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Seasonal Variations of Hard parts of *Dactylogyrus molnari*Ergens and Dulmaa, 1969 (Monogenea: Dactylogyridae) on the Gills of *Labeo rohita* (Rohu) in River Ganges Near Chandpur (U. P.), India

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Abstract:

There are several abiotic factors including temperature, oxygen, pH etc. that affect the phenotypic plasticity of parasites. During present study, the authors concentrated on seasonal variation in measurements of attachment apparatus and copulatory organ of Dactylogyrus molnariErgens and Dulmaa, 1969. Worms were collected from Labeo rohita at different water temperatures.Samples were taken at monthly intervals of about 30 (± 5) days during the period from January 2009 to March 2012. Main results indicate that the difference between winter and spring conditions cause significant changes in morphometrical variables of measurements of anchors, connective bar and copulatory organ.

Keywords*:* *Monogenea, Dactylogyrus molnari, attachment apparatus, copulatory apparatus, seasonality, morphometric variables.*

Introduction:

Dactylogyrus molnari has been abstracted from the fish *Labeo rohita* for the first time in India. This might have been transplanted to India with transplantation of *Cyprinuscarpio*. Earlier, this parasite has been reported from *Cyprinuscarpiohaematopterus* by Ergens and Dulma (1969) from Mangolia (China) and from Hungary by Molnar (1982).Highest prevalence of infection was recorded in period from January to March. There are several morphological and anatomical characteristics of monogenea that are used for species determination. Main morphological parameters are morphometric characteristics of attachment apparatus and copulatory organ. Species definition using only the shape and measurement of attachment apparatus and copulatory organ is difficult in similar species, because some measurements often overlap, while shape is variable. Because the mistaken description, of an already existing species as newly discovered occurs, it is important to know which factors affect morphometrical variation to avoid incorrect determination of species (Gusev 1985).

The aim of this study is to investigate the differences of measurements of attachment apparatus and copulatory organs between *D. molnari*individuals in different temperatures. It is expected to detect metrical variation in morphology of *D. molnari*considering seasonal changes in water temperature.

Materials and Methods:

Fishes for present investigation were collected from river Ganges at Chandpur.Total 12 specimens were examined. All specimens were used to study seasonal variations. Mizelle's (1936 and 1938) freezing technique was employed for collecting parasites. Parasites thus collected, were processed for morphometric studies.

Morphometric Analysis:

Worms were washed thoroughly several times with chilled distilled water, to remove any mucous or debris adhering to the parasites. These worms were fixed in hot 4% neutral formaldehyde for at least 8 hours. Worms were washed thoroughly with distilled water. For study of hard parts worms were mounted in glycerol. These worms were observed under microscopes and photographs of hard parts were taken.

Parameters that were measured for morphometric analysis and study of seasonal variation as suggested by Mo (1991) include 13 morphometric parameters of the attachment apparatus and two of the copulatory organ: total length of anchor - la; length of anchor shaft - las; length of anchor point - lap; length of anchor root - lar; length of transverse bar - lb; total basal width of transverse bar - tbwb;basal width of transverse bar - bwb;total median width of transverse bar - tmwb;median width of transverse bar - mwb;maximal distance between processes of transverse bar - mdpb;total length of marginal hooks - lmh;length of marginal hook handle - lh;length of marginal hook sickle - lsi;length of cirrus tube, and width of cirrus tube.

A total 50 specimens of *D. molnari*were measured and photographed with help of calibration tool of Motic DMB1 Microscope. It was not always possible to measure all 15 characters, because some prepared specimens were destroyed during the preparation or because with time had deteriorated. Some attachment apparatus had inconvenient position or were unsuccessfully compressed between the coverslip and the slide therefore, have not been recorded, all envisaged measurements. Unequal number of measurements and low number of specimens in autumn period was taken into account during statistical analysis. All data was processed using SPSS version 11 and a computer for calculating the mean and standard error. This data was also processed for probability coefficient and regression analysis. All results obtained were graphically presented. All the measurements are in microns (μ) in table 1 and 2. All temperature readings are in degree Celsius (ºC).

