may not occur during regular feeding. The proventriculus bears posteriorly directed ctenidia on its intimal surface. Posteriorly directed ctenidia also cover the channels between the major and minor plicae (Fig 18) and do not appear to be conducive to the flow of fluids in the anterior direction. Figure 19 shows the anterior section of the proventriculus of *Cicindela punctulata* Olivier. In this specimen the food material filled the lumen between the major and minor plicae blocking the passage, and the crop was similarly filled with food material which resembled a mass of tangled thread. This material was placed on a slide and viewed under 80X magnification. The food material appeared pastey and uniform in texture indicating that it had been extensively processed before entering the proventriculus. The remaining food material trapped in the proventriculus is likely subjected to digestive juices from the midgut, and might prevent loss of water which would probably occur during regurgitation of midgut

fluids during high day-time temperatures. Most straining of food likely occurs during the diurnal hunting periods. Movement of midgut fluids along the apodemes probably does not occur continuously, but at intervals, and possibly during periods of rest.

Figure 17. Proposed routes of liquid movement through the proventriculus: A,
apodeme; mP, minor plica; MP, major plica; F, food; MF, midgut
fluids.

Figure 17.

0.25 mm
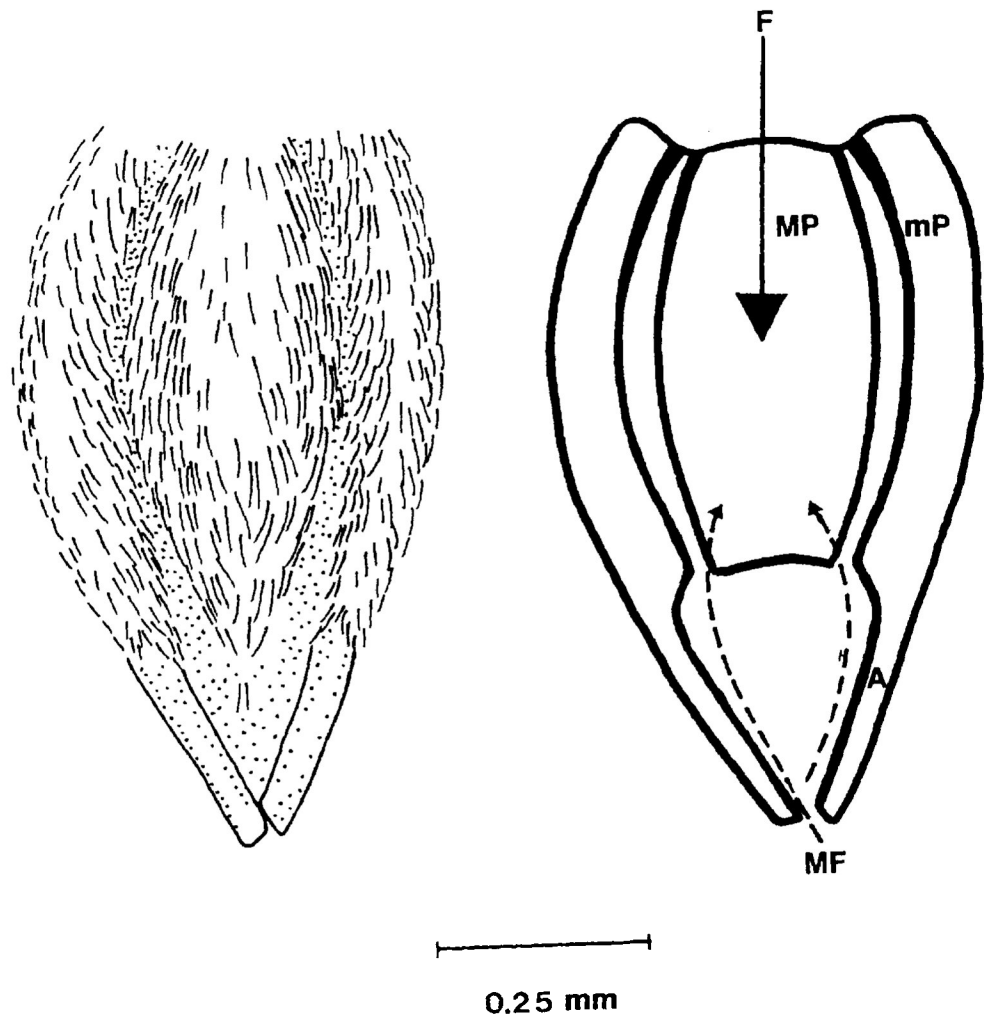
Figure 18. Intimal ctenidia of the channel between major and minor plicae.

Figure 19. Anterior cross section of the food filled proventriculus of *Cicindela*
punctulata: MP, major plica; F, food; C, ctenidium.

Figure 19. C, MP, F. 50 um

Figure 18. 30 um
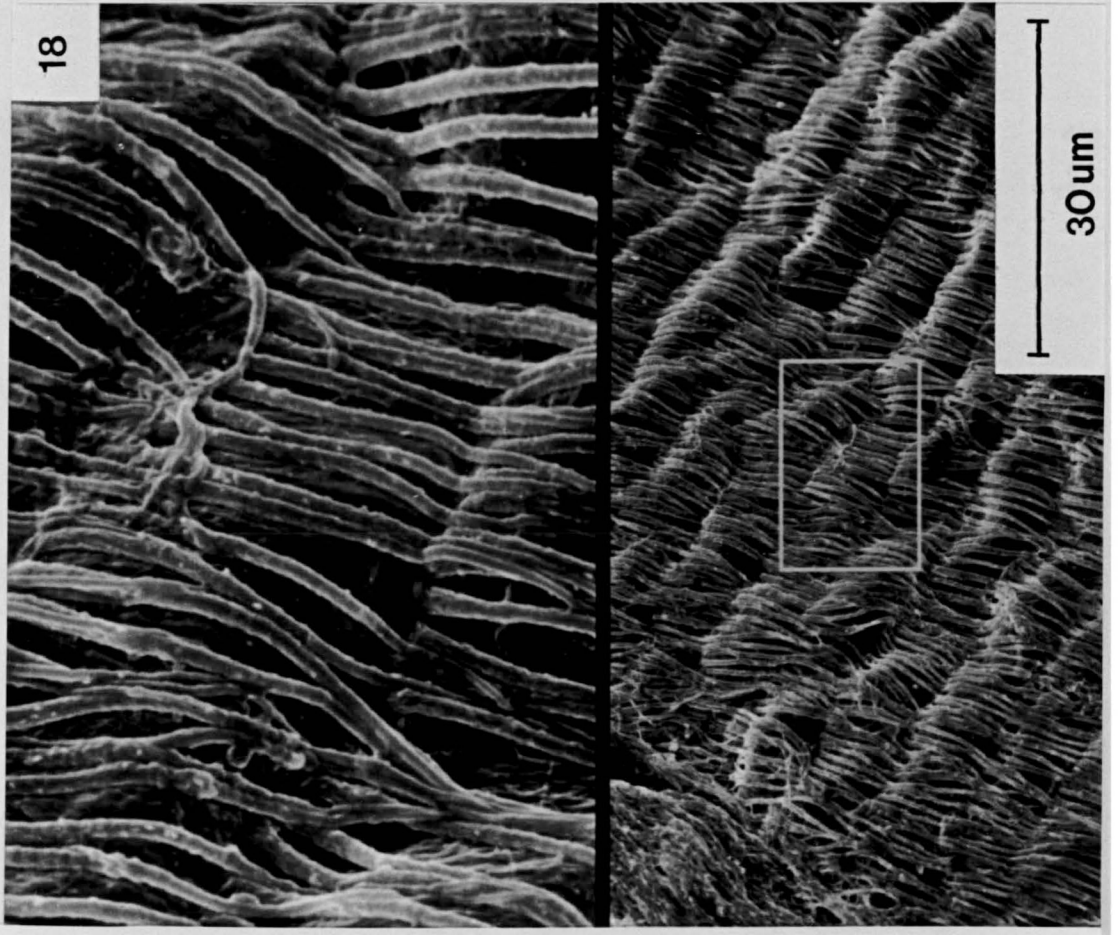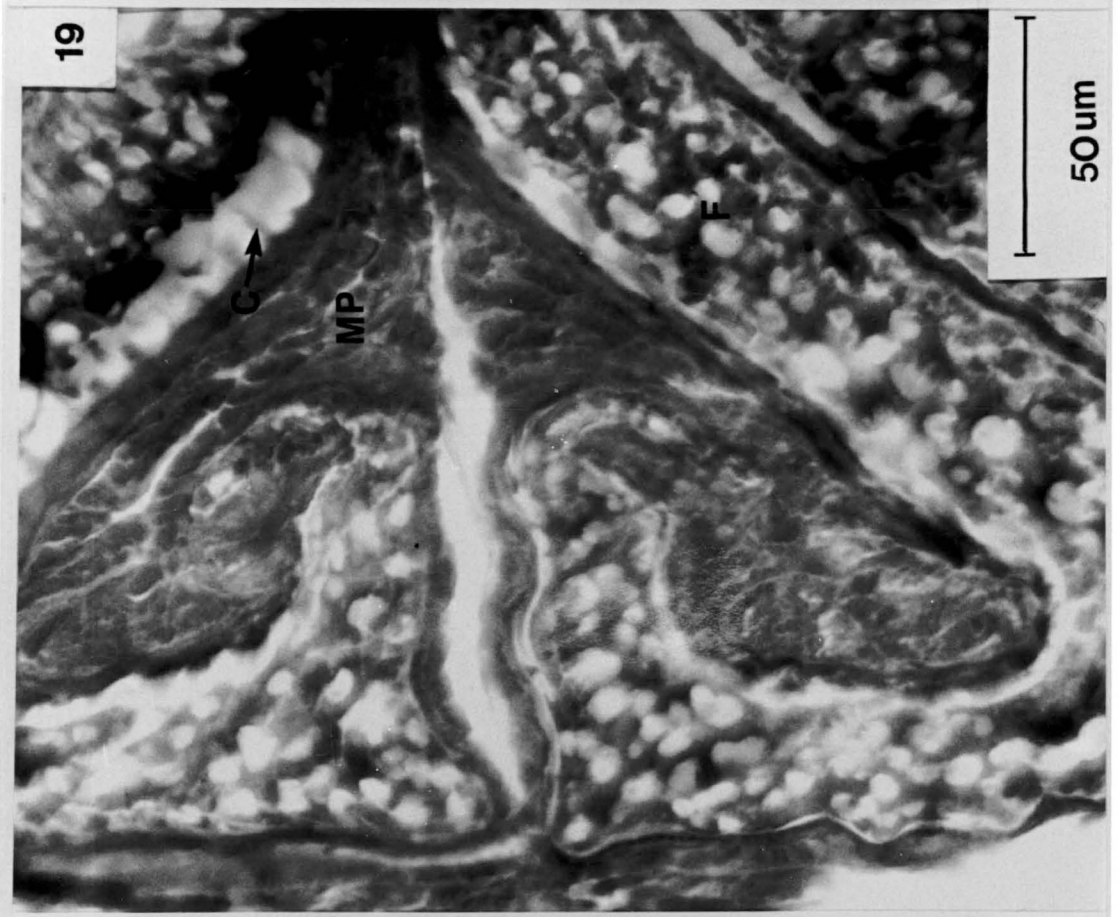
Morphology of the proventriculus: *Carabus nemoralis* Müller

