The Mindanao Forum, Vol XIII, No. 2 (December 1998)

Variability in Wild and Farmed Golden Rabbitfish *Siganus guttatus* Bloch (Perciformes: Siganidae) in Nothern Mindanao

JESSIE G. GOROSPE
CESAR G. DEMAYO

Abstract

Variations in wild and farmed golden rabbitfish were investigated based on qualitative and quantitative descriptions of internal body parts and morphomeristic characters. Thirty OTU's (operational taxonomic units) equally representing the different populations were collected and measured although only twenty (20) characters were indentified into quantitative morpological, meristic and qualitative morphological characters. Results of cluster analysis of the various characters show high variability was observed in wild than in farmed populations of the fish.

Introduction

The golden rabbitfish, *Siganus guttatus* (Bloch), locally known as "kitong" is a highly esteemed food fish in the Philippines and in the Indo-Pacific region. Siganids are an export item of the Philippines and they are considered an expensive fish in the local market with price ranging from P80-120;kg fresh fish and more valued when sold live. The demand of siganids as food fish is ever increasing with the growing population and the degradation of natural habitats such as coral reefs and seagrass beds. Aside from economic and cultural considerations, siganids are ecological efficient as they occupy a low trophic level specifically *S. guttatus* which is fast growing. The systematic study of *S. guttatus* natural populations in two areas of Iligan Bay and the farmed populations of Naawan will help expand the understanding of *S. guttatus* biology and may help improve aquacultrue of the golden rabbitfish.

Graduate student and Professor, Department of Biological Sciences, MSU-Iligan Institute of Technology, Iligan City

The Golden Rabbitfish or Golden Spinefoot is taxonomically classified to belong to:

Phylum : Chordata
Subphylum : Vertebrata
Grade: Pisces
Class: Osteichthyes
Subclass: Acteropterygii
Infraclass: Neopterigii
Division: Helecostomi
Subdivision: Teleostomi
Infradivision: Euteleostei
Superorder: Acanthopterigii
Order: Perciformes
Suborder: Acanthoroidea
Family: Siganidae
Genus: *Siganus*
Species: *guttatus*

The family Siganidae are represented by approximately 26 species, 15 of which are schooling and the rest inhabit coral reefs. In the Philippines, 17 species are known to occur. Rabbitfishes are widely distributed in the Indo-Pacific region ranging from the Indian Ocean coast of Africa to Polynesia and from southern Japan to northern Australia. Rabbitfish or Siganids are generally littoral to sublittoral fishes and inhabit coral reefs, seaweed and seagrass beds, mangrove swamps, estuaries, river mouths and lagoons.

Rabbitfishes are primarily herbivorous but are facultative feeders making them good candidates for aquafarming. Spawning of *S. guttatus* in the Philippines is year round with peak season during January to April and July to September. Spawning of Golden Rabbitfish exhibits lunar periodicity and coincides with the onset or 2-3 days after the First Quarter of the lunar phase.

Sexes in Rabbitfish are separate but they do not exhibit secondary sexual dimorphism such as coloration and external structures. Generally, males are slightly smaller than females but they can only be distinguished during the spawing stage of the fish when gonadal products ooze out from the urogenital pore with slight pressure on the abdomen.

The numerical phenetics study of *S. guttatus* in two wild populations that is ... opez-Jaena, Misamis Occidental and Kauswagan-Bacolod, Lanao del Norte and the farmed population in MSU-Naawan, Misamis Oriental was the subject of this study. The farmed population of *S. guttatus* at MSU-Naawan were already at the F5 and F6 generation which originally came from the wild population in Lopez-Jaena, Misamis Occidental in 1990.

Objectives

The primary objective of this study was to conduct a numerical phenetics study of the *S. guttatus* populations in two geographical areas in Northern Mindanao and the farmed population of S. guttatus at MSU-Naawan. Specifically, the study aims;

1. To determine quantitative and qualitative morphological and meristic characters of *S. guttatus*; and
2. To determine variability among, between and within *S. guttatus* populations.

Methodology

I Study Area

Wild sourced *S. guttatus* were collected in two study sites namely; Lopez-Jaena, Misamis Occidental and Kauswagan-Bacolod, Lanao del Norte. The farmed S. guttatus shall be collected from MSU-Naawan, Misamis Oriental.

II Collection, Identification and Preservation of Samples

S. guttatus were collected from fishermen in two collection sites and from MSU-IFRD Siganid Hatchery. Collected samples were placed in specimen jars and were preserved in 10% formalin solution. Identification were done in the laboratory following descriptions in FISHBASE97 computer software system.

III Numerical Phenetics Method

Numerical phenetics has developed an array of techniques for quantifying and displaying patterns of similarity among organisms (Sneath and Sokal 1973 as cited by Lundberg and McDade, 1990). However, it has been shown that different techniques applied to the same data may produce different results and this makes selection of methods difficult and arbitrary. Further, there is no general agreement on which phenetics methods are best for producing classifications. The method for numerical phenetics study of *S. guttatus* was done on the following methods:

a. Assembly of character state information of operational taxonomic units (OTUs). Quantitative characters (measurements and counts) were used directly. States of "qualitative" characters were transformed to numerical codes. Color intensity, however, was not considered as intensity was affected by factors related to physiological responses. Characters that can be present or absent or that have two alternative states, are coded 0 to 1. ordinated (graded) state series were coded additively to reflect relative similarity among character states.
b. Data sets containing characters measured on equal scales (mm in this study) were recomputed as ratio of standard length.
c. A measure of overall similarity or distance is computed by comparison of each OUT pair. These pairwise resemblance measures are entered in a matri of similarities or distances. The distance of coefficient sum differences in character states between two OTUs over all characters to provide a measure of distance. Frequently used coefficients is the Euclidean distance computed by an n-dimensional extension of the Pythagorean theorem (Sneath and Sokal, 1973) but in this study, chord distance was more often utilized as coefficient.
d. Finally, the original resemblances are summarized and displayed by cluster analysis

which create groups or clusters of OTUs by one of several criteria based on their mutual resemblances. The most commonly used approach is the unweighted pair group method with arithmetic averaging (UPGMA). In UPGMA, the most similar pairs of OTUs are linked at their observed level of resemblance to form the lowest level clusters. Unplaced OTUs are added sequentially to the "closest" clusters. The resemblance of an unplaced OUT to a cluster is the average of its pairwise resemblances to each cluster member. The results of cluster analyses are displayed in phenograms.

IV. Ecological characters

Some ecological characters such as sex-ratio, food habits, and habitat description in all collection sites were investigated.

V. Sample size and analysis

Ten (10) individuals per collection site were collected for taxonomic analysis considering the bulk of the work and time constraint. Multivariate statistics was applied to simultaneously analyze differences among many characters. ECOSTAT software run in QBASIC program was used in data processing and cluster analysis. Strategy used is the unweighted pair group method average (UPGMA) using Euclidean distance and chord distance as indices.

VI. Photography and Illustration

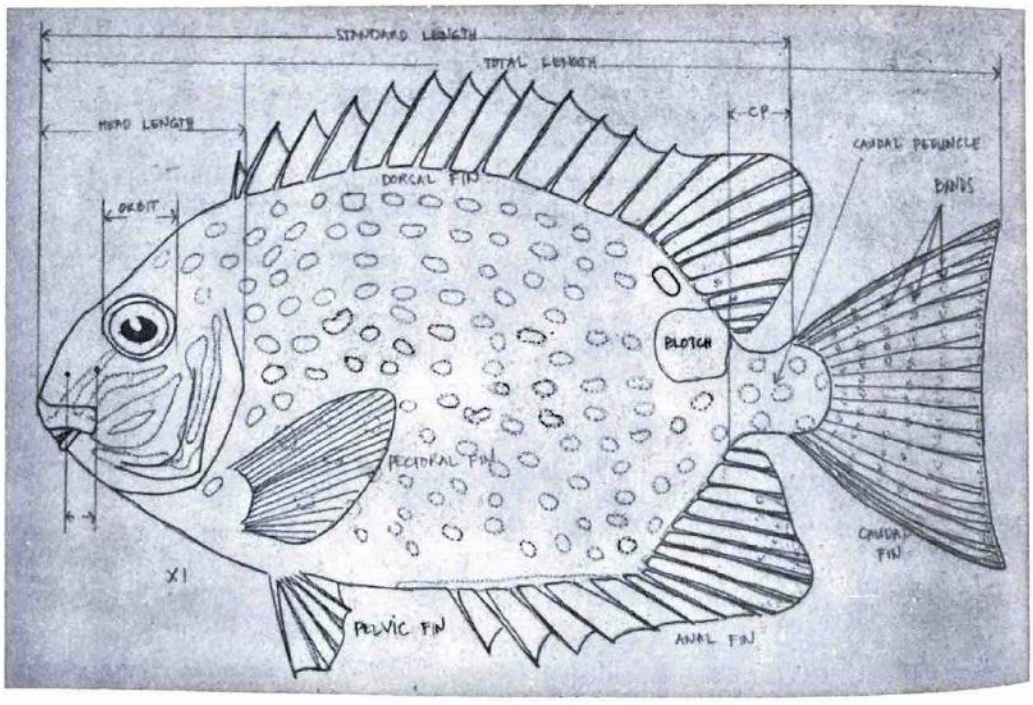


Figure 1. Representative Drawing of *S. guttarus*

Representative samples of *S. guttatus* from various collection sites were photographed. A representative sample was scientifically illustrated in a tracing paper (Fig. 1) showing the internal parts and morpho-meristic characters.