Results:

Site of infection in host	:Gills of*Labeorohita*(Ham)
No. of hosts examined	: 12
No. of hosts found infected	: 07
No. of total worms collected	: 50
Locality	: Chandpur (Meerut, UP) India

Worms were found parasitizing gills of *Labeo rohita*. Worms are elliptical in shape and measure 346.22 - 693.17 µ in length. Minimum and maximum width of parasite is 41.85 - 44.93 µ and 108.62 - 121.10 µ. Male copulatory complex consists of cirrus proper and an accessory piece. Cirrus is a double walled, 'Anchoratoid' type conical tube. Cirrus measures 19.09 – 37.85 µ in length. Diameter at base of cirrus is 5.91 – 10.65 µ. Cirrus measuring 16.25 – 30.52 µ in length (Table 1). Haptor is fairly set off from body proper and measures 39.70 – 101.00 µ in length and 16.17– 51.15 µ in width (Table 2). Body length to haptor ratio of parasites is approximately 4:1.

Table 1: Mean minimum and maximum length and width of cirrus of *D. molnari* (January 2009 – March 2012)

	Total length (µ)	**Width (µ)**
January (10.5°)	34.32, 35.90 (35.13)	10.15, 10.64 (10.37)
February (8.0°)	35.77, 37.85 (37.12)	9.83, 10.65 (10.16)
March (16.4°)	19.09, 28.01 (22.28)	5.91, 9.49 (7.13)

Table 2: Mean minimum and maximum length of haptoral hard parts of *D. molnari*(January 2009 – March 2012)

	Dorsal Anchor (µ)				**Dorsal Transverse Bar (µ)**		**Marginal Hooklets (µ)**	
	LA	**LAS**	**LAR-Out**	**LAR-Inr**	**LB**	**MWB**	**LMH**	**LH**
January (10.5°)	29.89, 35.24; (32.14)	24.76, 27.01; (26.17)	12.80; 19.49; (16.36)	12.80; 19.49; (16.36)	22.17, 28.00; (26.10)	4.08, 5.32; (4.77)	18.06, 20.12; (19.25)	14.33, 16.03; (15.51)
February (8.0°)	36.77, 37.75; (37.17)	36.98, 40.53; (48.46)	19.49, 23.25; (21.20)	14.50, 18.30; (15.94)	22.02, 25.00; (23.80)	4.11, 5.55; (4.76)	15.93, 21.16; (17.33)	13.72, 17.10; (15.03)

March (16.4°)	20.78, 26.65; (23.96)	16.80, 22.37; (18.77)	16.80, 22.37; (18.77)	13.40, 14.75; (14.06)	14.19, 25.42; (18.04)	4.12, 5.75; (4.92)	15.75, 17.84; (16.48)	12.75, 13.24; (12.99)

Table 3: Spss parameters and their outputs of *D. molnari*(January 2009 – March 2012)

Output a: Total length - Width of Cirrus (l - w)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	VAR00002[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: VAR00001

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.984[a]	.967	.935	2.05946

a. Predictors: (Constant), VAR00002

b. Dependent Variable: VAR00001

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	125.528	1	125.528	29.596	.116[a]
	Residual	4.241	1	4.241		
	Total	129.769	2			

a. Predictors: (Constant), VAR00002

b. Dependent Variable: VAR00001

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-8.778	7.500		-1.170	.450	-104.081	86.524
	VAR00002	4.370	.803	.984	5.440	.116	-5.836	14.576

a. Dependent Variable: VAR00001

Output b: Dorsal Anchor (la - las)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	VAR00002[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: VAR00001