The proventriculi of two adult *Carabus nemoralis* were examined mainly to assess the value of this organ as a taxonomic character among carabids and cicindelids. The proventriculus appeared bulky and incapable of efficient closure due to the large amount of sclerotization. There appeared to be no posterior apodemes as in *Cicindela*. Internally the longitudinal planes of the major plicae were examined by scanning electron microscope (Fig 20). Ctenidia arose from flat plates on the surface (Fig 20). The ctenidia branched apically, and some appeared to be setae arising from "sockets" (Fig 21).

Morphology of the proventriculus: *Omus audouini* Reiche

The excised proventriculus of *O. audouini* appeared cylindrical and fleshy, and had the four posterior apodemes characteristic of *Cicindela*. The crop however, was not large and sac-like as it was in *Cicindela,* rather it was cylindrical and slightly wider than the proventriculus. Also, the proventriculus was not deeply embedded into the posterior of the crop as it was in *Cicindela*. Internally, the plical patterns of *O. audouini* were similar to those of *Cicindela* (Fig. 22). The chitinous lining of the proventriculus was thicker and more robust

in *Omus*, and sparse ctenidia were also present on the inner surface. The basic

structural design of the proventriculus of *O. audouini*, resembled that of

Cicindela.

The Larval Gut of *Cicindela tranquebarica* Herbst: Morphology and Biology

The alimentary canal of *C. tranquebarica* (Fig 23), resembled a uniform

tube-like structure. The oesophagus widened posteriorly, and there was no

structure distinguishable as the proventriculus. Dissections of the gut released a

dark brown unidentified homogeneous liquid, which was probably food material.

The internal structure was similar along its entire length. A layer of granular

epithelium, probably secretory or absorptive in function (Snodgrass 1935), was

seen sloughed away from the internal intima (Fig. 24). Areas of cell

regeneration were scattered along the periphery, and the entire structure was

ringed by a layer of muscle. This general construction prevailed throughout the

gut and resembled that of *C. repanda* (Fackler 1918).

Adults and larvae of *Cicindela* are known to be predaceous, feeding on

similar prey items, primarily ants (Larochelle 1974, 1977). The tiger beetle larva

has been reported by Wigglesworth (1930) to employ extra-intestinal digestion,

regurgitating digestive fluids from the midgut onto prey and sweeping food

materials into the mouth with the labium. If both adults and larvae employ pre-

oral digestion of similar food items by regurgitating mid-gut enzymes, why is the

proventriculus present only in the adult stage?

The larvae of tiger beetles live in burrows, which is reflected by their external morphology (Horn 1915; Hamilton 1925; Gilyarov and Sharova 1954; Wallis 1961; and Palmer 1978). The soft bodied larvae are sheltered from desiccation by their cool subterranean environment and are probably dependent on the moisture available in their burrows for continued development. Shelford (1908) noted that larvae moult more rapidly under moist soil conditions. Hadley et al. (1990) found that larvae of Cicindela marutha Dow extract water from the soil in their burrows by injestion thus supplementing dietary water intake. Hori (1982) showed that moulting of larvae is dependent upon body weight and size and that the role of soil moisture is critical for the development of immature stages. Comparatively the diurnal activity of the adult on the ground surface subjects it to desiccation.

I have observed that no structure comparable to the adult proventriculus exists in the alimentary canal of the third instar larvae, and conclude that unhindered regurgitation from the midgut occurs regularly for digestion in the larvae.

I believe that the feeding methods of both adult and larvae may be viewed as adaptations to their environments which may be interpreted from the morphology of the digestive tract. Water conservation for larvae is less important because they live in moist burrows and supplement water needs with soil moisture. Erwin (1979) stated that the similar larval morphology of carabids

might " . . . be a general preadaptation for entry into diverse ecological zones,

permitting initial establishment of the species, and allowing time for selection to

act on the adult form while the new resident becomes fully adapted to the new

zone." The proventriculus is likely an adaptation to the adult's environment and

mode of life. Absence of this organ in the third larval instar may be explained by

it's generalized body type and may be linked to the constant environment

provided by the moist subterranean habitat.

Figure 20. Major plica of the proventriculus of *Carabus nemoralis*: C, ctenidium.

Figure 21. "Socketed seta" of the proventricular intima of *Carabus nemoralis*: S, seta.

Figure 22. Anterior cross section of the proventriculus of *Omus audouini*: MP, major plica; mP, minor plica.

Figure 23. Alimentary canal of the third instar larva, *Cicindela tranquebarica*: oe, oesophagus.

Figure 24. Cross section of the alimentary canal of the third instar larva, *Cicindela tranquebarica*: E, epithelium; CR, area of cell regeneration.

20

43 um

21

S

3um

22

mP

MP

100 um

23

oe

1mm

24

CR

E

100 um
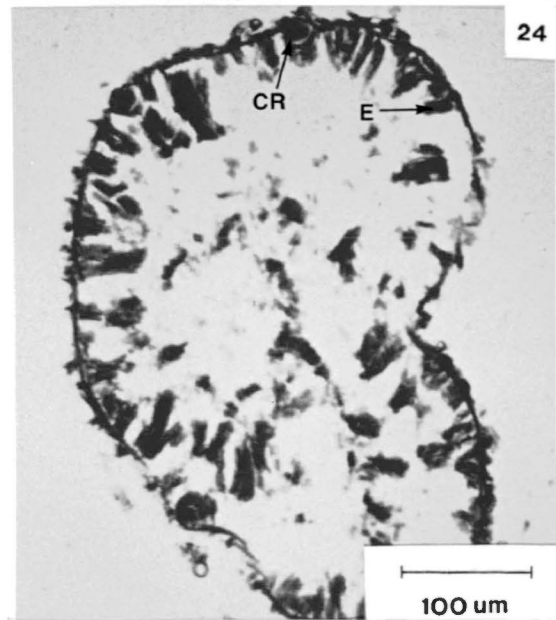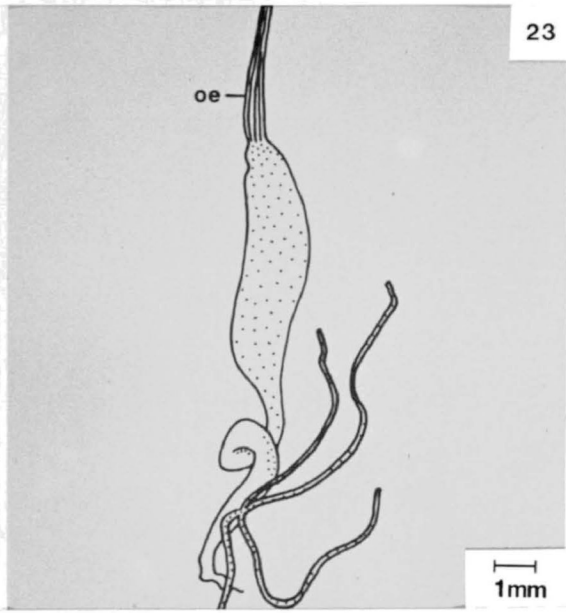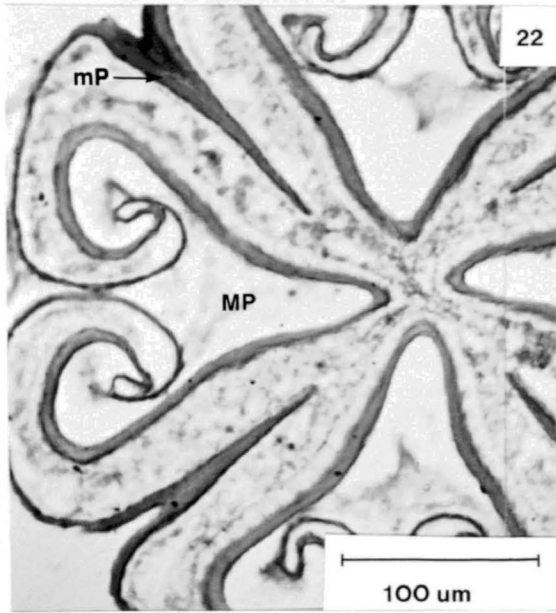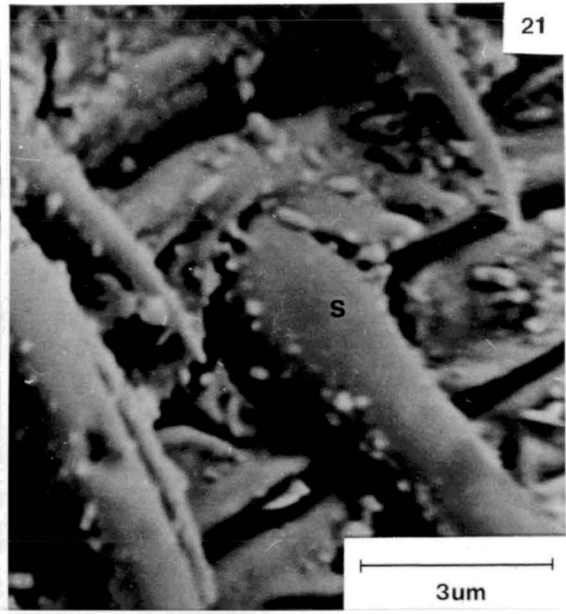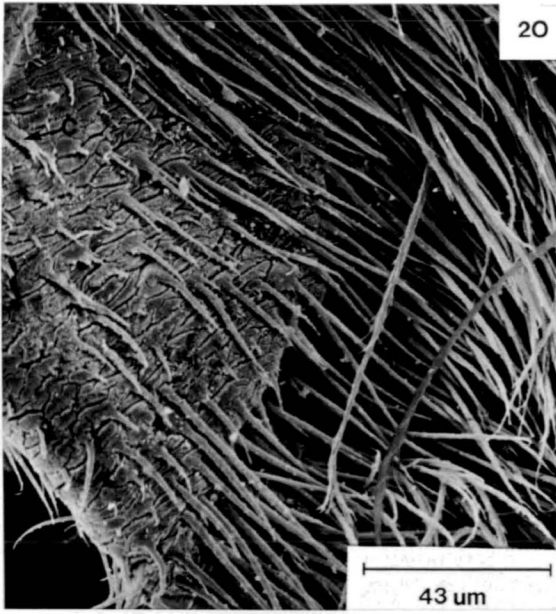
Biological and Systematic Implications