Results and Discussion

Thirty OTUS (Operational Taxonomic Units) equally representing Naawan, Lopez Jaena and Kauswagan-Bacolod (Fig. 2, 3 and 4) were collected and measured. About 24 characters were initially noted, however, three were found to be age and size dependent characters such as body weight, total length and standard length. Sex was considered a parameter but not included for morphological measurements such that 20 characters were identified for data processing purposes and were categorized into quantitative morphological characters, meristic characters and qualitative morphological characters.

Meristic characters are morphological characters that can be counted. In this study, meristic characters considered include number of Dorsal fin spines, dorsal fin rays, anal fin spine, anal fin rays, caudal fin bands caudal peduncle spots and number of pyloric caeca.

Quantitative morphological characters are characters that can be measured and in this study, it was expressed as a ratio of standard length (Cailliet et.al.,1986). Qualitative morphological characters are gradient characters or presence – absence data that are transformed into numerical codes. In this study, shape of the caudal blotch and position of gall bladder relative to the body cavity were considered.

Data were then arranged into a data matrix from for Q-mode resemblance function (Table 1) which will be used to measure similarity between OTUs in terms of their character composition. Euclidean distance coefficients such as Absolute distance also known as character difference in numerical taxonomy and mean Absolute Distance equivalent to mean character difference in numerical taxonomy (Sneath and Sokal, 1973) were initially used as an index of distance measure. However, analysis of output result show discrepancies were noted in cluster levels and cycles. Findings were concurred by evaluation of Ludwig and Reynolds (1988) which indicated that spurious results can occur especially when zero data are present. Ludwig and Reynolds (1988) however, recommended Chord distance as an index instead,. Chord Distance measure puts greater importance on the relative proportions of characters in OTUs and less importance on their absolute quantities. Technically, this is done by projecting the OTUs onto a circle of unit radius through the use of direction cosines (Ludwig and Reynolds, 1988) Chord distance is also identical to Ochial's coefficient when it involves the use of presence or absence data. Chord distance values ranged from 0 to $\sqrt{2}$.

Cluster analysis were performed separately on male and female siganids. The female siganid cluster analysis based on 19 OTUs and 20 characters indicated 4 major clusters based on 0.19-0.22 cluster level which were apparently influenced by posterior blotch shape and relative positions of gall bladder (Fig. 5). The four major clusters of female *S. guttatus* are presented in Table 2.

Result of the male golden rabbitfish cluster analysis based on 20 characters to 11 OTUs using group average strategy and chord distance as index showed two clusters at 0.29 cluster level composed of anteriorly and middle placed gall bladder groups (Fig. 6). All clusters were of mixed composition and represented by different sources. However, there

was an apparent clustering of OTUs (80%) from Naawan in the anteriority placed gall bladder cluster (Fig. 7, 8, 9 and 10). This character may be influenced by the effect of inbreeding, founders effect, genetic drift and mutations that result to homogeneity or a ratio approaching homozygosity. The phenotypic expression of Naawan siganid population is a result of selective breeding (non-random mating) over many generations inherent to farmed populations that resulted to homogeneity.

Table 1. Morphological characters measured in *S. guttatus*

	SAMPLE NUMBER														
	LJ16	LJ17	LJ18	LJ19	LJ20	KB21	KB22	KB23	KB 24	KB25	KB 26	KB27	KB28	KB29	KB30
A. Quantitative Morphological Characters															
Total Length (TL)mm	217	193	180	190	191	310	277	250	247	222	243	217	233	265	220
Standard length (SL)mm	175	157	142	157	147	249	215	203	205	179	192	163	197	230	175
Body weight(g)	200	165	140	160	165	600	320	315	260	260	280	220	320	450	250
Body width/SL	0.48	0.458	0.683	0.49	0.497	0.493	0.502	0.478	0.473	0.574	0.479	0.509	0.417	0.475	0.506
Orbit/SL	0.074	0.076	0.092	0.095	0.095	0.08	0.074	0.078	0.083	0.106	0.088	0.092	0.075	0.076	0.095
Anus-Anal fin distance/SL	0.131	0.115	0.113	0.121	0.136	0.108	0.116	0.123	0.132	0.095	0.125	0.135	0.107	0.108	0.09
Head length/SL	0.251	0.261	0.261	0.267	0.279	0.257	0.246	0.236	0.258	0.268	0.245	0.276	0.273	0.25	0.25
Gut length/SL	4.08	4.14	4.78	4.24	4.01	4.1	3.3	3.47	3.16	4.06	3.75	3.62	4.81	3.95	4.01
Nares distance	0.028	0.025	.039	0.025	0.023	0.024	0.023	0.029	0.034	0.031	0.026	0.03	0.024	0.029	0.03
Gall bladder to liver distance /SL\	0.0257	0.292	0.211	0.299	0.224	0.229	0.056	0.084	0.058	0.486	0.255	0.337	0.348	0.205	0.337
Stomach length/SL	0.314	0.343	0.486	0.363	0.374	0.385	0.288	0.33	0.327	0.24	0.245	0.325	0.257	0.325	0.245
Caudal peduncle Length/SL	0.068	0.063	0.056	0.038	0.068	0.04	0.046	0.039	0.043	0.067	0.052	0.067	0.064	0.04	0.065
Caudal peduncle width/SL	0.057	0.063	0.063	0.063	0.068	0.068	0.074	0.069	0.078	0.072	0.073	0.049	0.064	0.048	0.065
Pyloric caeca length/SL	0.268	0.255	0.26	0.274	0.238	0.249	0.172	0.197	0.181	0.179	0.197	0.233	0.241	0.245	0.175
B. Meristic Characters															
Dorsal fin Spine	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
Dorsal fin ray	10	10	10	10	10	10	10	10	10	10	10	10	11	10	10
Anal fin spine	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Anal fin ray	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Caudal fin bands	4	3	3	4	4	4	3	3	4	3	5	3	2	3	3
Caudal peduncle spots	2	5	4	3	5	7	5	7	7	6	7	6	4	7	6
Pyloric Caeca number	3	5	4	5	4	4	4	2	3	4	5	4	5	4	5
C. Qualitative Morphological Characters															
Shape of posterior blotch	3	3	3	1	1	1	1	1	1	1	1	1	3	1	1
Position of gall bladder relative to cavity	3	3	3	3	3	3	1	1	1	5	3	3	5	3	3

Table 2. Major clusters of female wild and farmed *S. guttatus*

CLUSTER	DISTINCT CHARACTER	COMPOSITION	OTUs
1	Irregular blotch shape Anterior to middle placed gall bladder	Farmed (Naawan)	1,2,4
2	Regular blotch shape Anterior and middle placed gall bladder	Mixed	3,12,18,7,19, 16,11,5,13,14
3	Irregular blotch shape Middle placed gall bladder	Wild (Lopez Jaena)	6,9,10
4	Posterior	Lopez Jaena Kauswagan Bacolod	8,17,15

* OTUs 1-5 Naawan
6-11 Lopez Jaena
12-19 Kauswagan-Bacolod

Table 3. Major clusters of male wild and farmed *S. guttatus.*

CLUSTER	DISTINCT CHARACTER	COMPOSITION	OTUs*
1	Anteriority placed gall bladder	Mixed	1,3,4,5,6,7,10
2	Middle placed gall bladder	Mixed	2,9,11,8

* OTUs 1-5 Naawan
6-9 Lopez Jaena
10-11 Kauswagan-Bacolod

The male and female OTUs were combined and cluster analyis was performed on 30 OTUs. Results show for quantitative morphological characters cluster analysis indicated that there was no pronounced separation of sexes which may imply that no secondary sexual dimorphism do exist based on 20 characters and there was no trend between farmed and wild populations. The distribution of three populations were interspersed among clusters and no pronounced separation of characters of the three populations were observed. All OTUs formed one group (Fig. 11).

