Bull. Inst. Zool., Academia Sinica 6: 77~86 (1961)

A HISTOLOGICAL STUDY OF DEVELOPMENT OF THE THORACIC MUSCULATURE FROM LARVAE TO ADULTS IN *TENEBRIO MOLITOR* L. (TENEBRIONIDAE, COLEOPTERA, HEXAPODA).[1]

I. A STUDY OF THE THORACIC MUSCULATURE OF ADULTS STAGE.

WEN-YUNG LEE[2], KUO-KOUNG CHANG[3]

Received for Publication, Nov. 20, 1967.

ABSTRACTS

The histological study of the thoracic musculature of the adults in *Tenebrio molitor* L. has been investigated with the methods of the paraffin section and teasing muscles.

All muscles of thoracic segments were examined. Three histological types of muscles were found: the general, the tubular general and the fibrillar. Most muscle fibers are muscles of the general type; only a few muscles are the muscles of the tubular general type, and the direct and the indirect flight muscles in the metathorax are the fibrillar type.

The muscles are attached to the cuticle through the epidermis; some muscles are attached to the internal skeleton such as the phragma or the furca; most of the leg muscles are attached to the integument by a long tendon, and most of the flight muscles are attached to the sclerite discs.

The tracheoles were found as the intercellular of the fibers. The tracheal system helps in binding together of the flight muscle fibers.

INTRODUCTION

While working on the internal anatomical development of the thoracic musculature during metamorphosis in *Tenebrio molitor* L. (6), the changes of the musculature were found as follows: (1) majority of the larval muscles are transformed into the adult muscle, (2) Some muscles are gradually degenerated and finally disappeared and (3) new muscles are developed during the prepupal and the pupal stages. Consequently during metamorphosis of insects both macro and microscopic changes are involved anatomical and histological. The histological differentiation of the thoracic muscles in *T. molitor*, so far remain unknown. The purpose of this study is to clarify the histological structures of thoracic muscles in *T. molitor* during the adult stage. It is intended to lay the foundation for the further study of the histological changes of the thoracic musculatures during metamorphosis.

1 This investigation was supported by a research grant from NCSD.

2 Associate Research Fellow, Institute of Zoology of Academia Sinica.

3 Research Assistant, Institute of Zoology of Aca demia sinica.

Little work has been done concerning the histological study of the musculatures of the beetle. Breed (1) studied the changes of the musculature of the beetle, *Thymalus marginticollis* Chevr. during metamorphosis. He gave a detailed description of the anatomical and the histological changes of the musculatures in the pupal stage of the beetle.

Kielich (5) worked on the structure of the insect muscles, and reported that the leg muscles of *Dytiscus* are the tubular muscles.

ACKNOWLEDGEMENT

The authors wish to express their thanks to Mr. Lin, Fei-Jann of Institute of Zoology, Academia Sinica for his great help in the photographs of this investigation.

MATERIALS AND METHODS

Specimens for this investigation were reared in the laboratory in the glass jars at room temperature. The larvae and the adults were fed on a medium (whole wheat flour 300 grams, yeast 15 grams, corn meal 30 grams). Some vegatables were added ocassionally to provide moisture.

The newly emerged adults and the about five days old adults were used. The specimens were fixed in Bouin-Duboscq solution (3) for 24 hours and washed in 70% alcohol for 12 to 24 hours to remove the excess picric acid (8). The muscles were cut into small pieces and were dehydrated *via* the series (7), embedded in tissue mat (m. p.) 60°C and cut at 6 to 10 u. The sections were stained with Harris-hematoxylin and 0.5% eosin in 95% alcohol. The striations of the muscle fibers and the nuclei were obviously identified by the blue color.

The preparations were examined with the 40X plan achromatic and 100X plan achromatic oil immersion lens of Olympus research microscope. The microphotographs were made with SV Asahi Pentax Camera. The drawings were made through the Cameralucida.

RESULTS

Histology:

There are three kinds of muscles, the general type of muscles (4), the tubular general type and the fibillar muscles (11) throughout the thoracic segments of *T. molitor* adults.