Introduction

Hengeveld (1980) reviewed the literature concerning phylogenetic relationships among carabids based on preferred foods. He concluded that relationships among the taxa of this group can be based on feeding habits and that the subfamily Carabinae [including Cicindelinae] comprises food specialists. "Consequently, food specialization within this family should be seen as an ultimate phylogenetic rather than a proximate ecological process caused by competition for food". Smrz (1982) also concluded that the structure of the proventriculus is not influenced by the type of nutrition, and that proventricular type is characteristic within a tribe. Nevertheless he also concluded that this organ can be used for phylogenetic studies because of its slight adaptability, and he constructed an anageny of the internal intimal proventricular structures for Adephagans.

In a systematic context I compared the structural variations of proventriculi between the carabid *Carabus nemoralis* and Cicindelinae, the cicindelid *Omus audouini* and members of *Cicindela* as well as among subgenera and species groups of *Cicindela*, as a method of evaluating this organ as a taxonomic character and as a phylogenetic analysis of tiger beetles.

Subfamilies

According to Theile (1977) the subfamilies Carabinae, containing

Carabus nemoralis, and Cicindelinae, containing the tiger beetles, are both

members of the Carabidae. Pearson (1988) treated the tiger beetles as the

family Cicindelidae, and noted that considerable controversy surrounds the

placement of the group, though few morphological characters distinguish

carabids from cicindelids.

Reichenbach-Klinke (1953) distinguished families of Adephagans by

proventricular cross sectional patterns. Recently it has become apparent that

the intimal structures of this organ may also provide distinguishing familial

characters. Smrz (1982) indicated that the level of evolution can be deduced not

only from "gross differentiation" but also from the type of structures that cover

the proventricular intima. In examining the internal structure of the

proventricular intima, Smrz (1982) noted that cicindelids have long hair ctenidia

while members of Carabini have long plumose hairs.

As discussed earlier, the overall appearance and the intimal structures of

the proventriculus of *Carabus nemoralis* distinguish it from the cicindelids.

Figure 21 displays the "setae" which appear to arise from " sockets". These

structures do not occur in the cicindelids examined, and they may provide an

additional structural means of distinguishing the carabids and cicindelids.

Genera of Cicindelinae

The primitive tiger beetle *Omus* resembles carabid beetles in its habitat and habits. In general, species of the genus *Omus* are considered to be crepuscular or nocturnal (van den Berghe 1990), unlike the diurnal *Cicindela* (most species), and specific life styles are indicated. For example adults of *Omus audouini* prefer forest/meadow ecotones, and show a preference for centipedes as a food source (Maser and Beer 1971). Because adults of the genus *Omus* differ in body form so significantly from the diurnal adults of *Cicindela*, the proventriculus was examined to determine if a complementary level of variation in it exists between these two taxa.

The proventriculi of *Omus audouini* and *Cicindela* are similar in external structure, cross sectional plical pattern and ctenidial structure. However the crop of *O. audouini* was not large and sac-like as that described for *Cicindela*. Measurements of two posterior ctenidia of *O. audouini* averaged 10.5 um which appears to be considerably shorter than those listed for species of *Cicindela* examined (Appendix B). Although the species of *Omus* differ in overall appearance, and habitat, the overall structure of the proventriculus remains similar to that of the *Cicindela* species examined, indicating that this organ nicely displays the phylogenetic relationship between *Omus* and *Cicindela*. Further examination of the intimal ctenidia of *Omus* may yield additional structural variations that can distinguish cicindelid genera.

Cicindela: Ctenidia of the Posterior Apodemes

The apodeme ctenidia are structurally similar in *Cicindela (Cicindela) repanda* (Fig. 25), *Cicindela (Pachydela) scutelaris* Say (Fig. 26) and *Cicindela (Cicindelidia) tenuisignata* Leconte (Fig. 27) The ctenidia lie flat to the surface of the apodeme with ctenidial fingers extended in the anterior direction. The fingers arise directly from the apodeme surface and the number of fingers per grouping varies from three to ten. In contrast, *Cicindela (Habroscelimorpha) dorsalis* Say (Fig. 28) and *Cicindela (Ellipsoptera) marginata* Fabricius (Fig. 29), have ctenidial fingers that arise from a definite rounded plate which is slightly raised above the surface of the apodeme. The number of fingers per grouping varies from eight to fifteen. The plates from which the fingers arise stretch into each other, and a few create a row (Fig. 28). These conditions exist at the margin of the apodeme bordering the posteriorly directed ctenidia. Hence two conditions of the apodeme ctenidia exist among the subgenera examined.

I assumed that members of the primitive subgenera will more likely exhibit the primitive ctenidial condition, and derived subgenera the derived ctenidial condition. Primitive and derived states of apodeme ctenidia were determined based on the phylogenetic arrangement of taxa provided by Boyd and Associates (1982). As expected the relatively primitive, *Cicindela, Pachydela* and *Cicindelidia* are more similar to each other in apodeme structure than to either *Habroscelimorpha* or *Ellipsoptera*. In addition the subgenera *Habroscelimorpha* and *Ellipsoptera,* both relatively derived subgenera, resemble

each other in their form of the apodeme ctenidia.

Figure 25. Apodeme ctenidia of *Cicindela repanda*: C, ctenidium.

Figure 26. Apodeme ctenidia of *Cicindela scutelaris*.

Figure 27. Apodeme ctenidia of *Cicindela tenuisignata*.

Figure 28. Apodeme ctenidia of *Cicindela dorsalis*: C, ctenidium; R, row.

Figure 29. Apodeme ctenidia of *Cicindela marginata*: C, ctenidium.

25

26

7.5 um

7.5 um

27

28

c

6 um

10 um

29

8.6 um
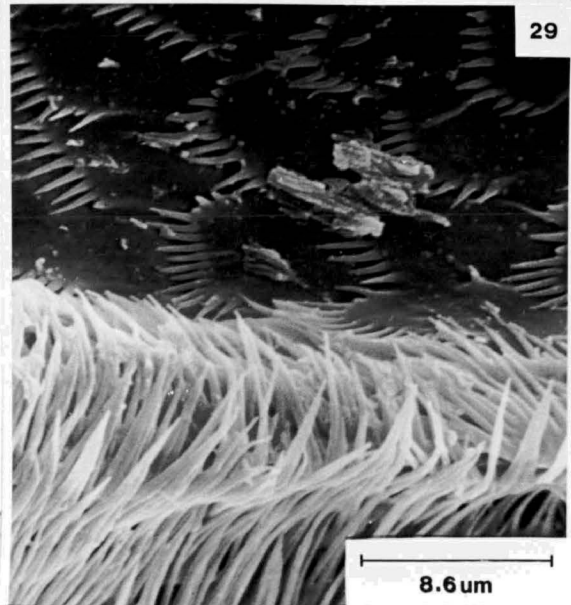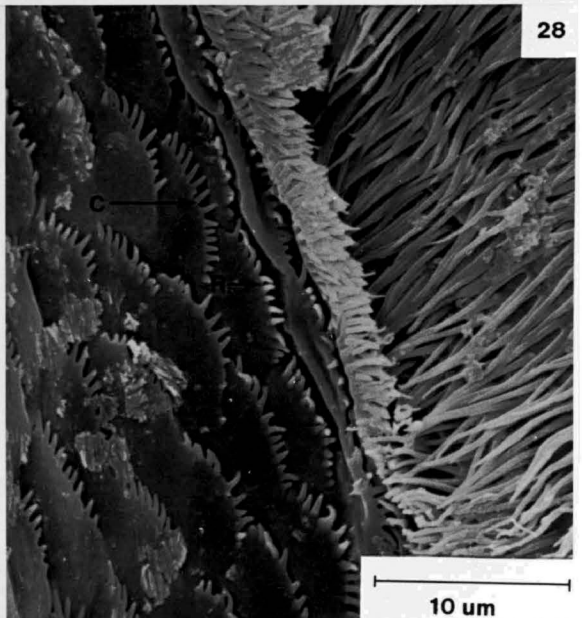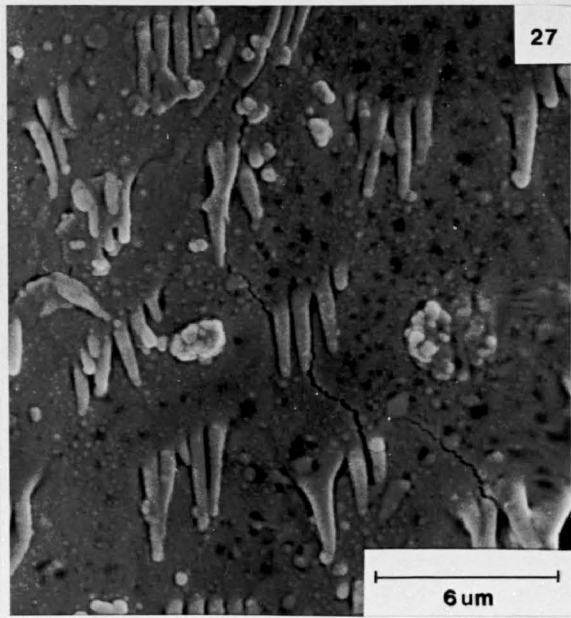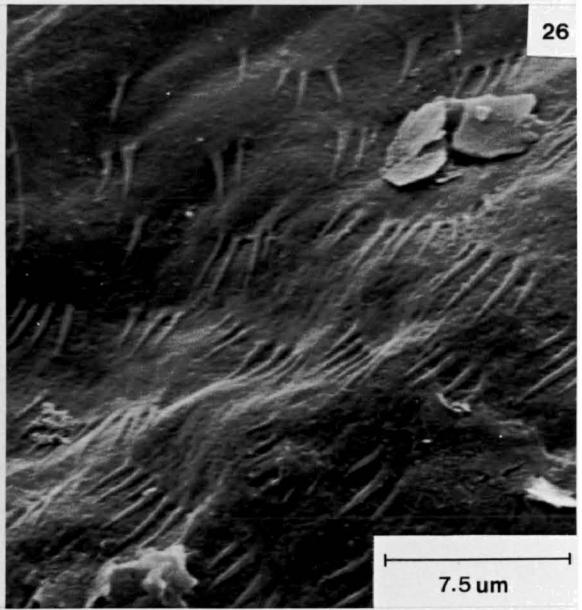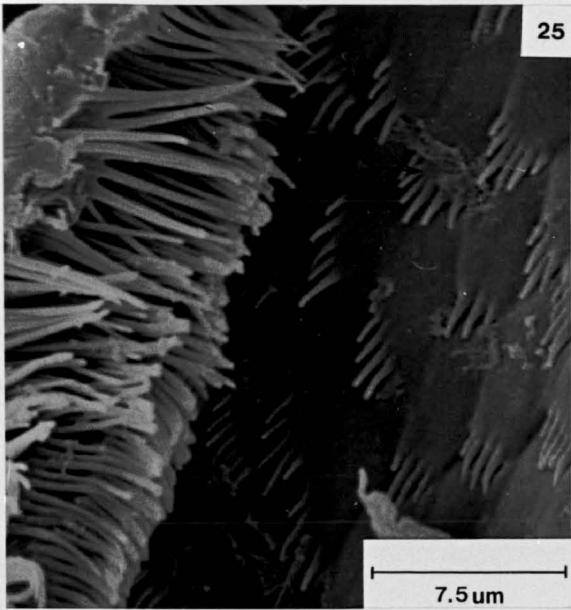
Cicindela: Ctenidia of the Major Plicae