Cluster analysis performed based on seven meristic characters indicated that the grouping arrangement was complex and that some clusters were influenced by number of pyloric caeca and caudal peduncle spots (Fig. 12).

Cluster analysis for qualitative morphological characters indicated five distinct groupings at 0.0 cluster level (Fig. 13, Table 3).

Table 4. Clustering arrangement of wild and farmed *S. guttatus* based on qualitative morphological characters.

Cluster	Distinct Character	Composition	OTUs*
1	Irregular blotch shape Anteriority placed gall bladde	Farmed (Naawan)	1,3,4,8
2	Irregular blotch shape Middle placed gall bladder	Lopez Jaena Naawan	2,6,11,16,17,18
3	Regular blotch shape Middle placed gall bladder	Mixed	5,10,12,19,20,21, 26,27,29,30
4	Regular blotch shape Anteriority placed gall bladder	Mixed	7,9,14,15,22,23,24
5	Posteriority placed gall bladder	Wild	13,28,25

* OTUs 1-10 Naawan (farmed)
11-20 Lopez jaena (Wild)
21-30 Kauswagan-Bacolod (Wild)

Cluster analysis for the combined morphological and meristic characters (20) in all 30 OTUs indicated multiple clusters at various cluster levels (Fig. 14). There are two cluster if 0.25 cluster level is considered. However, if cluster level range 0.16 – 0.22 is considered, there are five clusters (Table 5) with distinct identities mainly influenced by qualitative morphological characteristics such as positioning of the gall bladder and shape of the posterior blotch.

Table 5. *Clustering arrangement of wild and farmed S. guttatus based on 20 characters.*

Cluster	Distinct Character	Composition	OTUs
1	Irregular blotch shape Anterior to middle placed gall bladder	Farmed (Naawan)	1,3 4 8, 2 6
2	Regular blotch shape Middle placed gall bladder	Mixed	5,21,29,12,30, 27,20 26,10,19
3	Regular blotch shape Anteriority placed gall bladder	Mixed	7,9,24,23,15,14,22
4	Irregular blotch shape Middle gall bladder	Lopez Jaena only	11,17,18,16
5	Posteriority placed gall bladder	Wild	13, 28 25

* OTUs 1-10 Naawan (Farmed)
11-20 Lopez Jaena (Wild)
21-30 Kauswagan-Bacolod (Wild)

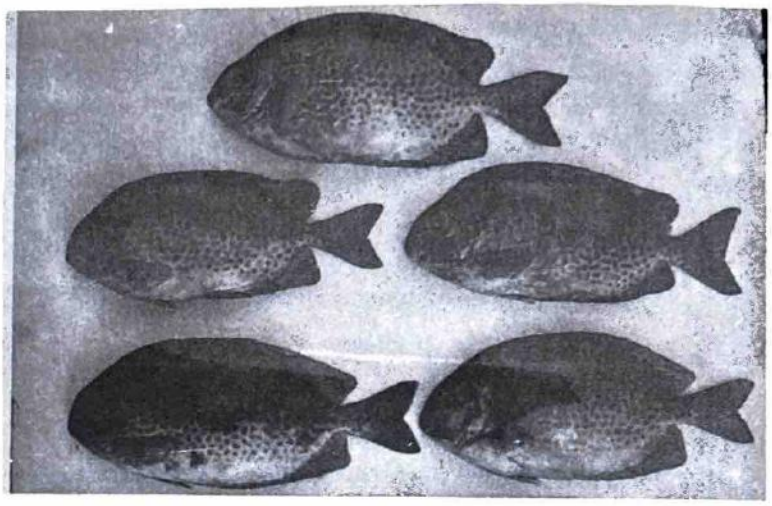


Figure 2. Farmed *S. guttatus* samples from MSU-Naawan Fish Hatchery Naawan, Misamis Oriental

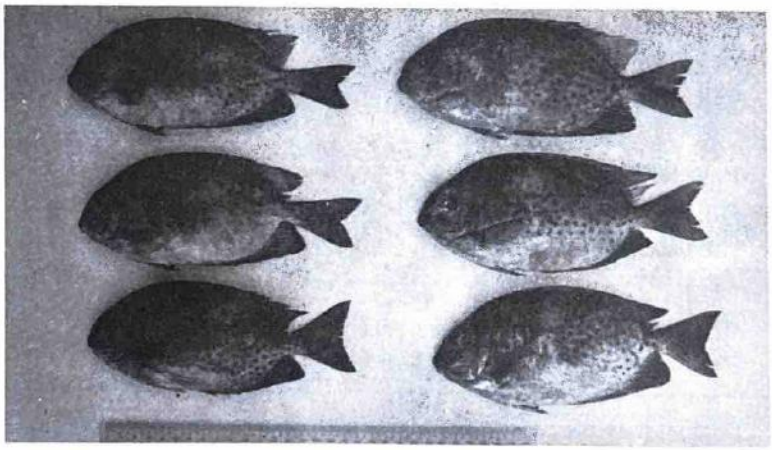


Figure 3. Wild sourced *S. guttatus* samples from Lopez Jaena, Misamis Occidental

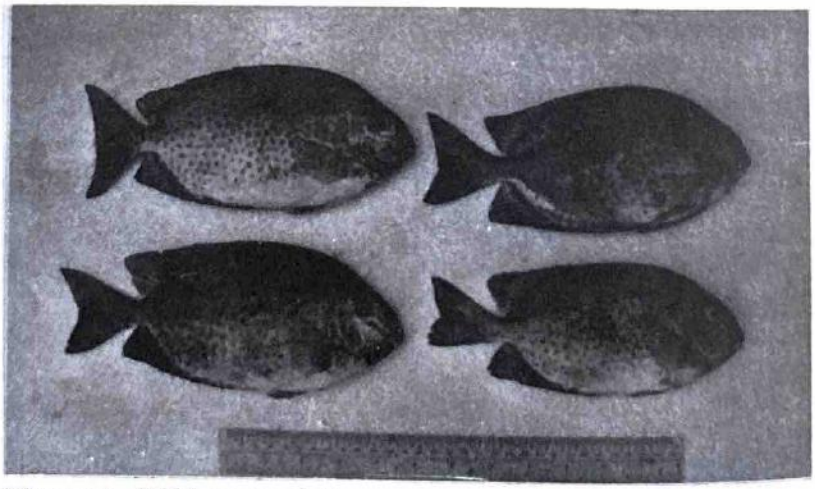


Figure 4. Wild sourced *S. guttatus* samples from Kauswagan-Bacolod area Lanao del Norte