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.961[a]	.924	.848	2.59874	.924	12.164	1	1	.178

a. Predictors: (Constant), VAR00002

b. Dependent Variable: VAR00001

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	82.152	1	82.152	12.164	.178[a]
	Residual	6.753	1	6.753		
	Total	88.906	2			

a. Predictors: (Constant), VAR00002

b. Dependent Variable: VAR00001

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	13.175	5.351		2.462	.246	-54.816	81.167
	VAR00002	.644	.185	.961	3.488	.178	-1.703	2.992

a. Dependent Variable: VAR00001

Output c: Dorsal Transverse Bar (lb-mwb)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	VAR00007[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: VAR00006

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.944[a]	.891	.782	1.93855	.891	8.174	1	1	.214

a. Predictors: (Constant), VAR00007

b. Dependent Variable: VAR00006

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	30.719	1	30.719	8.174	.214[a]
	Residual	3.758	1	3.758		
	Total	34.477	2			

a. Predictors: (Constant), VAR00007

b. Dependent Variable: VAR00006

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95% Confidence Interval for B Lower Bound	95% Confidence Interval for B Upper Bound
1	(Constant)	233.261	73.674		3.166	.195	-702.851	1169.373
	VAR00007	-43.726	15.294	-.944	-2.859	.214	-238.052	150.600

a. Dependent Variable: VAR00006

Output d: Marginal Hooklet (lmh - lh)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	VAR00009[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: VAR00008

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics R Square Change	F Change	df1	df2	Sig. F Change
1	.782[a]	.611	.222	1.12327	.611	1.572	1	1	.429

a. Predictors: (Constant), VAR00009

b. Dependent Variable: VAR00008

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.983	1	1.983	1.572	.429[a]
	Residual	1.262	1	1.262		
	Total	3.245	2			

a. Predictors: (Constant), VAR00009

b. Dependent Variable: VAR00008

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95% Confidence Interval for B Lower Bound	95% Confidence Interval for B Upper Bound
1	(Constant)	7.008	8.638		.811	.566	-102.742	116.759
	VAR00009	.744	.594	.782	1.254	.429	-6.798	8.287

a. Dependent Variable: VAR00008

Table 4: Significant Pearson correlation of *D. molnari*

Hard parts having bivariate correlation	***D. molnari***
Total Length of cirrus - width of cirrus	0.984
La - las of Dorsal anchor	0.961
Lar out – Larinr of Dorsal anchor	0.981
Lb - mwb of Dorsal Transverse Bar	-0.944

Regression Analysis:

Table 3; Output a - d obtained from regression analysis of *D. molnari* to find out their linear mathematical relationships with all hard parts of cirrus and haptor are as follows:

Cirrus

Length of cirrus = -8.778 + 4.370 × Width of cirrus

Dorsal anchor

Length of dorsal anchor = 13.175 + 0.644 × Length of shaft

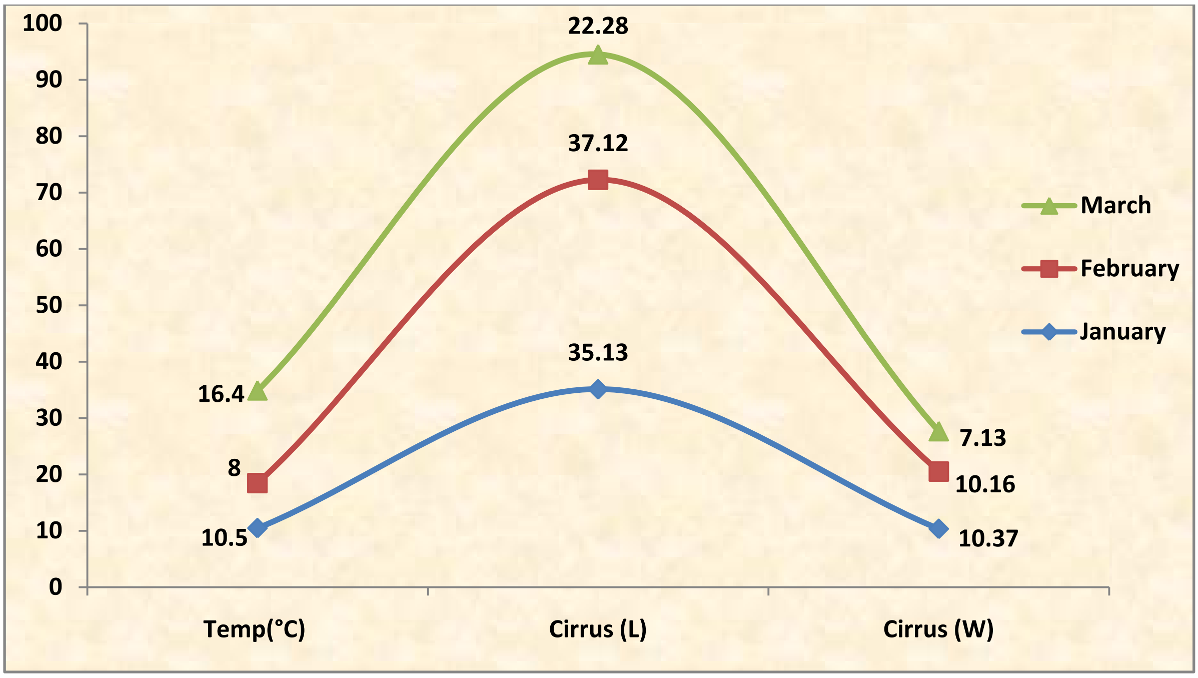
Dorsal transverse bar

Length of dorsal transverse bar = 233.261 - 43.726 × Median width of bar

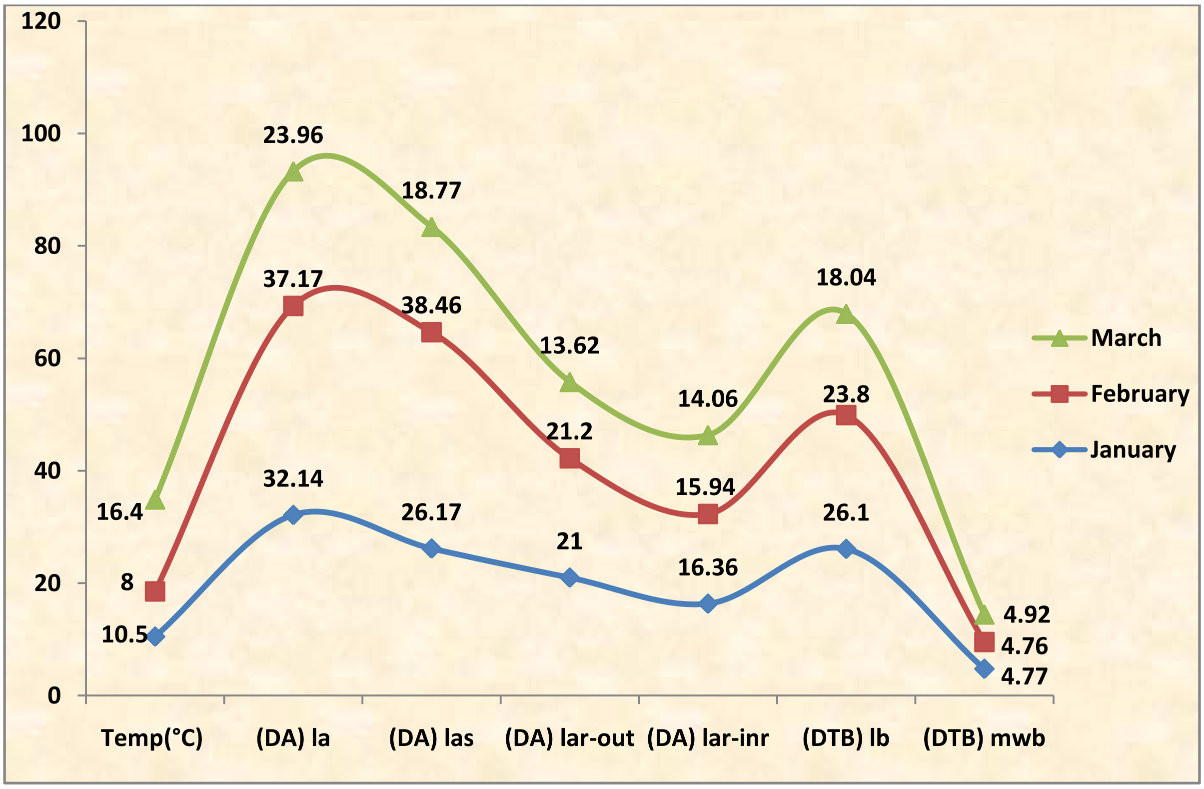
Marginal hooklet

Length of marginal hook = 16.671 -0.458 × Length of hook handle

Line Graph:



Graph 1: Mean variations in size of cirrus of *D. molnari*with temperature (°C) from January 2009 – March 2012.



Graph 2: Mean variations in size of dorsal anchor and dorsal transverse bar of *D. molnari*with temperature (°C) from January 2009 – March 2012.

Discussion:

All 15 characters (including cirrus) measured on each of the 07 samples were normally distributed. From 12characters 08 show positive correlations.Length and width of cirrus is inversely related to water temperature. When temperature falls from January to February, length of cirrus shows slight increase. While, as temperature doubles in March length of cirrus decreases significantly. In dorsal anchor, length of shaft and length of point is inversely related to temperature. Length of outer root and inner root is dependent on water temperature. Length of dorsal transverse bar is independent of water temperature. Median width of bar is temperature independent.

Pearson correlations were used to determine if hard armature of *D. molnari* is significantly correlated or not. Table 3 shows all spss outputs of cirrus and haptoral hard partsof *D. molnari*. In case of length - width of cirrus there is positive correlation in January and March, $r = 0.789$, $r = 0.891$ respectively. Obtained correlation value is significant at 0.05 and 0.01 level respectively. In February