The internal structure of the cicindelid proventriculus displayed two distinct regions, the anterior region where major plicae extend deeply into the lumen and are spade shaped (Fig 7), and the posterior region where major plicae are shorter and rounder (Fig 9). This general construction existed for all species of *Cicindela* examined (Appendix B).

Using the anterior plical heights and corresponding ctenidial lengths Appendix B, a regression analysis (Fig 30) indicated that no significant relationship existed between plical height and ctenidial length with $r^2=0.009$ and $p=0.4751$. Similarly the posterior ctenidial lengths and plical heights are unrelated $r^2=0.002$, $p=0.7228$ (Fig 31). Since the ctenidial lengths appear to vary independently of the plical height, the relationship between the anterior and posterior regions of the organ of these specimens may be described using the slope. The slope expresses the changing internal structure of the proventriculus in terms of both the major plicae and the ctenidia. Most slopes (displayed in Table 1) are negative, and a few are positive and flat. This indicates that generally the ctenidia of the posterior region are longer than those of the anterior region. Slopes appear to become flatter as ctenidial lengths of the anterior and posterior regions become similar in length. Slope may therefore be an indicator of variability occurring within the proventricular structure from the anterior to posterior regions.

The cicindelid taxa examined in this study have been arranged from

primitive to advanced according to Boyd and Associates (1982) in Table 1. the

calculated slopes in this table appear to become flatter in the derived species.

Because carabids are thought to have evolved from an aquatic ancestor that

radiated into new environments (Erwin 1979), the trend in the slopes which

accompanies the taxonomic arrangement has been discussed in terms of the

microhabitats of the species in the following sections. It is evident however that

the function of the proventriculus links its structure with the biology of the beetle.

Because the exact function of the cicindelid proventriculus has not been

determined, I have attempted to describe some of the variation of this organ in

terms of the microhabitat occupied by the species in which the slope is shown to

vary. These variations may be useful in determining the efficiency of the organ

which may be related to the microhabitat. Additionally, since microhabitats may

be useful for distinguishing groups of cicindelids in future studies, they have

been distinguished and compared in the following sections.

Figure 30. Scattergram of the anterior plical height and corresponding ctenidial length with regression line for all specimens listed in Appendix B.

Figure 31. Scattergram of the posterior plical height and corresponding ctenidial length with regression line for all specimens listed in Appendix B.

y = 0.017x + 12.08, r² = 0.009

Figure 30.

y = 0.012x + 27.60, r² = 0.002
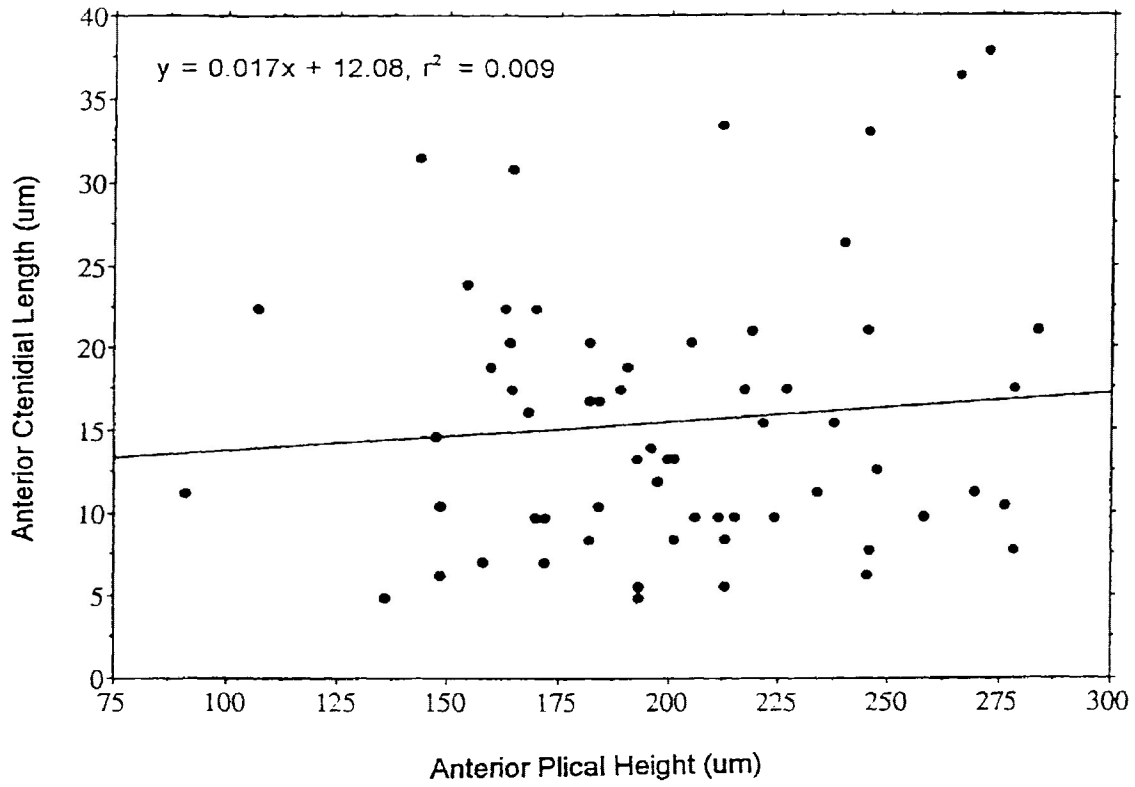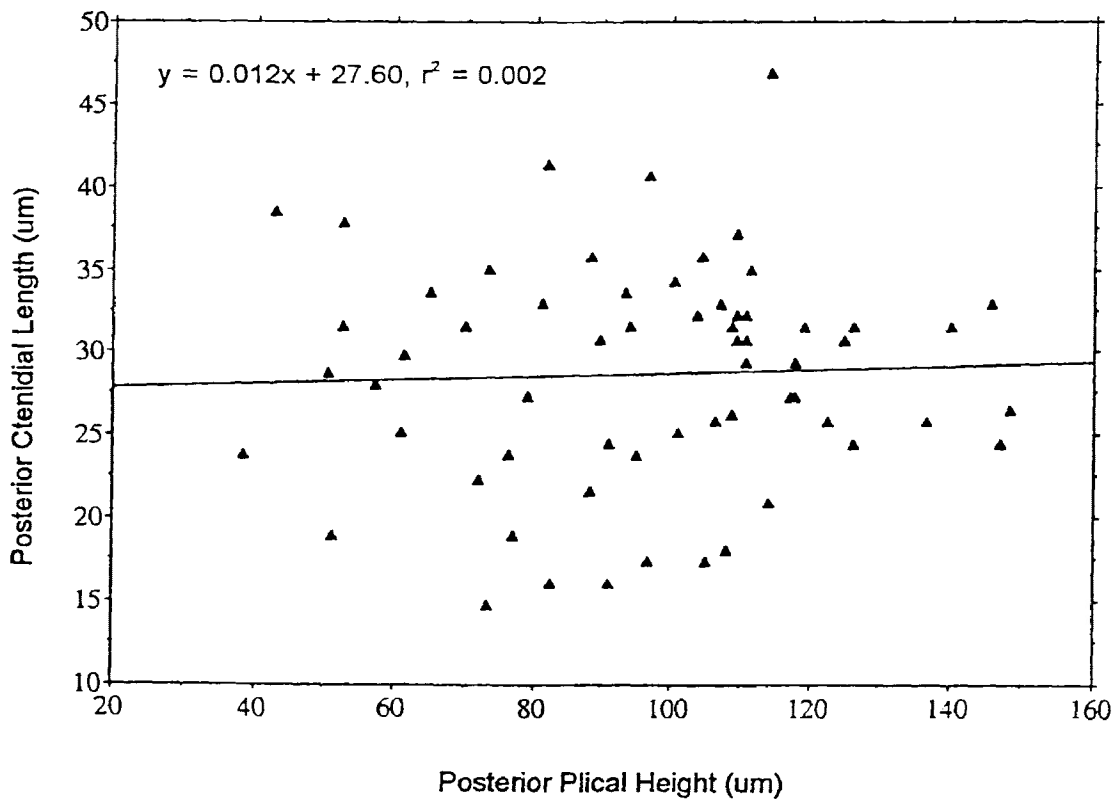
Figure 31.