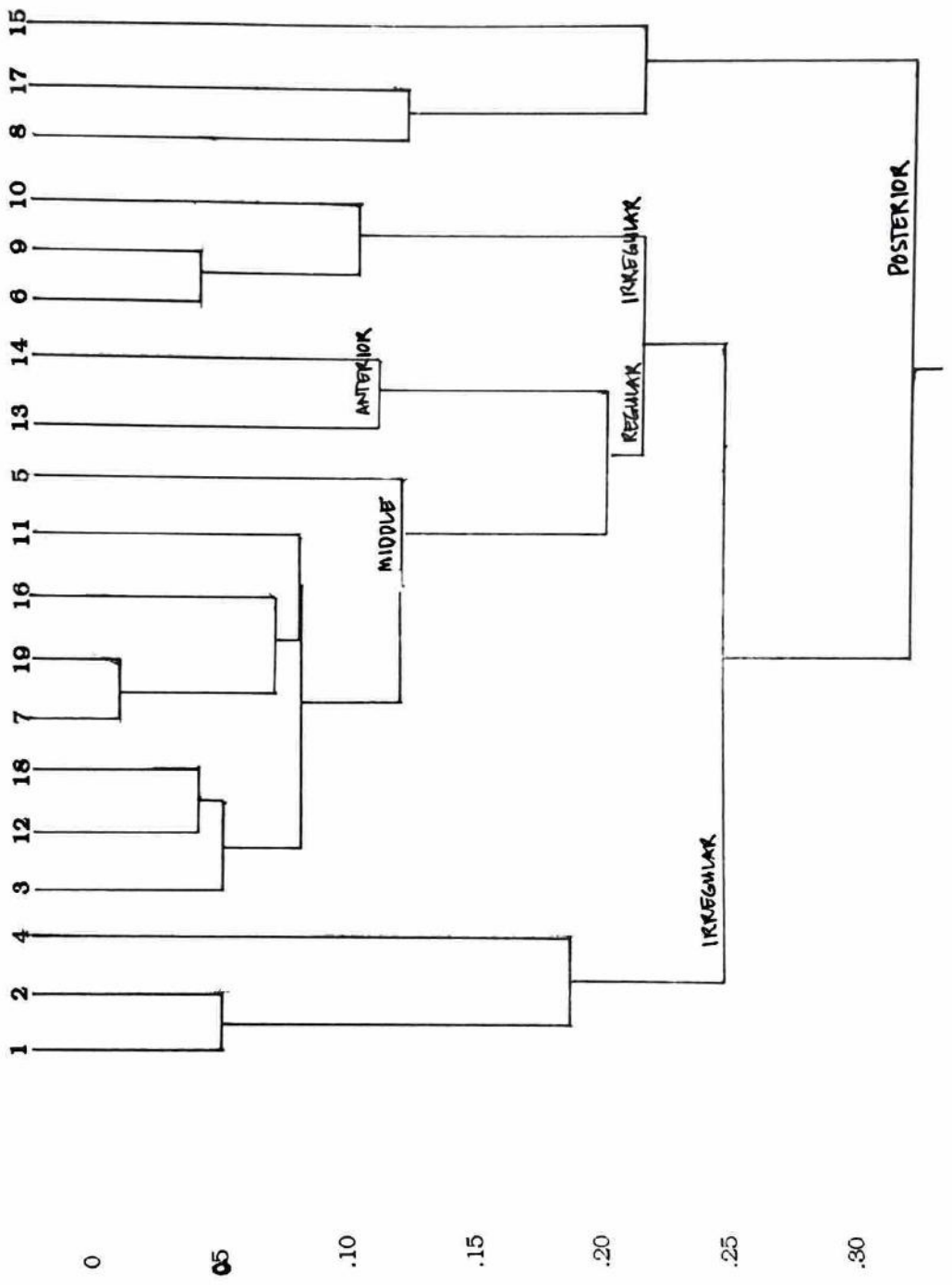


Figure 5. Phenogram for morphological characters of female *S. guttatus* Clustering is by the Group Average Strategy (Unweighted Pair Group Method) Index is Chord Distance

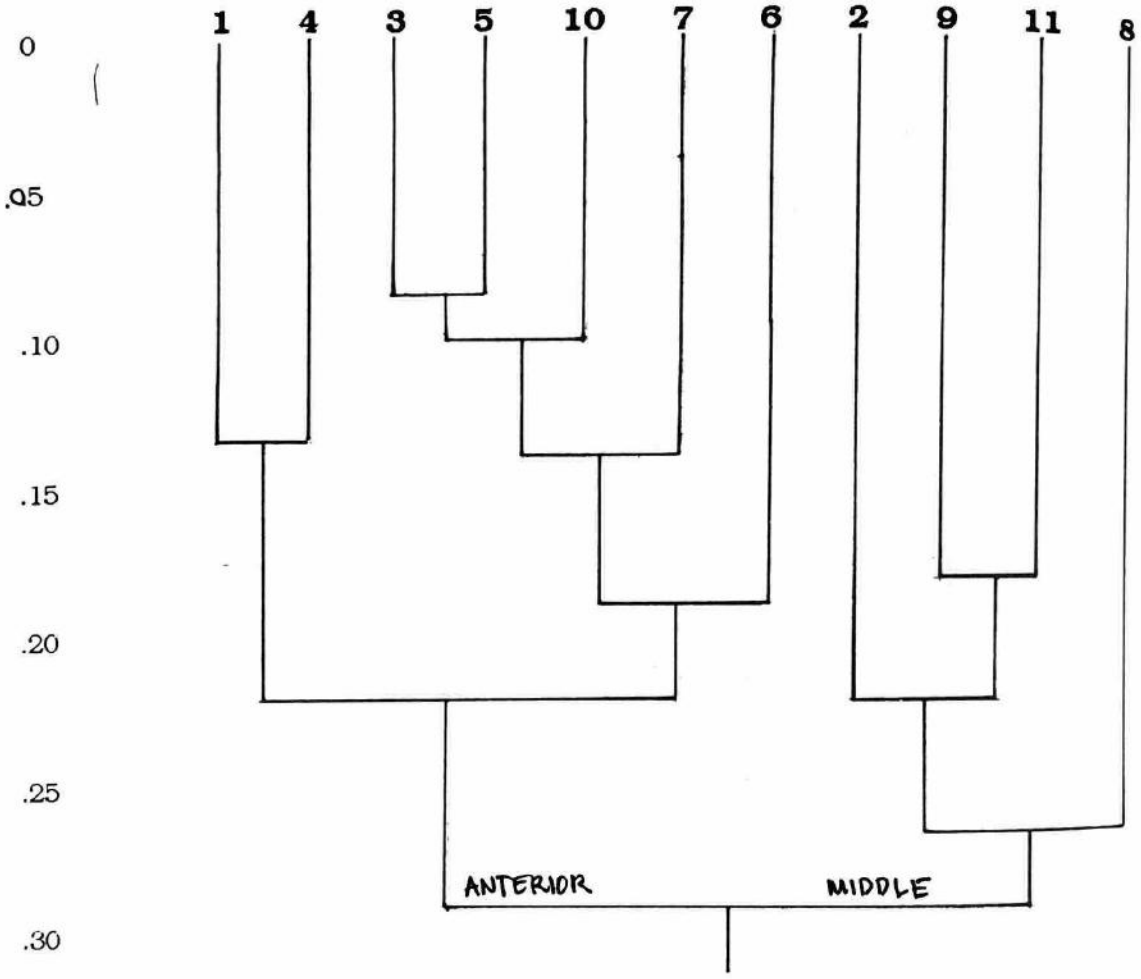


Figure 6. Phenogram for morphological characters of male *S. guttatus.* Clustering is by the Group Average Strategy (Unweighted Pair Group Method) Index is Chord Distance

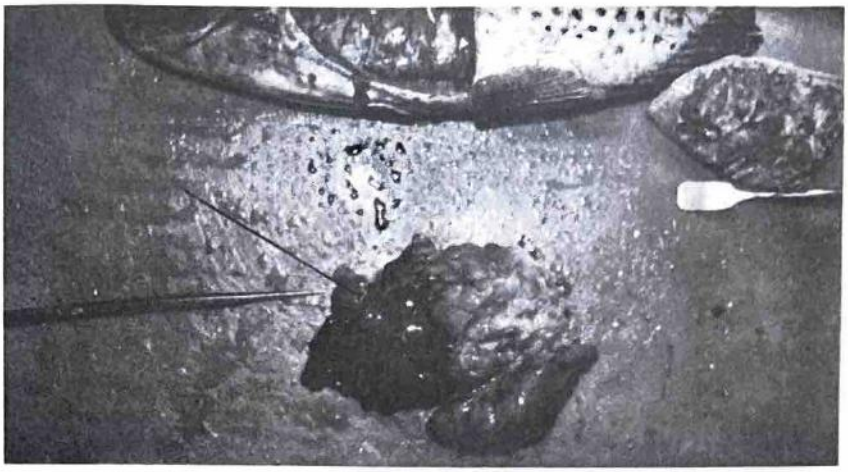


Figure 7. Female *S. guttatus* showing Anterior position of gall bladder relative to the body cavity.

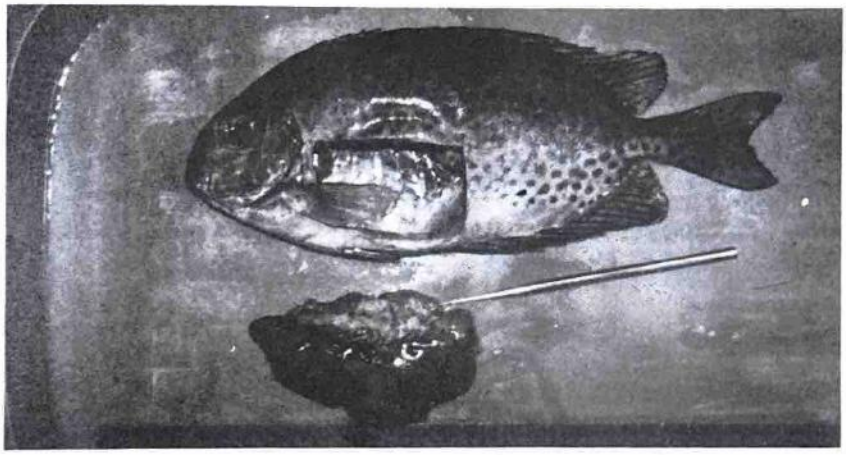


Figure 8. Female *S. guttatus* with gall bladder is the middle position relative to the body cavity.