The general type of muscles has different kinds of shape and includes all muscles throughout the thoracic segments excepts the flight muscles of the metathorax. The leg muscles are composed of numerous small fibers arranged in a penniform or bipenniform manner and attached by a common tendon. The dorsal and the ventral longitudinal muscles of the prothorax and the mesothorax. The muscles are attached to the endoskeleton, such as the phragma or the furca. These muscles are well striated similar to the striated "skeletal muscles" found elsewhere in the animal kingdon. The *Figure 1* and the *Figure 2* faintly show that the fiber is enshealthed by a relatively thick membrance, the sarcolemma (L). The elongated ovoid shaped nuclei (N) are scattered in sarcoplasm (P) at the periphery of the fiber.

Some muscles in *T. molitor* adults the cross striation of the muscle is well marked, the nuclei arrange in a row along the axis of the fibres (*Fig. 3*) instead of scattering distributed. The cross section of the muscle showed in the *Figure 4*, the fibrils of the muscle do not arrange as the lamellar type and the nucleus lies in a matrix of sarcoplasm. However, a few nuclei are found around the fiber. These muscles are termed the tubular general type of muscles.

The fibrillar muscles, in contrast, are characterized primarily by the relatively enormous diameter of the fibrils when are compared with those of the general muscles. The *Figure 5* shows that several

hundred of fibrils are bundled to form one muscle fiber which appears polygonal in transverse examination. In the *Figure 6*, the longitudinal section shows that ovoid nuclei of the muscles irregularly arranged within the fibrils. The striations are not well remarkable as those of the general muscles, only the telophragma (Z) can be seen under the light microscope with oil immersion lens.

Most of the fibrillar muscles are attached to the sclerite disc which is a small chitinous plate (*Fig. 9* bad, sad and cd) projecting downwards from the anterior edge of the prescutum and the posterior edge of the postnotum repectively (*Fig. 8*). Some are attached to the phragmae (*Fig. 7* dlm_3 and *Fig. 8* ph_2, ph_3).

The tracheoles of the fibers are intercellular or invaginated into the fiber surface. The tracheoles lie on the surface of the sarcolemma or enter into all depths of the fiber concentric sheath of the fiber membrane. The tracheal system helps in binding together of the muscle fibers. The fibrillar muscles are rich in tracheal supply.

Myology:

The names of the muscles adopted for this study were from those which are termed by Lee (6). The anatomy of the thoracic musculatures of *T. molitor* adults are briefly studied:

(I) The prothorax:

The prothoracic muscles may be divided into five categories:

(1) Dorsal muscles: There are two kinds of muscles, consisting of dorsal longitudinal median muscle and external dorsal longitudinal muscle in the dorsal muscles. The dorsal longitudinal median muscle (*Fig. 7*, dlm_1) extends from the postoccipotal ridge to the first phragma (ph_1). This muscle is the muscle of tubular general type.

The external dorsal longitudinal muscle (*Fig. 7*, edm_1) extends from the anterio-median part of the mesal area of the pronotum to the first phragma (ph_1). This muscle is the tubular general muscle.

(2) Ventral muscles: The ventral longitudinal muscle (*Fig. 2*, vlm_1) stretches between the cervical membrane and the profurcal arm (f_1). This muscle is the general type.

(3) Tergopleural muscle: The tergopleural muscle (*Fig. 2*, $tplm_1$) extends from the lateral portion of the pronotum to the trochantinal plate (tp). This muscle belongs to the general type of muscles.

(4) Sternopleural muscles: The sternopleural muscles have four muscles:

The sterno-dorsal muscle (*Fig. 9*, sdm_1) arised from the anterior lateral margin of the prostermum and inserts on the postocciput.

The anterior tergo-ventral muscle (*Fig. 8*, Atv) extends from the anterior lateral part of the pronotum to the postocciput.

The posterior tergo-ventral muscle (*Fig. 8*, Ptv) is attached to the median portion of the pronotum and to the postocciput.

The ventral phragma muscle (*Fig. 7*, vphr) passes from the postocciput to the first phragma.

All these muscles are the general type of muscles.

(5) Leg muscles: The leg muscles of the prothorax consist of four different muscles:

The tergal promotor muscle (*Fig. 9*, tpm_1) arises from the pronotum and is attached to the trochantin by a small long tendon.

The tergal remotor muscle of the coxa (*Fig. 8*, trm_1), a large fan-shaped muscle, extends from the pronotum to the posterior coxal rim by a wide tendon.

The pleural abductor muscle of the coxa (*Fig. 9*, pab_1) extends from the trochanter to the anterior lateral rim of the coxa by a tendon.

The extra coxal depressor muscle of the trochanter (*Fig. 9, and 10*, $edtr_1$) consists of internal and external muscle bundles. Both of them are attached to the trochantinal plate and to the trochanter through a single large apodeme forming a shape of a large fan.