Table 1. Specimens and Associated Proventricular Slopes, Classification based on Boyd (1982) and Rivalier (1954).

Subgenus	Species Group	Species	# of Specimens	Sex	Slope
Cicindela	sylvatica	C. longilabris	1	male	-0.5833
					-0.4667
			1	male	-0.0505
					-0.1695
Cicindela	sylvatica	C. nebraskana	1	female	-0.2093
					-0.2179
Cicindela	maritima	C. repanda	1	female	-0.3054
					-0.2803
			1	male	-0.2863
					-0.1573
Cicindela	maritima	C. duodecimgutatta	1	female	-0.2206
					-0.2174
			1	male	-0.1128
					-0.2381
Cicindela	maritima	C. oregona	1	female	-0.1498
					-0.1944
			1	male	-0.1359
					-0.2647
Cicindela	maritima	C. limbata	1	female	-0.2
					-0.2013
			1	male	-0.6429
					-0.2439
Cicindela	formosa	C. formosa	1	female	0.0881
					0.0043
			1	male	-0.1271
					-0.1392
Cicindela	splendida	C. limbalis	1	male	-0.1429
					-0.1344
			1	male	-0.1663
					-0.1545
Cicindela	purpurea	C. purpurea	1	female	-0.0692
					-0.25
Cicindela	purpurea	C. decemnotata	1	male	-0.1
					-0.0833
Cicindela	purpurea	C. sexguttata	1	male	-0.0743
					-0.0562
			1	male	-0.244
					-0.2857
Cicindela	purpurea	C. denikei	1	female	-0.1011
					-0.0636
			1	female	-0.0882
					-0.0811
Pachydela	scutelaris	C. scutelaris	1	female	-0.0963
					-0.2183
			1	female	-0.1515
					-0.1052
Tribonia	tribonia	C. longi	1	female	-0.1549
					-0.1599
			1	male	-0.1087
					-0.1005
Tribonia	tribonia	C. tranquebarica	1	female	-0.0865
					0.0079
			1	male	-0.0253
					-0.1007
Cylindera	terricola	C. terricola	1	female	0.0673
					-0.0185
			1	male	-0.0595
					-0.0756
Ellipsoptera	hamata	C. hamata	1	female	-0.1355
					-0.044
			1	female	-0.1948
					-0.0272

Total Number of Individuals = 31
Total Number of Slopes = 62
Mean Slope = -0.1549

Subgenera of *Cicindela*

Among the subgenera of *Cicindela* an upward trend is evident towards zero in the slope of the proventriculus (Fig 32). The subgenera are arranged in order of their traditional classification from primitive to advanced in Table 1. The upward trend indicates that derived subgenera have flatter proventricular slopes. Because the slope shows a continual trend, it cannot be used as a taxonomic character to distinguish among the subgenera of *Cicindela*, but it reflects the current notion of the phylogenetic relationships of the subgenera and points out the possible variation of this organ with the habitat.

Table 2 summarizes the results of the two-tailed Mann-Whitney U test conducted among subgenera. With the exception of *Ellipsoptera*, significant variation (p<0.05) occurs between the most distantly related subgenera. According to Willis (1971), the subgenus *Ellipsoptera* contains species which inhabit coastal beaches and salt flats which he considers to be the derived habitat condition within this subgenus, and this may be the derived habitat condition for all cicindelids. *Ellipsoptera* also contains numerous species which are linked to habitats with fresh water sources (the primitive habitat condition). It is apparent that a variety of habitats are occupied by the species within the subgenera, and evolution within a particular habitat may be evident in the proventricular structure, that is the steep proventricular slope/fresh water primitive habitat, or flat proventricular slope/dry or salt flats derived habitat.

Figure 32. Mean proventricular slopes of cicindelid subgenera.
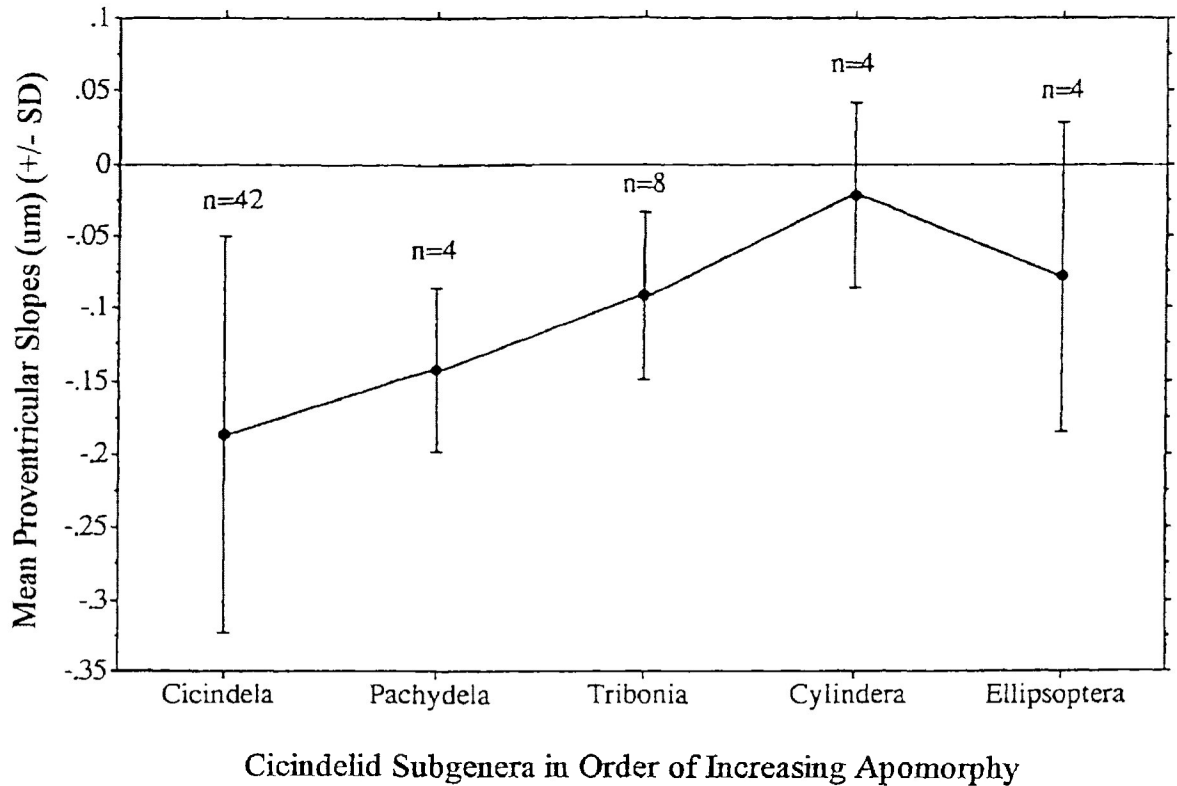
Figure 32

Table 2. Summary of Results of the Mann Whitney U Test Between the Subgenera of *Cicindela*.

Subgenera	Pachydela	Tribonia	Cylindera	Ellipsoptera
Cicindela	U'=98 n=46 p=0.5852	U'=249 n=50 p=0.0321	U'=154 n=46 p=0.0064	U'=126 n=46 p=0.1016
Pachydela		U'=22 n=12 p=0.3082	U'=16 n=8 p=0.0209	U'=11 n=8 p=0.3865
Tribonia			U'=27 n=12 p=0.0617	U'=16 n=12 p=1
Cylindera				U'=11 n=4 p=0.3865

Species Groups of *Cicindela*

Figure 33 illustrates the ctenidial slopes of cicindelid species groups which have been arranged according to traditional classification in order of increasing apomorphy (Table 1). The trend is similar to that shown for subgenera. Ctenidial slopes show an upward trend being steep in the primitive taxa, and becoming flatter in the more derived taxa except *formosa*. Table 3 depicts the results of a two-tailed Mann Whitney U test conducted for the species groups. Tiger beetles have been reported by Palmer (1978) to use substrate moisture and to drink available habitat water thus being connected to water availability in the environment. Because cicindelids occur in a variety of habitats, published habitat information was examined in an attempt to understand the observed variation of proventricular slope among the species groups, and to explore any possible relationship between the habitat, taxonomic arrangement and slope. The groups which vary significantly in Table 3 are discussed in terms of their habitats and the availability of moisture to the beetles in each habitat.

From Table 3, it is evident that the *sylvatica* group represented by *C. longilabris* Say and *C. nebraskana* Casey, varies significantly from the *tribonia* group (*C. tranquebarica* and *C. lengi* W. Horn), and also from *C. terricola* Say. Spanton (1988) discussed the phylogeny of the *sylvatica* complex, and indicated that the collective ranges of the species of this group are restricted to temperate regions, and that *sylvatica* is a northern lineage; the ancestor of the group was

proposed to have a Holarctic or Palearctic distribution. Schultz et al. (1992) proposed that the evidence of the northern history of this lineage exists in the physiological attributes of *C. longilabris*. The basking, foraging and stilting body temperatures of *C. longilabris* were reported to be lower than those recorded for other cicindelid species. *C. longilabris* is often found in dry sandy areas, (Leng 1902; Hooper 1969; Freitag and Tropea 1969, 1971; Larochelle 1972). Activity at cooler temperatures and occupation of cooler habitats with sufficient rainfall, likely reduce the water loss of *C. longilabris* and therefore inhabits such areas.

The *tribonia* species group contains the species *C. lengi* and *C. tranquebarica* and varies significantly in slope from *sylvatica* (Table 3). *C. lengi* has been reported to prefer loose dry sandy areas (Hooper 1969; Rotger 1974; Lawton 1974; Rogers 1974; Johnson 1975; Hilchie 1985; Acorn 1991). *C. tranquebarica* is less discriminating in its habitat preference as it has been observed in alkaline mud flats, sandy blowouts, prairie grasslands and boreal forest trails. Hilchie (1985) noted that this species can be found in almost any tiger beetle habitat. Similarily, Acciavatti (1992) reports *C. tranquebarica* residing in sand quarries, strip mines and dirt roads. Most areas with loose dry sand are apparently suitable habitats for *C. tranquebarica* (Freitag and Tropea 1969, 1971; Hooper 1969; Willis 1970; Larochelle 1971, 1972; Lawton 1971, 1972, 1974; Boyd 1973; Johnson 1975; Wilson 1978; Wilson and Brower 1983; Dunn 1981; Maser and Beer 1984). The proximity of *C. tranquebarica* habitat to fresh water varies, and this species can also be found on gravel (Choate 1975),

clay (Willis 1971; Lawton 1971), and alkaline soils (Willis 1970). The great

variety of habitats occupied by *C. tranquebarica* may be partially due to

temperature tolerance and physiological requirements. Schultz & Hadley (1987)

described *C. tranquebarica* as having tolerance of high temperatures, exhibiting

activity at warmer temperatures and having a lower water loss rate than the

riparian *C. oregona*.