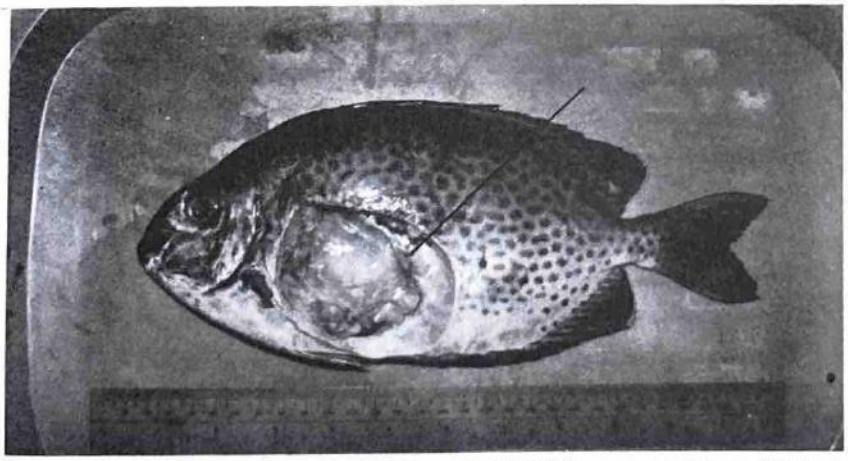


Figure 9. Farmed *S. guttatus* from MSU-Naawan with gall bladder positioned posterior relative to the body cavity and showing light color of internal organs.

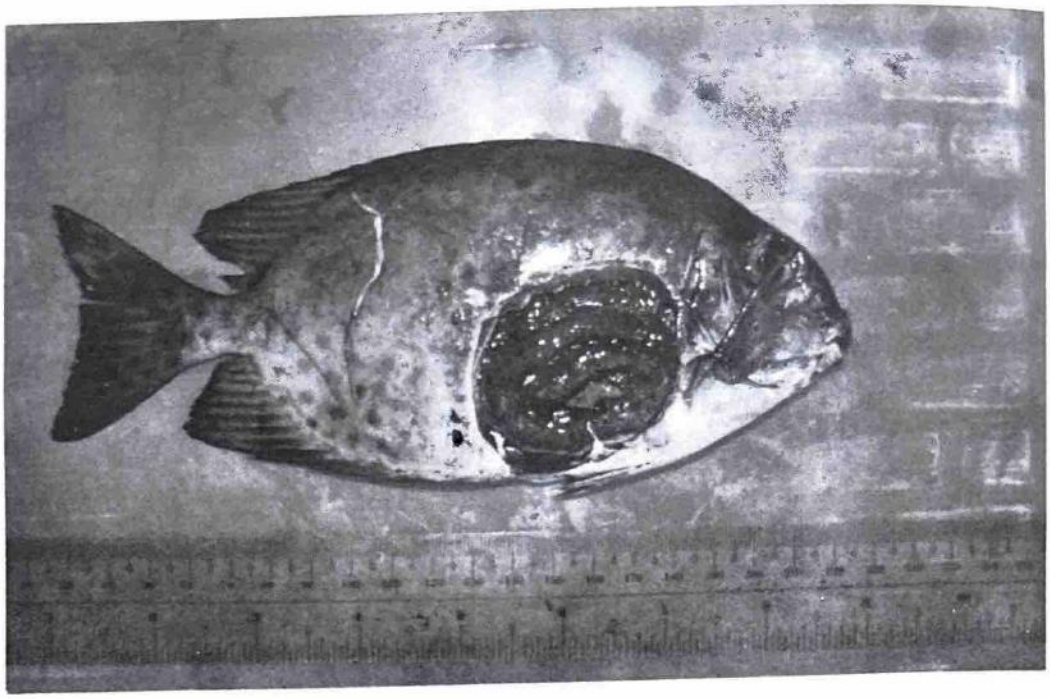


Figure 10a. *S. guttatus* from the wild with dark color of internal organs

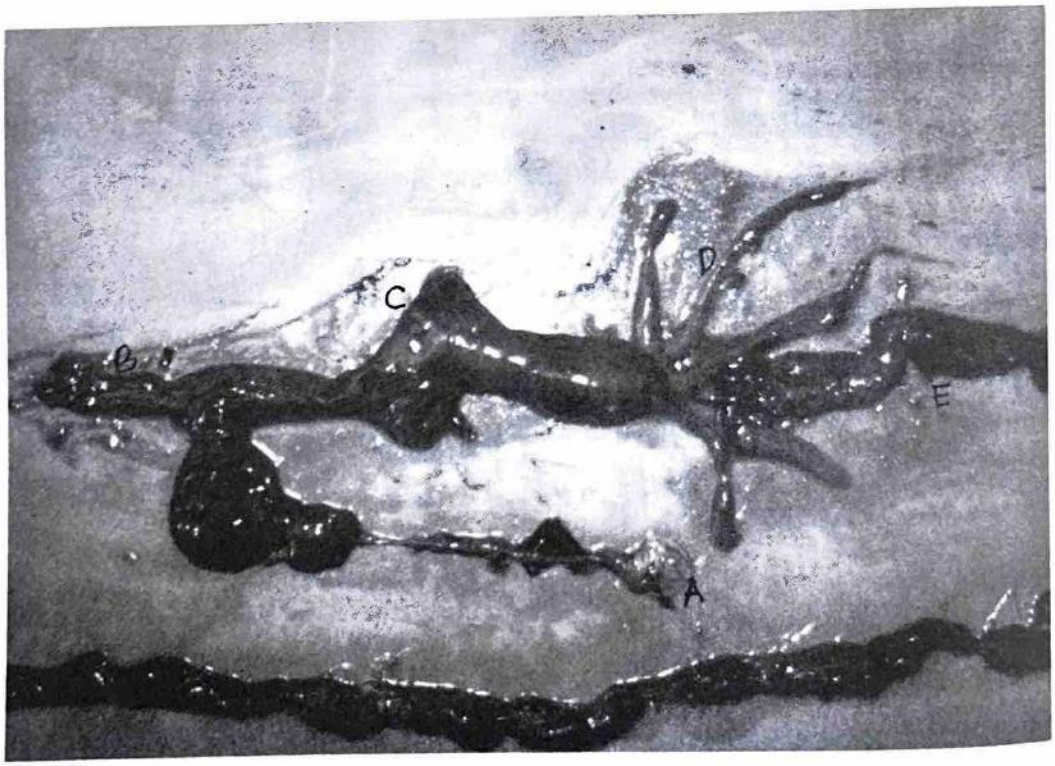


Figure 10b. Digestive tract of *S. guttatus* showing A. gall bladder, B. esophagus, C. stomach, D. pyloric caeca; and E. intestine