In these leg muscles, except the pleural abductor muscle of the coxa is the tubular general muscle, all the muscles are the general type of muscles.

(6) Intersegmental muscles: The intersegmental muscles are included three muscles as following:

The dorsal intersegmental muscle (*Fig. 2*, dim_1) is a fan-shaped muscle which extends from the pronotum to the posterior occipital ridge.

The lateral intersegmental muscle (*Fig. 7*, Lim_1) extends from the profurca (f_1) to the first phragma (ph_1).

The dorsal ventral intersegmental muscle (*Fig. 8*, dvi_1) is a large triangular shape of muscle which is attached to the pronotum and to the intersegmental membrane.

The lateral intersegmental muscle is the tubular general muscle, and the dorsal intersegmental muscle and the dorsoventral intersegmental muscle are the general type of muscles.

(*II*) *The mesothorax:*

In Coleoptera, the front wings have been modified into a protective elytra. The phasic flight muscles, therefore, are only found in the metathorax. The mesothoracic muscles are divided into five groups:

(1) Dorsal muscles: The dorsal muscles are involved two numbers of muscles as following:

The dorsal longitudinal median muscle (*Fig. 7*, dlm_2) extends from the first phragma (ph_1) to the second phragma (ph_2).

The lateral dorsal oblique muscle (*Fig. 7*, Ld_2) is a triangular shape attaching to the mesoscutum and to the mesopostscutellum by a small very long tendon.

(2) Ventral muscles: The ventral longitudinal muscle (*Fig. 7*, vlm_2) is attached to the profurcal arm (f_1) and to the mesofurcal arm (f_2).

(3) Tergopleural muscles: There are four muscles included in this group of muscle.

The levator muscle of the elytron (*Fig. 7*, Lc_2) extends from the prescutum to the soatulate sclerite of the mesoepisternum.

The pleural axillary muscles (*Fig. 8*, pam_2) consist of two narrow muscle bundles attaching to the third axillary sclerite and to the mesoepisternum or to the mesoepisternum or to the pleural ridge.

The episternal axillary muscle (*Fig. 8*, eam_2) stretched from the spatulate sclerite of the mesoepisternum to the third axillary sclerite.

The pleural subalar muscle (*Fig. 8*, pam_2) is a small triangular muscle attaching to the subalar sclerite and to the spiracle by a small tendon.

(4) Leg muscles: The leg muscles of the mesothorax consist of six muscles:

The tergal remotor muscle of the coxa (*Fig. 7*, trm_2) extends from the mesoprescutum and the mesoscutum to the coxal rim by a strong tendon.

The sternal remotor muscle of the coxa (*Fig. 8*, $strm_2$) is a fan-shaped muscle attaching to the mesofurcal arm (f_2) and to the coxal rim by a tendon.

The pleural abductor muscle of the coxa (*Fig. 10*, pab_2) is bipenniform lying on the mesoepisternum and ending at the coxal rim by a tendon.

The coxal basalar muscle (*Fig. 10*, cbm_2) extends from the basalar sclerite to the anterior coxal rim by a tendon.

The extra coxal depressor muscles of the trochanter (*Fig. 9*) consist of two muscle bundles, the episternal coxal depressor muscle of the trochanter ($edtr_2$) and

the sternal extra-coxal depressor muscle of the trochanter ($sdtr_2$). The former arises from the mesoepisternal flange, the later from the mesofurca. Both of them insert on the trochanter by a strong tendon.

All dorsal muscles, ventral muscles, tergopleural muscles and leg muscles of the mesothorax are the general muscles.

(5) Intersegmental muscle: The lateral intersegmental muscle (*Fig. 7*, Lim_2) extends from the second phragma (ph_2) to the mesofurcal arm (f_2). This muscle is the tubular general muscle.

(III) The metathorax:

The metathoracic muscles can be divided into five categories:

(1) Flight muscles: The flight muscle consist of four numbers of the indirect flight muscles (9), and three numbers of direct flight muscles. All muscles of this type are the fibrillar muscles.

(A) Indirect flight muscles:

The dorsal longitudinal median muscle (*Fig. 7*, dlm_3) extends from the second phragma (ph_2) to the third phragma (ph_3).

The anterior leteral muscle (*Fig. 8*, alm) is attached to the preepisternum at the base of the discriminal ridge and to the lateral muscle plate.