C. *terricola* also varies significantly from the *sylvatica* group (Table 3). C.

terricola occupies a variety of habitats including sand (Freitag and Tropea 1969;

Lawton 1971), clay (Willis 1971; Johnson 1975, 1990; Hilchie 1985), alkaline

soil (Wallis 1961; Lawton 1974; Maser and Beer 1984). This species can be

found in numerous habitats, and does not appear to be directly related to

freshwater sources. The *tribonia* and *terricola* species groups do not vary

significantly by slope; both have flat slopes.

Figure 33 shows the *terricola* group with a flatter slope than the *splendida*,

purpurea and *scutelaris* species groups, and Table 3 indicates that the slope

varies significantly between *terricola* and these groups. According to Schincariol

and Freitag (1991), the *splendida* group has likely evolved as an inhabitant of

cool temperate riparian areas. This is reflected by the species *C. limbalis* Klug

which represents the *splendida* group in this study. *C. limbalis* is an inhabitant of

clay banks often near rivers (Freitag and Tropea 1969; Hooper 1969; Lawton

1972; Wilson and Brower 1983). The present habitats and geographical

distributions of these species may indicate that the *terricola* lineage was not

temperate and riparian as were the ancestors of *C. limbalis*.

The slope of the *purpurea* group, which varies significantly with *terricola* (Table 3), is represented in this study by that of *C. purpurea*, *C. decemnotata* Say, *C. sexguttata* and *C. denikei* W. Brown. Several races of *C. purpurea* live in Canada (see Wallis 1961 for distribution) and is often found on clay soils (Willis 1971; Lawton 1971, 1972, 1974; Larochelle 1972; Hilchie 1985). *C. decemnotata*, has also been associated with clay soils by Hooper (1969) and silt Leffler (1975).

Similar to *C. purpurea*, the species *C. sexguttata* is found on hard packed clay soil (Kaulbars and Freitag 1993; Nelson and LaBonte 1989), and shows no particular association with water sources (King, 1988). The species *C. denikei* has been reported from sandy till areas with extensive rocky outcroppings in Northwestern Ontario (Freitag and Tropea 1971; Kaulbars and Freitag 1993), and I have obtained this species from bare gravel. Members of the *purpurea* group appear to be non-riparian and, with the exception of *C. denikei*, prefer clay soils. These present habitats are probably much the same as those in which the *purpurea* group has evolved. Most members of the *purpurea* group are associated with clay soils which provide moist habitat conditions as compared to the drier bare sand or gravel preferred by the *terricola* group.

The *scutelaris* group also varies significantly in slope with *terricola*; it is not associated with moist habitat conditions. *C. scutelaris* is generally found in

sand (Hooper 1969; Willis 1970, 1971; Freitag and Tropea 1971; Larochelle

1972, 1974; Boyd 1973; Lawton 1974; Choate 1975; Dunn 1981; Roman 1988;

Acorn 1991). Acciavatti (1992) suggests that *C. scutelaris* is generally found

away from water and it has been considered an upland species with no

particular association with water (Graves and Pearson 1973).

The *formosa* group varies significantly from the essentially riparian

species groups *splendida* and *maritima* (Table 3), and exhibits a flat slope

(Figure 33); however it is positioned among the less derived cicindelid taxa. *C.*

formosa is generally associated with dry sandy areas (Willis 1971; Lawton 1971,

1972; Larochelle 1972; Boyd 1973; Johnson 1975; Dunn 1981; Wilson 1983;

Nelson and LaBonte 1989; King 1988; Acorn 1991, 1992) and according to

Vaurie (1950), surface water is not required by this species. The subspecies *C.*

formosa gibsoni Brown used in this study, inhabits sand dunes (Gaumer 1977).

The very wide elytral maculation of *C. formosa gibsoni* was viewed by Acorn

(1991) as a physical adaptation for thermoregulation in the hot dry environment,

and indicates an adaptation to hot, dry, sandy environments. All members of

formosa are known to inhabit dry sandy areas and the flat proventricular slope of

C. formosa gibsoni may reflect the evolution of the *formosa* group in dry habitats.

The *maritima* group varies in slope with all other species groups except

sylvatica (Table 3). Representatives of the *maritima* group in this study are *C.*

repanda, C. duodecimguttata Dejean and *C. oregona* LeConte which are known

to be riparian species, and *C. limbata* Say which inhabits very dry sandy areas.

The soil of the *C. repanda* habitat has been described by Freitag and Tropea (1969), Hooper (1969), Larochelle (1971, 1972), Willis (1971), Lawton (1972, 1974), Boyd (1973), Rogers (1974), Johnson (1975), Choate (1975), Leffler and Pearson (1976), Wilson (1978), Dunn (1981), Maser and Beer (1984), Nelson and LaBonte (1989), and King (1988). Generally *C. repanda* may be found occupying sandy areas very near a constant supply of fresh water.

Similar to *C. repanda,* is *C. duodecimguttata* which also inhabits sandy-clay soils in riparian areas (Freitag and Tropea 1969; Willis 1970; Freitag and Lee 1972; Larochelle 1972; Boyd 1973; Wilson 1978). *C. duodecimguttata* has also been described occurring on darker soils which likely bear a higher content of organic material (Freitag 1965; Boyd 1973; Hilchie 1985).

Closely related to *C. duodecimguttata* is the western riparian species *C. oregona* which commonly occupies sand (Freitag and Tropea 1971; Lawton 1972, 1974; Johnson 1975; Maser and Beer 1984). This species can often be found in gravel and stony waterside habitats (Willis 1971; Leffler 1975). Although *C. oregona* is usually associated with rivers and streams I have collected this species from coastal beach areas where no fresh water supply is evident. Although Schultz et al. (1992) describe *C. oregona* as requiring a cool riparian habitat, the species may not be tied to areas with fresh water supplies along the Pacific Coast where cool temperatures and abundant rainfall exists. The *maritima* group also contains the species *C. limbata* which occurs in very dry sandy areas remote from fresh water. For habitats of *C. limbata* see Hooper

(1969), Willis (1970, 1971), Lawton (1972, 1974) and Acorn (1991).

Freitag (1965) described the ancestor of the *maritima* group as a cool-temperate riparian species. The primitive cool-temperate riparian habitat is occupied by *C. repanda, C. duodecimguttata*, and *C. oregona* and the derived warm and dry habitat is occupied by *C. limbata*.

It can be concluded from these data that a general trend from a steep proventricular slope to a flatter one is correlated with increasing apomorphy among the species groups of the subgenus *Cicindela*, and with a decreasing presence of fresh water in the habitat. This corroborates a study by Willis (1971), who used habitat as a character in his cladistic analysis of the *Ellipsoptera* group. Habitats near fresh water were considered primitive while areas with little fresh water such as sand and coastal beaches were considered derived. It is likely that this is true for all cicindelids and that the ability of the beetle to attain and retain water are affected by the microhabitat.

Figure 33. Mean proventricular slopes of species groups of *Cicindela*.

Species Groups in Order of Increasing Apomorphy
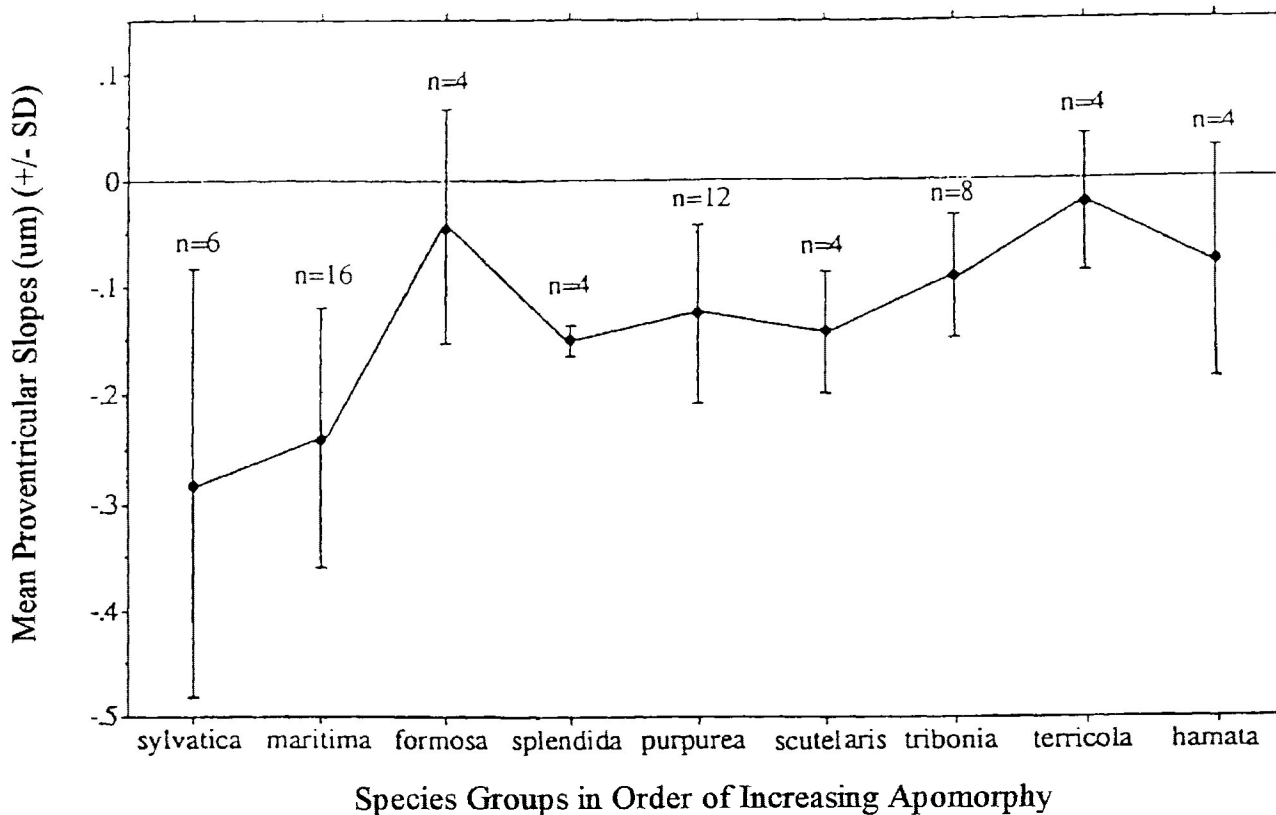
Figure 33.