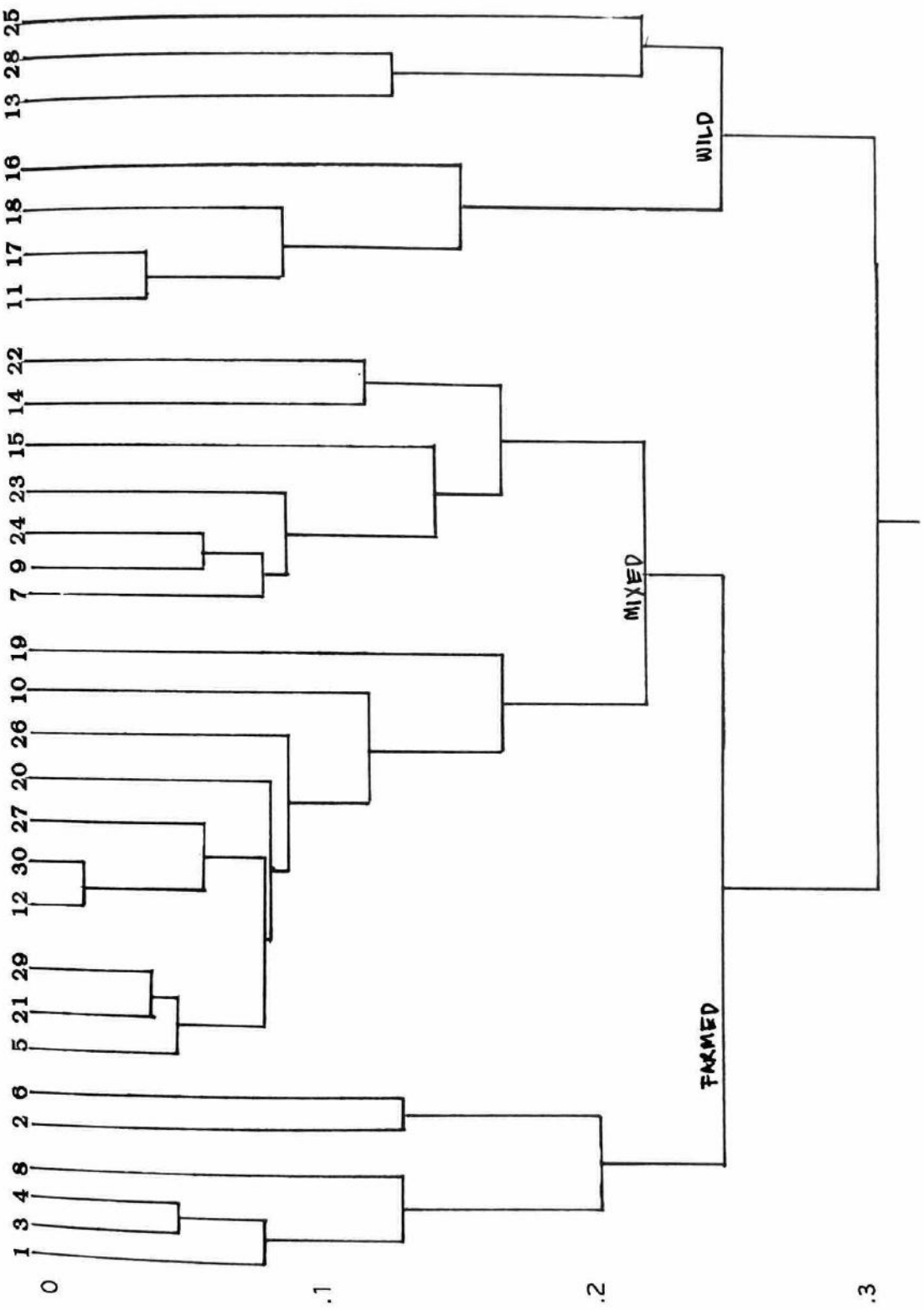


Figure 11. All Morphological Characters of *S. guttatus*. Clustering is by the Group Average Strategy (Unweighted Pair Group Method). Index is Chord Distance

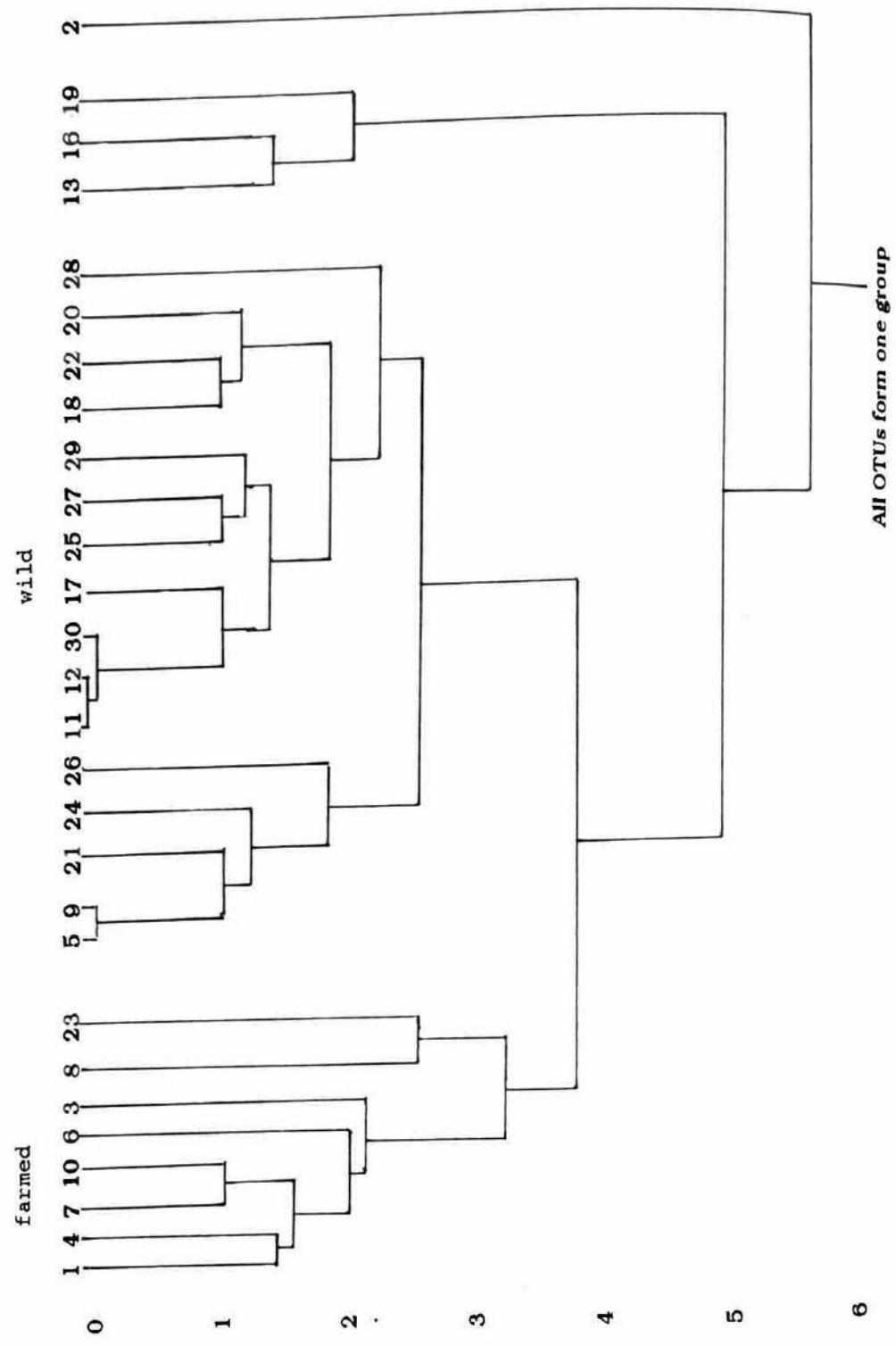


Figure 12. Meristic Morpholocial Characters of *Siganus guttatus*. Clustering is by the Group Average Strategy (unweighted Pair Group Method). Index is Euclidean Distance.