The median lateral lavator of the wing (*Fig. 8*, mlw) extends from the metascutum to the coxal muscle disc.

The lateral dorsal oblique muscle (*Fig. 8*, Ld_3) extends from the third phragma (ph_3) to the metascutum.

(B) Direct flight muscles:

The anterior extensor of the wing (*Fig. 9*, aew) originates on the preepisternum and inserts on the basalar disc.

The coxal basalar muscle (*Fig. 9*, cbm^3) is attached to the basalar disc and to the anterior rim of the coxa.

The posterior extensor of the wing (*Fig. 9*, pew) extends from the subalar disc to the muscle disc of the hind coxa.

(2) Axillary muscle: The axillary muscle is the flexor muscle of the wing (*Fig. 9*, fw). This muscle is attached to the metapleuron and consists of two parts: One inserts on the anaepisternum and other on the pleural ridge. These two muscles are the general type of muscles.

(3) Tergopleural muscles: This muscle has three number of muscles: The prealar muscle (*Fig. 9*, $pram_3$) is a small fan-shaped muscle extending from the prealar muscle disc to the pleural ridge.

The first tergopleural muscle (*Fig. 9*, $ltplm_3$) is attached to the lateral metascutum and to the dorsal surface of the basalar disc.

The second tergopleural muscle (*Fig. 9*, $2tplm_3$) extends from the metascutum to the anaepisternum.

These three muscles are the general muscles.

(4) Sternopleural muscle: The sternopleural muscle (*Fig. 7*, $stplm_3$) is a triangular muscle which extends from the mid-coxal socket to the metafurcal arm (f_3) by a small and long tendon. This muscle is the muscle of the general type.

(5) Leg muscles: The leg muscles of the metathorax consist of five muscles: The pleural abductor muscle of the coxa (*Fig. 9*, pab_3) arises on the anaepisternum and inserts on the ventral coxal rim by a slender tendon.

The sternal promotor muscle of the coxa (*Fig. 8*, $stpm_3$) is attached to the metafurcal arm (f_3) and to the ventral coxal rim by a very small tendon.

The sternal remotor muscle of the coxa (*Fig. 8*, $strm_3$) extends from the metafurcal arm (f_3) to the dorsal coxal rim.

The sternal extra coxal depressor muscle of the trochanter (*Fig. 8*, $sdtr_3$) is attached to the metafurcal arm (f_3) and to

the trochanter process.

The tergal remotor of the coxa (*Fig. 9*, trm_3) is triangular shape extending from the postnotal wing process and to the dorsal rim of the coxa.

All these muscles belong to the general type of muscles.

(IV) Discussion:

In the present study of the thoracic musculature in *Tenebrio molitor* adult, most of the muscle fibers are the general type of muscles in the prothorax, also the muscle fibers in the mesothorax. The muscle fibers were generally of small in diameter and show clearly the regions and all lines described for striated muscle. Only a few of the leg muscles, the intersegmental muscles and the dorsal longitudinal muscles in the prothorax and the intersegmental muscle of the mesothorax are the tubular general muscles which the nuclei of the muscle line up in the center of the fibers. However, the striations of the muscle fibers are still remarkable and the fibrils are not arranged as the lamellar. Imms (4) states that the general type of fibers is present in the leg and the abdominal muscles of the beetles and other insects; the muscle fiber has cross striations and the nuclei are scattered throughout the surface of the fiber or desposal beneath the sarcolemma. Breed (2) found that all the skeletal muscles of a beetle, *Thymalus marginicollis*, except the flight muscles and the abdominal muscles are the muscle of the second, or leg, type which has well striations and the nuclei are found at the surface of the fibers between the fibrils. This is similar to the general type of muscles in the thoracic segments of *T. molitor* adult. Kielich (6) studied the structure of the insect muscles and found that the leg muscles of *Dytiscus* are the tubular muscle which the nuclei are arranged in rows along the axis of the fiber. However, in *T. molitor* adult, all leg muscles are the general type of muscles except the pleural abductor muscle of the coxa in the prothorax. Such difference can not be ascribed to function, and yet there is no evidence to explain this difference.