Table 3. Summary of Results of the Mann Whitney U Test Between the Species Groups of *Cicindela*.

Species Groups	maritima	formosa	splendida	purpurea	scutelaris	tribonia	terricola	hamata
sylvatica	U'=49 n=22 p=0.9412	U'=22 n=10 p=0.33	U'=20 n=10 p=0.0881	U'=51 n=18 p=0.1601	U'=17 n=10 p=0.2864	U'=34 n=13 p=0.0404	U'=22 n=10 p=0.033	U'=21 n=10 p=0.055
maritima		U'=61 n=20 p=0.0061	U'=54 n=20 p=0.0376	U'=157.5 n=28 p=0.0043	U'=53 n=20 p=0.0472	U'=121 n=24 p=0.0005	U'=64 n=20 p=0.0025	U'=58 n=20 p=0.014
formosa			U'=15 n=8 p=0.0433	U'=30 n=16 p=0.4669	U'=12 n=8 p=0.2482	U'=19 n=12 p=0.6104	U'=9 n=8 p=0.7728	U'=10 n=8 p=0.5637
splendida				U'=36 n=16 p=0.1456	U'=10 n=8 p=0.5637	U'=26 n=12 p=0.0894	U'=16 n=8 p=0.0209	U'=11 n=8 p=0.3865
purpurea					U'=34 n=16 p=0.2253	U'=49 n=20 p=0.9385	U'=43 n=16 p=0.0212	U'=30 n=16 p=0.4669
scutelaris						U'=22 n=12 p=0.3082	U'=16 n=8 p=0.0209	U'=11 n=8 p=0.3865
tribonia							U'=27 n=12 p=0.0617	U'=16 n=12 p=1
terricola								U'=11 n=8 p=0.3865

Species of the *C. maritima* group

The *maritima* group provides an opportunity to examine the effect of microhabitat on the slope of the proventriculus because it is represented in this study by both riparian and non-riparian species with a shared ancestry. The proventricular slopes of the species representing the *maritima* group are displayed in Figure 34. Although *C. limbata* inhabits dry areas, the proventricular slope is not flatter than those of the riparian species *C. repanda*, *C. duodecimguttata*, and *C. oregona*. Table 4 also shows that *C. limbata* does not vary significantly from the riparian species. This indicates that the dry habitat characteristic of *C. limbata* has not influenced the slope of the proventriculus, and this species retains the steep slope shown by the riparian members of the group. Thus the slope of the proventriculus indicates phylogenetic relationships and ancestral habitats more strongly than habitat adjustments within a species group.

Figure 34. Mean proventricular slopes of the species of the *maritima* group.
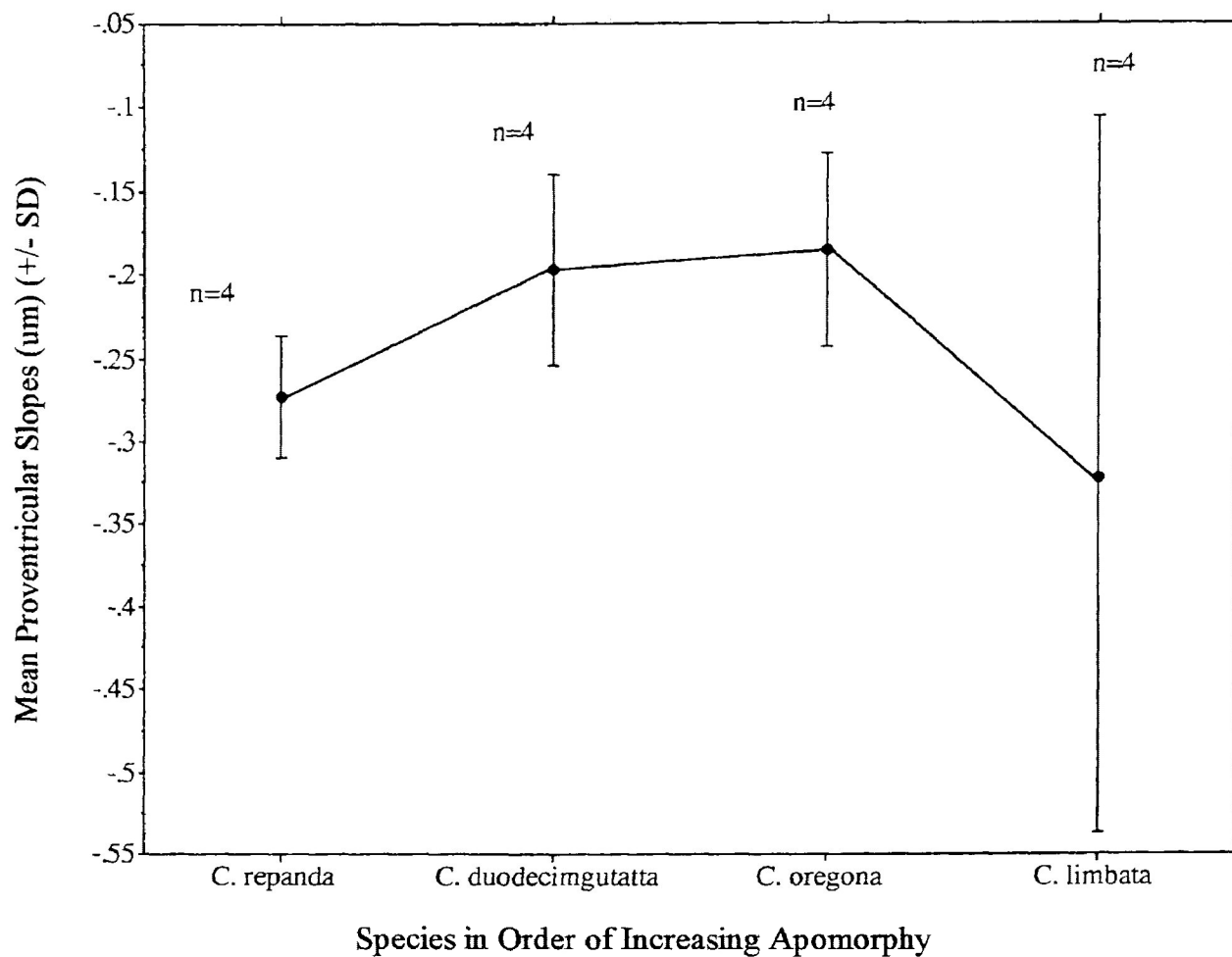
Figure 34.

Table 4. Summary of Results of the Mann Whitney U Test Between the Species of the maritima group.

Species	C. duodecimgutatta	C. oregona	C. limbata
C. repanda	U'=14.5 n=8 p=0.0591	U'=15 n=8 p=0.0433	U'=11 n=8 p=0.3865
C. duodecimgutatta		U'=9 n=8 p=0.7728	U'=10 n=8 p=0.5637
C. oregona			U'=13 n=8 p=0.1489

Habitat Soil and Water Associations

Tiger beetles occupy a great variety of habitats and their distributions have often been linked to habitat soil type and proximity to water. Shelford (1911) originally associated soil moisture and egg laying of females. However the importance of adult use of habitat moisture has not yet been determined. Erwin (1979) indicated that carabids on the whole are thought to have evolved from an aquatic ancestor and through taxon pulses have moved linearly along pathways radiating into new environments. Above I consider the cool-temperate riparian habitat as being primitive among cicindelids, and that the primitive habitat is reflected by a steep slope of the proventriculus. Erwin (1979) also stated that "Rates of evolution are quite different for different groups and determined by where groups live in both the micro and macrohabitat sense." Thus I attempted to determine if the slope condition reflects the availability of moisture in the habitats occupied by each species in this study.

The calculated mean value of slopes was used to divide them into steep and flat categories. These two slope categories were then subjected to Chi-square tests of independence. The first test relates the proventricular slope to the distance from water. Table 5 summarizes the frequencies of the variables, with n=62, X^2=2.954, p=0.228, and df=2. The observed frequencies are therefore not dependent on the distance to a fresh water source. Although this analysis did not reveal a significant relationship, it is valuable in determining the distribution of tiger beetle habitats in relation to water sources. According to

Schultz and Hadley (1987), riparian species such as *C. oregona* require habitats close to water because they are not tolerant of high temperatures and have greater rates of water loss than *C. tranquebarica* which also inhabits the same general area. However, *C. tranquebarica* remains away from the water on the upper beach. These authors have suggested that it is the physiological requirements of the species which determine their distributions on the same beach. Thus, on a smaller scale, proximity to water may be valuable in determining distributions of tiger beetles.

The second Chi-square analysis tested the association between the proventricular slope and the surface soil type. The observed frequencies are shown in Table 6, and although the frequencies are unconventionally small, this analysis was significant at a 90% confidence level, n=62, X^2=8.79, p=0.0619 and df=4. This may indicate a dependency of the slope on the soil type and the relationship may be strengthened by the addition of more samples. The relationship more likely reflects the primitive and derived conditions of the soil microhabitat.

Table 5. Chi-square 2 X 3 Contingency Analysis of Proventricular Slope and Proximity to Fresh Water.

	< 20 m	20 - 200 m	> 200 m	Totals:
Steep	10	6	13	29
Flat	14	2	17	33
Totals:	24	8	30	62

Table 6. Chi-square 2 X 5 Contingency Analysis of Proventricular Slope and Soil Particle Size.

	Clay	Clay/Sand	Sand	Sand/Gravel	Gravel	Totals:
Steep	7	0	13	7	1	28
Flat	3	6	17	5	3	34
Totals:	10	6	30	12	4	62

Conclusions

The conclusions listed here are based on the data derived in this study as well as the methodology which has been applied.