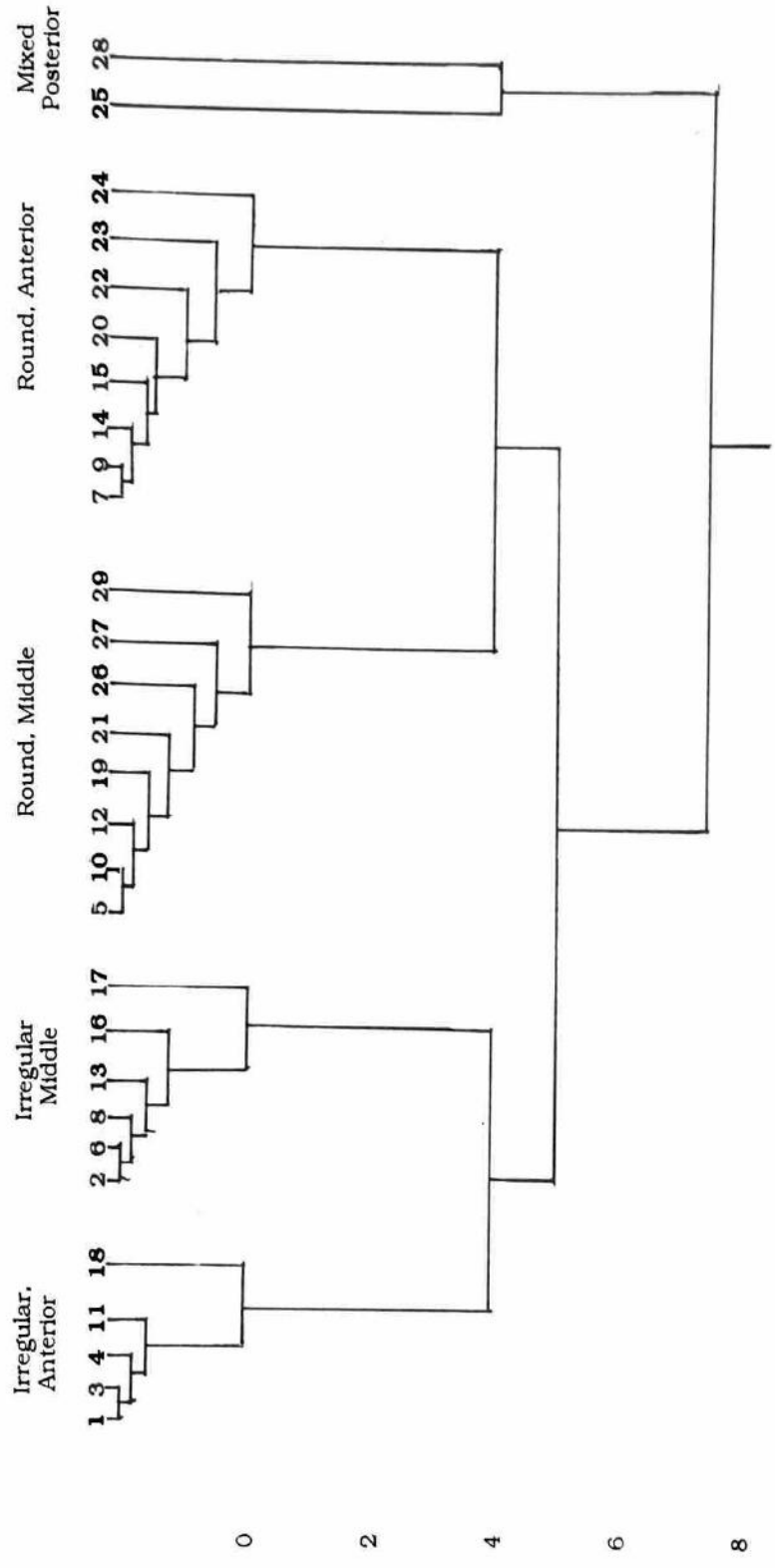
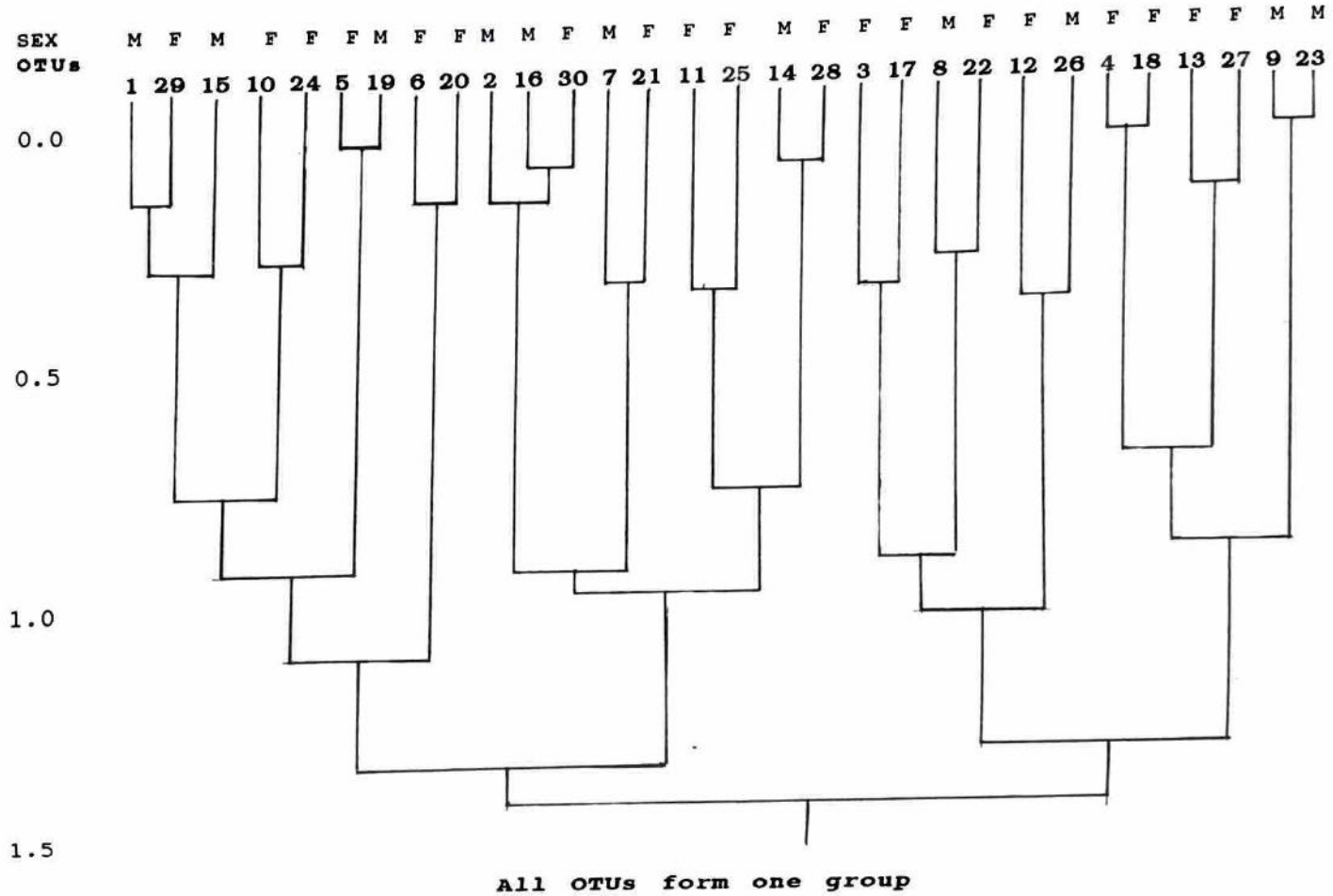


Figure 13. Qualitative Morphological Characters of *S. guttatus*. Clustering is by the Group Average Strategy (Unweighted Pair Group Method). Index is Euclidean Distance

Figure 14. Quantitative Morphological Characters of *Siganus* guttatus. Clustering is by the Group Average Strategy (unweighted Pair Group Method). Index is Chord Distance.