The flight muscles in *T. molitor*, both the indirect flight muscles and the direct flight muscles are the fibrillar type of muscles. Daly (2) states that three histological types of the muscles are present in the Neuropteroidea: Tubular, close-packed and fibrillar; the first type is found throughout the body while the last two are restricted to the flight mechanism. He found that only two genera of Hymenoptera out of 54 genera which he observed, have the close-packed type in the flight muscles, the other are the fibrillar type. Williams and williams (12) reported that the flight muscles of the fruit fly, *Drosophila repleta*, their indirect muscles are composed of the fibrillar muscles and the direct flight muscles consist of the tubular muscles. Breed (1) found that the flight muscles including the indirect and the direct flight muscles are the fibrillar muscles in the beetle, *Thymalus marginicollis*. Kielich (5) states that the flight muscles are the fibrillar muscles in *Dytiscus* also. These are similar to the flight muscle in *T. molitor*, the type of their muscles is composed of the large and near cylintrical fibers with nuclei scattered throughout their substance; numerous tracheoles are intercellular of the fiber and bound the fibers of the muscles.

LITERATURE CITED

1. BREED, R.S. (1903). The Changes which occur in the muscles of a beetle, *Thymalus marginicollis* Cheve. during metamorphosis. Bull. Mus. Comp. Zool. at Harvard College. **60(7)**: 1-382.
2. DALY, H.V. (1963). Close-packed and fibillar muscles of the Hymenoptera. Ann. Ent. Soc. Amer. **56(3)**: 295-306.

3. GURR, E., (1956). A practical manual of medical and biological staining technique (2nd). Leonard Hill (Books) Limited.
4. IMMS, A.D. (1957). A general textbook of entomoloy. 9th ed. Rev. by O.W. Richards and R.G. Davies. Methuen and Co. Ltd. p. 63–65.
5. KIELICH, J. (1918). Beitrage zur kenntnis der Insektenmuskeln. Zool. Fb., Anat. U. Ontog., **40**: 515–536.
6. LEE, WEN-YUNG (1964). A study of the development of the thoracic musculature from the larva to the adult in *Tenebrio molitor* L. (Tenebrionidae, Coleoptera). Ph. D. thesis in the University of Minnesota, U.S.A.
7. LEES, B. (1950). The microtomists' Vademecum (11th ed.) J. B. Blakston Co., Phil. p. 95.
8. LILLIE, R.D. (1953). Histopathologic technic and practical Histochemistry. The Blakiston Division, McGraw-Hill Book Co., p. 45–47.
9. ROCKSTEIN, M. (1965). The physiology of insect. Vol. II. Academic Press. p. 303–309.
10. SMITH, D.S. (1964). The structure and development of flightless Coleoptera, A light and electron microscopic study of the wings, thoracic exoskeleton and rudimentary flight musculature. J. Morph. **114**(1): 107–191.
11. WIGGLOSWORTH, V.B. (1962). The principles of insect physiology. (6th ed.). Methuen p. 95–97.
12. WILLIAMS, C. M. and M. V. WIALLIAMS (1943). The flight muscles of *Drosophila replela*. J. Morph. **72**: 589–597.

LEGEND OF FIGURES

Fig. 1 Longitudinal section of the extra coxal depressor of the trochanter of prothorax—the general muscle. 1300X

Fig. 2. Cross section of the same muscle. 1300X

Fig. 3. Longitudinal section of the dorsal longitudinal muscle of the prothorax—the general tubular muscle. 1300X

Fig. 4. Cross section of the same muscle. 1300X

Fig. 5. Longitudinal section of the median lateral levator of the wing of the metathorax—the fibrillar muscle. 1300X