Length of cirrus with width shows negative correlation, $r = -0.384$. On the basis of these spss outputs the authors have drawn data Table 4 showing correlation between variables. Results of data obtained are significantly correlated to each other. In case of dorsal anchor the character length of anchor with length of shaft is positively correlated, $r = 0.969$ and significant at 0.01 level.Length of outer rootwith inner root is positively correlated to each other, $r = 0.956$ significant at 0.01 level. Dorsal transverse bar of *D. molnari* shows positive correlation in March. Its length shows high positive correlation with median width of bar, $r = 0.787$ significant at 0.05 level. Marginal hooklet show strong positive correlation. Length of marginal hook shows positive correlation with length of hook handle in all months, $r = 0.823$ significant at 0.05 level, $r = 0.967$ significant at 0.01 level, $r = 0.785$ significant at 0.05 level. In case of length of hook handle with length of sickle it shows negative correlation, $r = -0.738$ in January which means increase or decrease in the value of corresponding length of hook handle variable will either decrease or increase the value of variable length of sickle respectively.

Regression analysis of *D. molnari*helps to find out a linear mathematical relationship between two variables or characters of hard parts of cirrus. Length of cirrus and length of dorsal anchor, length of dorsal transverse bar and length of marginal hooks have been chosen as independent variable because they can be measured easily. Here total width of cirrus and length of anchor shaft, median width of dorsal transverse bar and length of marginal hook handle are dependent variables whose values have to be determined.