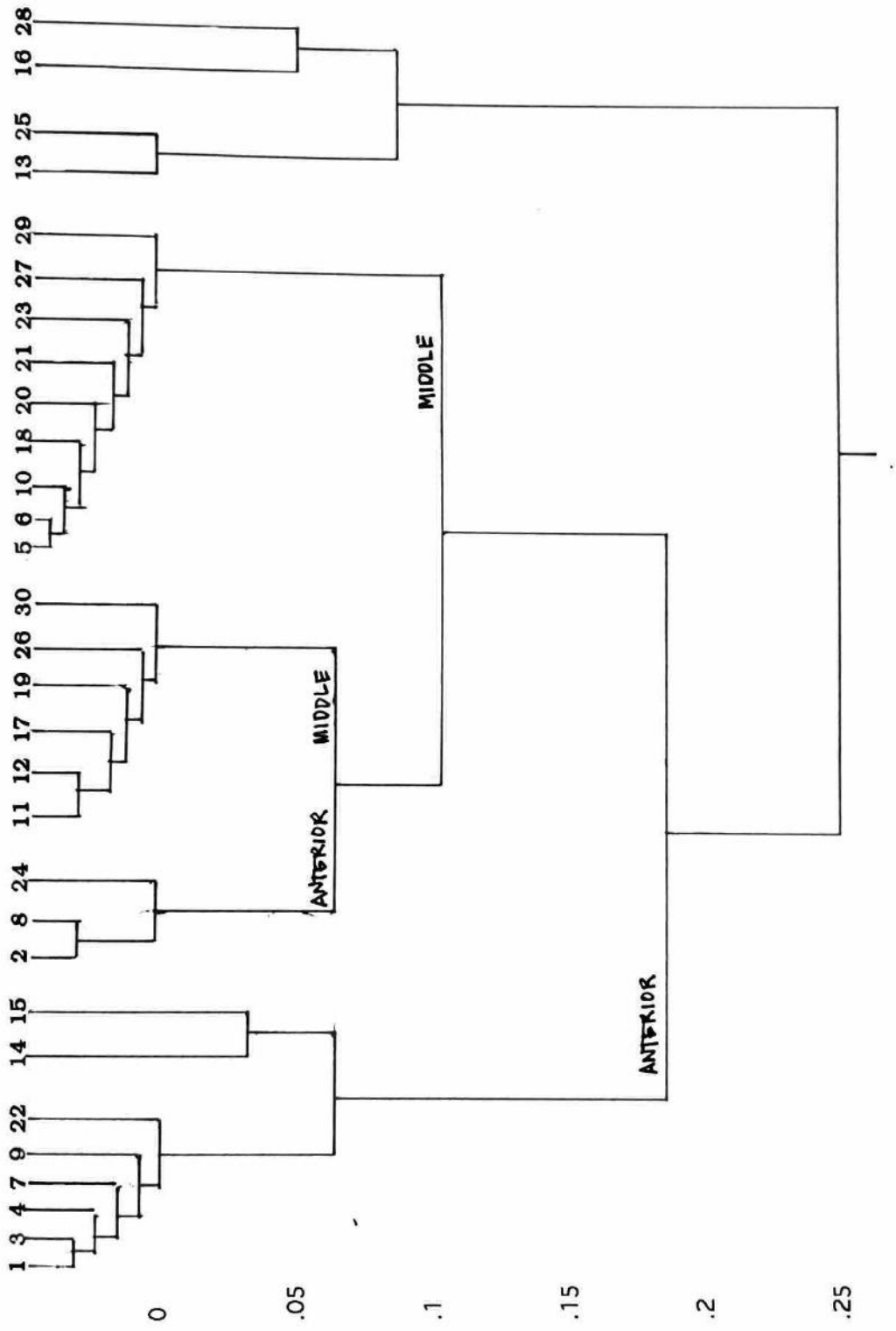
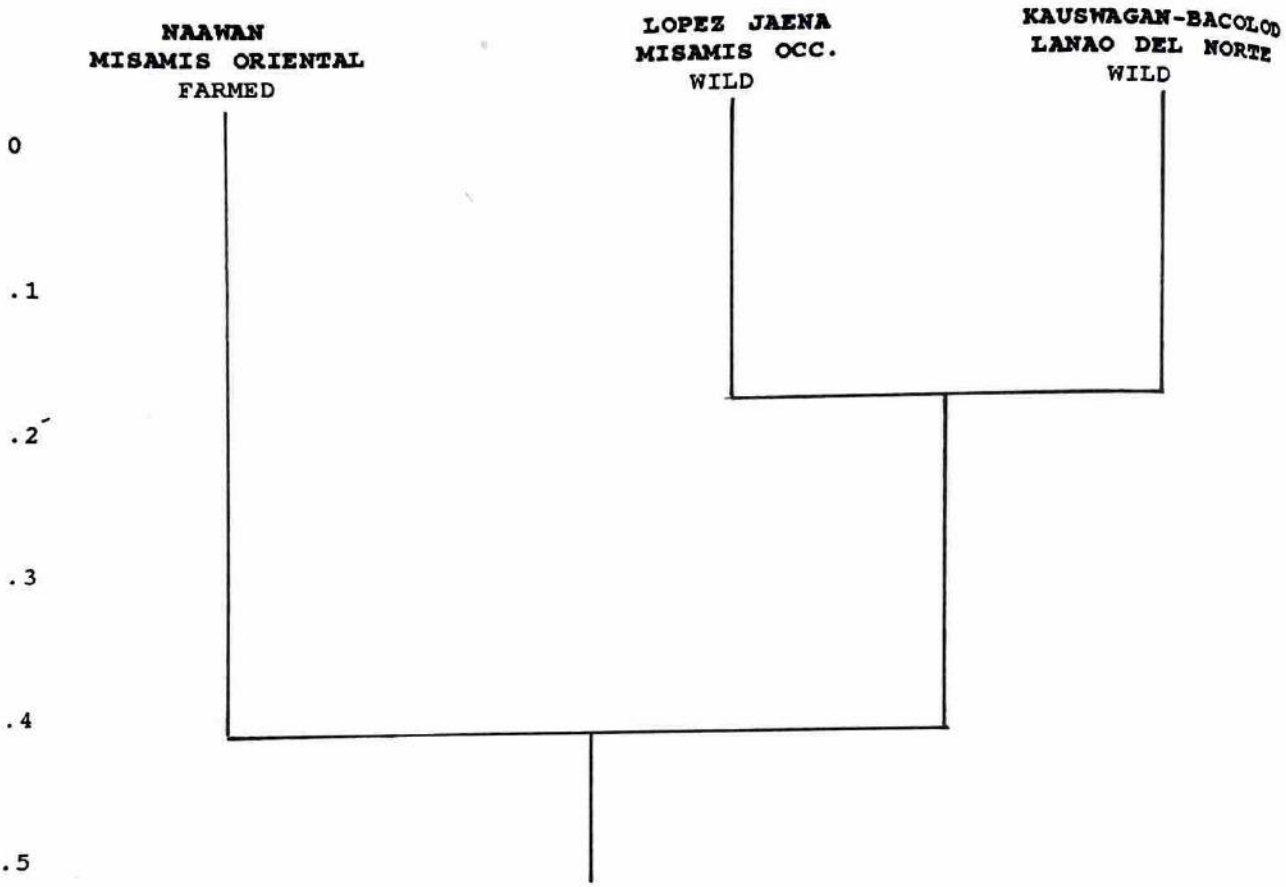


Figure 15. Pyloric Caeca Number & Gall Bladder Position of *S. guttatus*. Clustering is by the Group Average Strategy (Unweighted Pair Group Method). Index is Chord Distance.

Figure 16. Cluster Output Analysis between three populations of *S. guttatus*. Clustering is by the Group Average Strategy (Unweighted Pair Group Method). Index is Chord Distance



The digestive system strategy of the three *S. guttatus* populations was investigated based on the following five characters namely; gut length, stomach length, maximum pyloric caeca length, number of pyloric caeca and positioning of the gall bladder relative to the liver. Cluster analysis was performed. Result of the cluster analysis using chord distance as index and clustering by group average strategy indicated three groups at cluster level between 0.11 – 0.15 (Fig. 15) and all cluster from one group at 0.31 cluster level. Clustering among OTUs was influenced by pyloric caeca number and gall bladder positioning.

Cluster analysis was also performed between three populations based on mean values from respective OTUs (Fig. 16). Clustering was executed by group average strategy using chord distance and Euclidean distance as indices and results revealed all populations form one group. However, populations from the wild, Lopez Jaena, Misamis Occidental and Kauswagan-Bacolod, Lanao del Norte showed more similarity than the farmed population in Naawan, Misamis Oriental (Fig. 9 and 10). Ecological data on the three above-mentioned population are presented in Table 6 below.

Although there is big difference in size ranges these do not matter in character measurements as measurements were expressed in ratios to standard length. Differences in sizes may be affected by sampling errors as this indicate variation of mesh sizes used in catching *S. guttatus* by fishermen in the two wild populations.

The two wild populations (Lopez-Jaena and Kauswagan-Bacolod) indicate polymorphism in their expression of various characters which may be the result of various factors associated with natural selection and random mating. The outcome of natural selection is the elimination of alleles which are under selection pressure thus those that survive in the wild are ecologically fit. On the other hand, the farmed siganid population in Naawan is a product of selective breeding and culture under controlled environmental conditions and feeding, regimes. Thus, it is observed that years of breeding have encountered presence of deformities as a result of mutation and faint natural selection pressure in the population. Further inbreeding for the next generation will increase homogeneity within the population of siganids in Naawan Siganid Hatchery.

It can be seen from this study that variability was high in all characters in the wild

Table 6. Ecological description of three populations of *S. guttatus*.

PARAMETERS	NAAWAN	LOPEZ JAENA	KAUSWAGAN-BACOLOD
OTUs	10	10	10
Male:Female ratio	1:1	2:3	1:4
Salinity	28-31 ppt.	26-31 ppt	29-31 ppt
Temperature	31C	30C	30C
Food Habit	Commercially formulate diet +algae	algae	algae
Size range			
TL	240-285 mm	180-214 mm	217-310 mm
BW	300-520 G	140-200g	250-600g
Habitat	concrete tanks	coral reef & sea grass beds	coral reef & sea grass beds

population. The diversity may be the result of interaction of random effects of natural selection pressure in all developmental stages and random mating in the large-wild population such that the ratio may follow the hardy-Weinberg equation.

The low variability of morphological characters in the farmed population of *S. guttatus* at MSU-Naawan is a result of inbreeding, selective mating (non-random mating) and controlled environmental conditions inherent to farmed population resulting to homozygosity as a genetic consequences of inbreeding.

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