1. Slopes calculated from measurements of anterior plicae and ctenidia, and posterior plicae and ctenidia, provided a method of quantifying variations that exist in the internal proventricular structure among species of *Cicindela*.

2. Third instar larvae may be easily obtained from their burrows by placing golf tees in the opening. Movement of the tee indicates the presence of the larva at the top of the burrow at which time it may be extracted from the soil with a spade.

3. The soil microhabitat index was created to generally assess the microhabitat. A need for a continuous more detailed index exists for the assessment of tiger beetle microhabitats.

4. The proventriculus of *Cicindela* is located in the first three abdominal segments.

5. Externally, the cicindelid proventriculus appears fleshy and funnel shaped narrowing posteriorly.

6. Internally, the proventriculus exhibits four major plicae which appear spade shaped in cross section. The major plicae alternate with four minor plicae which are narrow and needle-like in cross section. The internal intima of the proventriculus is covered by comb-like structures known as ctenidia. The minor plicae become integrated into the apodemes and a large circular muscle ring is

located at the posterior end of the organ.

7. Posterior apodemes bear ctenidia which are directed anteriorly and these demonstrate some structural variation among subgenera of *Cicindela*.

8. The structure of the tiger beetle proventriculus described in this study, supports the discussions by other authors of this organ's function as a filter. At the hind end of the proventriculus, apodemes probably serve as tracks to anterior movement of midgut fluids. When and how midgut fluids move anteriorly to the crop through the proventriculus remains unknown.

9. Third instar larvae of *C. tranquebarica* do not have a structure comparable to the adult proventriculus in the alimentary canal.

10. *Carabus nemoralis* exhibits structures which appear to be "socketed setae", which may be useful for distinguishing carabids from cicindelids.

11. *Omus* and *Cicindela* exhibit a phylogenetic relationship based on the overall structure of the proventriculus; the posterior ctenidia appear to be shorter in *Omus*. Also the crop of *Omus* is cylindrical and slightly wider than the proventriculus, in contrast to that of *Cicindela* which is sac-like and much larger than the proventriculus.

12. Among *Cicindela* the proventricular slopes exhibit a phylogenetic trend which is correlated with the traditional taxonomic arrangement of the group, and primitive and derived microhabitat conditions. The steep and flat proventricular slopes appear to be consistently related to wet and drier habitats respectively; for all examined taxa.

13. Small scale measurement of habitat proximity to water may be valuable in determining distributions of Cicindelid species within microhabitat.

Future Studies

Investigations of proventricular structure in remaining cicindelid and carabid genera may yield additional phylogenetic information. Within *Cicindela*, taxonomic revisions of *C. tranquebarica* and *C. terricola* are required to determine ancestry, distributions and subspecies. Analysis of cicindelid microhabitats may also provide additional characters. Finally, the functional hypothesis of the proventriculus proposed in this study requires testing to determine the role of this organ in the digestive process.

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APPENDIX A

Specimens Collected for Histological Examination Summer 1992:

Cicindela longilabris:	(2 male) June 2 10:00 AM - 10:45 PM 5 km east of Kenora, Ontario. Small gravel road on HWY 1 to pit.
Cicindela nebraskana:	(female) June 12 5:00 PM - 5:50 PM 8.4 km South of Maple Creek, Saskatchewan. Side of Hwy 21
Cicindela repanda:	(female) June 8 10:30 AM - 12:30 PM White Bear Reserve, Saskatchewan Sand pit at the shore of Lake Carlyle. (male) June 1 12:00 PM - 1:00 PM English River, Ontario Sand pit beside small marshy lake along HWY 1.
Cicindela duodecimguttata:	(female & male) June 1 12:00 PM - 1:00 PM English River, Ontario Sand pit beside small marshy lake along HWY 1.
Cicindela oregona:	(female & male) June 18 1:15 PM - 2:30 PM Yoho National Park, British Columbia. Sandy shore of Columbia River near HWY 1.
Cicindela limbata:	(female & male) July 13 1:00 PM - 1:45 PM 1 km east of Redwater Alberta HWY 644. Sandy patch in hay field.

Cicindela formosa: (female & male)
June 11 9:20 AM - 11:00 PM
Douglas Provincial Park, Saskatchewan.
Sand dunes.

Cicindela limbalis: (2 male)
May 21 2:00 PM - 4:00 PM
Thunder Bay, Ontario.
Rosslyn Brickyard.

Cicindela purpurea: (female)
June 9 11:30 AM
Fort Qu'Appelle, Saskatchewan.
Bank of Qu'Appelle River.

Cicindela decemnotata: (male)
June 12 5:00 PM - 5:50 PM
8.4 km south of Maple Creek, Saskatchewan.
Sandy area along HWY 21.

Cicindela denikei: (2 female)
July 21 4:00 PM - 5:20 PM
22 km west of Kenora, Ontario
Rice Lake Road and HWY 1 intersection.
Gravel pipeline route.

Cicindela scutelaris: (2 female)
June 7 12:10 PM - 1:30 PM
Aweme, Manitoba
Sandy area beside Criddle Homestead.

Cicinela lengi: (female & male)
June 11 9:20 AM - 11:00 AM
Douglas Provincial Park, Saskatchewan.
Sand dunes.

Cicindela tranquebarica: (female)
June 1 12:00 PM - 1:00 PM
English River, Ontario.
Sand pit beside HWY 1 next to marshy lake.

(male)
June 11 4:30 PM - 5:40 PM
Saskatchewan Landing Provincial Park.
Beach of South Saskatchewan River.

Cicindela terricola: (female & male)
June 19 12:30 PM - 1:30 PM
Revelstoke, British Columbia.
Spillpond of Columbia River.

Specimens Collected for Histological Examination Spring 1993:

Cicindela sexguttata: (2 male)
May 14 2:00 PM - 3:00 PM
Watkins Mill State Park.
Lawson, Missouri.
Lawn of park.

Cicindela hamata: (2 female)
May 11 1:15 PM - 2:30 PM
35 km east of Freeport, Texas along road to
Galveston.

Gulf beach area below toll bridge.

Cicindela tranquebarica: (4 third instar larvae)
Tysoski Rd. Sand Pit
Thunder Bay, Ontario

Specimens Received From Other Collectors for Histological Examination:

Cicindela punctulata:	U.S. A. Florida Flager County, Palm Coast. 18X 1992 P. Skelley
Omus audouini:	(male) University of Washington Campus Seattle, Washington 13 - VI - 1992 S. R. Leffler

Specimens Used for Examination by Scanning Electron Microscopy from R. Freitag Collection, Lakehead University:

Cicindela repanda:	(male) HWY 77 Republican River, Kansas V 7 . 70
Cicindela oregona:	(male) Sand Cr 13 mi off HWY 191 St. Anthony, Idaho V I 3. 70
Cicindela scutelaris:	(female) June 7 Aweme, Manitoba Criddle Farm 11 km from Treesbank
Cicindela marginata:	(male) FL. Monroe Co. Big Torchkey 2. V I 1986 M. Kaulbars

Cicindela tenuisignata: (male)
Morelos Dam Colo R., Ca 5 mi S Yuma, Arizona
V I 15. 63

Cicindela dorsalis: (male)
June 11/69 Jekyll Beach, G. A

Carabus nemoralis: Thunder Bay, Ontario
Summer 1971
V 20
W. Mercer & A. Smith

APPENDIX B

Specimens and Corresponding Measurements.

SPECIES	# OF SPECIMENS	SEX	ANTERIOR PLICAL HEIGHT (um)	ANTERIOR CTENIDIAL LENGTH (um)	POSTERIOR PLICAL HEIGHT (um)	POSTERIOR CTENIDIAL LENGTH (um)	SOIL INDEX SCORE
C. longilabris	1	male	91	11	83	16	3.4
			147	15	126	25	
C. longilabris	1	male	258	10	105	18	3.4
			200	13	117	27	
C. nebraskana	1	female	201	13	111	32	3.4
			197	12	88	36	
C. repanda	1	female	193	5	104	32	2.1
			193	6	93	34	
C. repanda	1	male	158	7	65	34	1.3
			182	8	57	28	
C. duodecimguttata	1	female	213	8	118	29	1.3
			206	10	109	31	
C. duodecimguttata	1	male	148	6	74	15	1.3
			136	5	77	19	
C. oregona	1	female	278	8	119	32	1.4
			211	10	111	29	
C. oregona	1	male	234	11	90	31	1.4
			196	14	148	27	
C. limbata	1	female	247	13	146	33	3.3
			215	10	111	31	
C. limbata	1	male	160	19	140	32	3.3
			148	11	91	25	
C. formosa	1	female	272	38	137	26	3.3
			265	36	104	36	
C. formosa	1	male	278	18	97	41	3.3
			212	33	114	47	
C. limbalis	1	male	221	15	109	32	2.1
			237	15	107	33	
C. limbalis	1	male	224	10	94	32	2.1
			213	6	95	24	
C. purpurea	1	female	144	32	52	38	2.3
			107	22	43	39	
C. decemnotata	1	male	184	17	79	27	3.4
			190	19	106	26	
C. sexguttata	1	male	165	18	61	25	1.1
			162	22	50	29	
C. sexguttata	1	male	182	17	125	31	1.1
			193	13	109	37	
C. denikei	1	female	246	8	114	21	1.2
			245	6	91	16	
C. denikei	1	female	245	21	126	32	1.2
			217	18	109	26	
C. scutellaris	1	female	189	18	61	30	3.3
			169	10	70	32	
C. scutellaris	1	female	227	18	111	35	3.3
			201	8	108	18	
C. lengi	1	female	172	7	72	22	3.3
			184	11	53	32	
C. lengi	1	male	276	11	147	25	3.3
			269	11	123	26	
C. tranquebarica	1	female	219	21	81	33	3.2
			240	26	101	25	
C. tranquebarica	1	male	165	31	109	32	1.3
			284	21	82	41	
C. terricola	1	female	169	22	97	18	1.3
			164	20	88	22	
C. terricola	1	male	168	16	39	24	1.3
			172	10	51	19	
C. hamata	1	female	182	20	74	35	3.5
			245	33	118	27	
C. hamata	1	female	154	24	100	34	3.5
			205	20	76	24	

Total Number of Individuals = 31