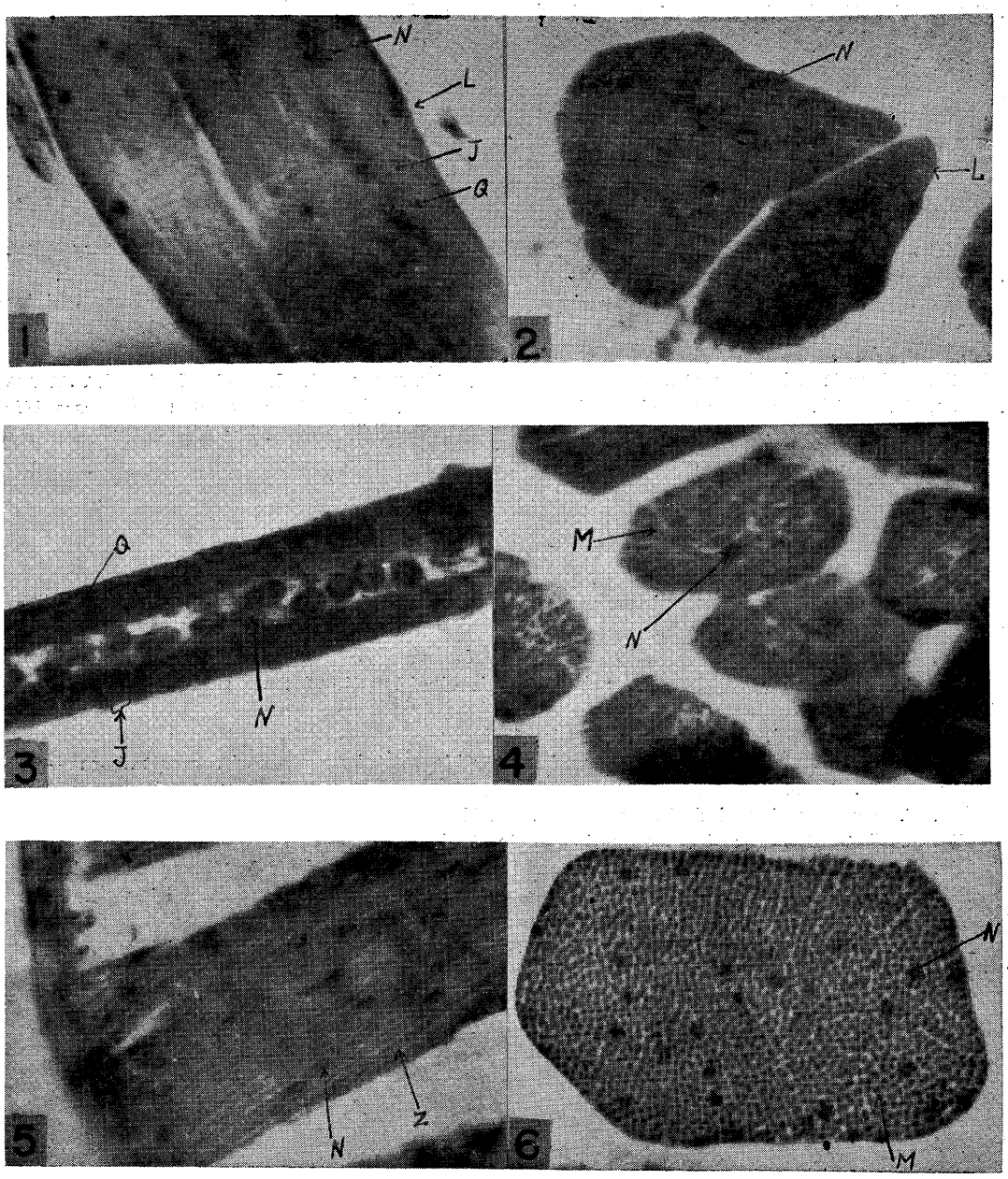
Fig. 6. Cross section of the same muscle. 1300X

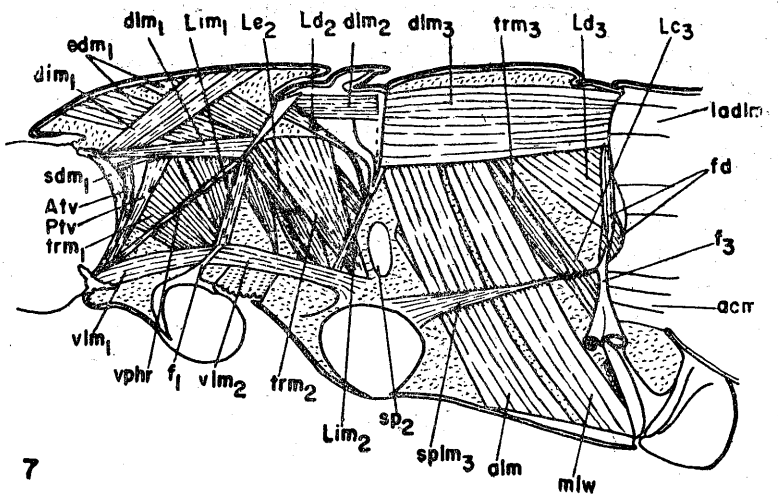
Fig. 7. Lateral aspect of the thoracic muscle. 10X

Fig. 8. Lateral aspect of the thoracic muscle, all the muscles of the first layer removed. 10X