Line graphs of cirrus and haptor were obtained and represented in Graph 1 and 2of three months. Length and width of cirrus is inversely related to water temperature. When temperature falls from January to February by approximately 1.5°C, length of cirrus shows a slight increase. While, as temperature doubles in March length of cirrus decreases significantly. Most line graphs show a wide range of variations in size and shape of cirrus and haptoral hard parts of *D. molnari* with temperature (ºC). In dorsal anchor length of shaft inversely related to temperature. When temperature falls from January to February length of shaft shows a slight increase. While, as temperature doubles in March length of shaft decreases significantly. Length of outer root and inner root is dependent on water temperature. When temperature decreases from January to February outer root of anchor shows a slight increase. While, as temperature rises in March length of outer root decreases significantly. Length of dorsal transverse bar is independent on water temperature because as temperature decreases in February, length of transverse bar decreases. As temperature doubles in March the length of bar decreases. When temperature decreases in February median width of bar shows slight decreases while as temperature increases in March median width of bar increases slightly.

Conclusion:

From this study it is concluded that anchors show a slim and long appearance in colder months of January and February. Whereas, in warm spring month, anchors appear short, stout and curved. Dorsal transverse bar also appears long and slender in colder months and short and stout in warm month. Study also conclude that continuous analysis of haptoral hard parts in different seasons provide great details of those variations which are shown by opisthaptoral hard parts of monogenean parasites. Parasites belonging to different genera also differ in their hard parts. Present study also describes that size of hard parts (cirrus and haptor) of a parasite depend upon seasonal temperature. These seasonal variations increase our knowledge and are very important for improving species descriptions. It helps in resolving some disparity in taxonomy of monogeneans.This study concluded that correlations between hard parts of haptor cannot be used as characteristic feature to differentiate between two genera. In addition regression analysis is carried out to find out a mathematical linear relationship between two variables (hard parts). One variable is independent variable i.e. its value is known. Other variable is dependent i.e. whose value is to be determined. Obtained equations help to find out unknown values of different hard parts of haptor with known value. Results from present statistical study of correlation and regression analysis indicate towards common purpose of degree and direction of relationship between different hard parts of cirrus and haptor. And it also indicates to differentiate among monogeneans genera.

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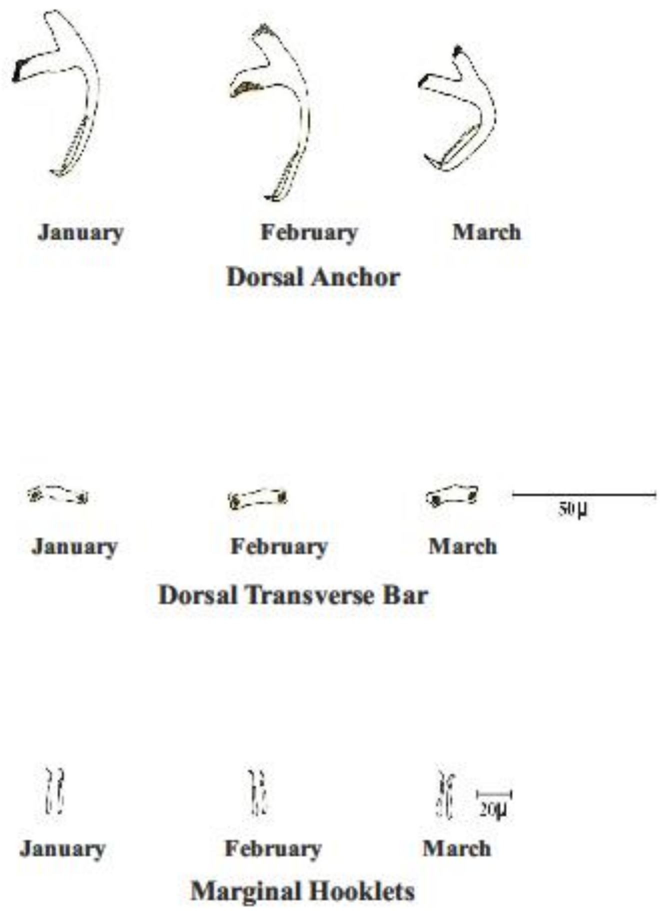

Haptoral Hard Parts of
***Dactylogyrus molnari* Ergens and Dulmaa, 1969**