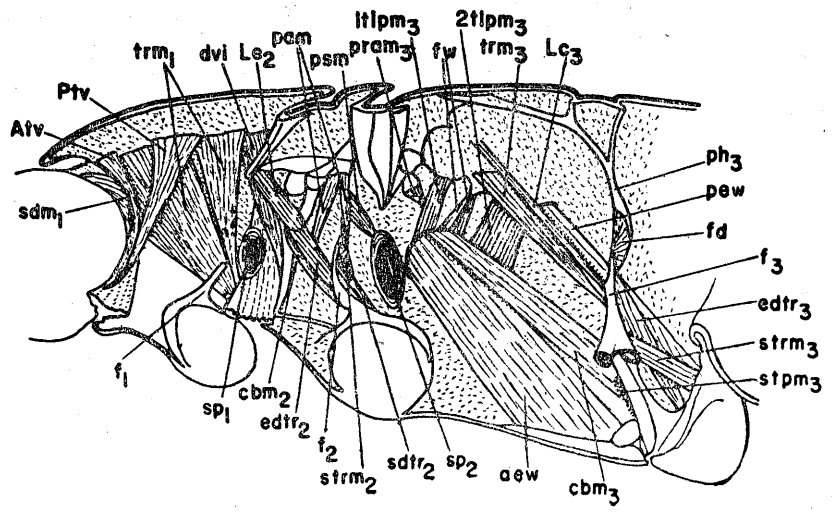
Fig. 9. Lateral aspect of the thoracic muscles, all the muscles of the second layer and the furcae removed. 10X

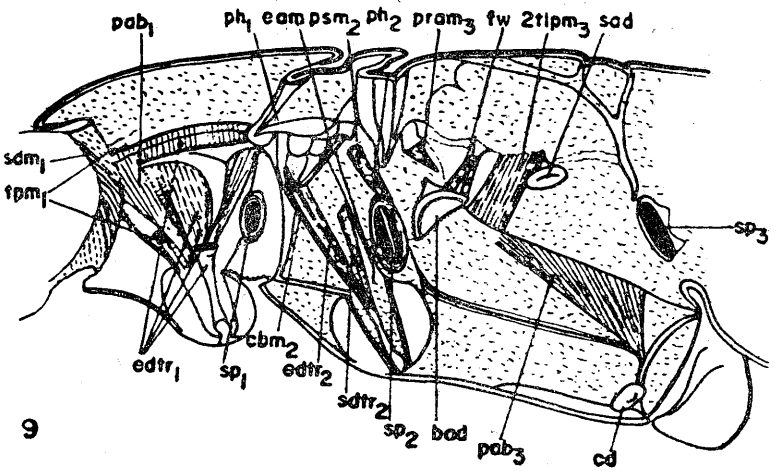
Fig. 10. Lateral aspect of the mesothoracic muscles, the muscles of the third layer and the furca removed. 10X


N
L
J
Q
1
N
L
2
Q
J
N
3
M
N
4
N
Z
5
N
M
6

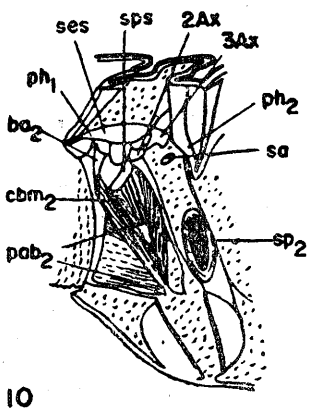

dim1
Lim1
Le2
Ld2
dim2
dim3
trm3
Ld3
Lc3
edm1
dim1
ladm
fd
sdm1
Atv
Ptv
trm1
f3
acr
vlm1
vphr
f1
vlm2
trm2
sp2
Lim2
spim3
alm
mlw

7


trm1
dvi
Le2
pcm
psm
itipm3
pram3
2tipm3
fw
trm3
Lc3
Ptv
Atv
sdm1
ph3
pew
fd
f3
edtr3
strm3
stpm3
f1
sp1
cbm2
edtr2
f2
strm2
sdtr2
sp2
aew
cbm3


pab1
ph1
eam
psm2
ph2
pram3
fw
2tipm3
sad
sdm1
fpm1
sp3
cbm2
edtr1
sp1
edtr2
sdtr2
sp2
bad
pab3
cd

9


ses
sps
2Ax
3Ax
ph1
ph2
ba2
sa
cbm2
sp2
pab2

10