



UNIVERSITI PUTRA MALAYSIA

**SOME ASPECTS OF THE BIOLOGY AND CONTROL OF
QUADRIACANTHUS KOBIENSIS HA KY, 1968 (MONOGENEA:
DACTYLOGYRIDAE) FROM *CLARIAS BATRACHUS* (LINNAEUS) IN
INDONESIA**

OMAN KOMARUDIN

FPSS 1989 1

SOME ASPECTS OF THE BIOLOGY AND CONTROL OF *QUADRIACANTEUS*
KOBIENSIS HA KY, 1968 (MONOGENEA: DACTYLOGYRIDAE) FROM
CLARIAS BATRACHUS (LINNAEUS) IN INDONESIA

By

OMAN KOMARUDIN

A Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of Master of Science
in the Faculty of Fisheries and Marine Science
Universiti Pertanian Malaysia

October 1989



ACKNOWLEDGEMENTS

The work described herein could not be completed without the assistance, friendship, support and contribution of many individuals most of whom are associated with the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia, and the Research Institute for Freshwater Fisheries, Bogor, Indonesia. Some of these people deserve particular recognition and thanks. It is to them that I wish to express my gratitude:

To my supervisor, Associate Professor Dr. Mohamed Shariff, for his encouragement, patient advice and assistance during the whole period of this study.

To my co-supervisor, Dr. Faizah Shaharom for her advice and comments.

To the director of RIFF, Bogor, Dr. Atmadja Hardjamulia for his providing me the study leave to complete this work.

To the coordinator of Fish Disease Section, Dr. Akhmad Rukyani for his invaluable advice and assistance during my country research in Indonesia.

To Miss Retna Utami and Mr. Abdul Wahid for their assistance on water quality observation.



To Mr. Komar for his assistance during my country research and Mr. Rosli Aslim for his assistance in histological study.

To my loving wife, Adhe Rumsiah for her encouragement, support and endurance during separation.

I also thank International Development Research Centre, Canada for providing me the fellowship and research funding for the project.



TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	ix
LIST OF PLATES	x
ABSTRACT	xi
ABSTRAK	xiii
CHAPTERS	
I GENERAL INTRODUCTION	1
Background	1
Disease Problems in Cultured Fish	2
Objectives	4
II IDENTIFICATION OF MONOGENETIC TREMATODE FROM <i>CLARIAS BATRACHUS</i> (LINNAEUS)	6
Introduction	6
Materials and Methods	7
Results	8
Comments and Remarks	11
III EFFECT OF <i>QUADRIACANTHUS KOBIENSIS</i> HA KY, 1968 ON THE GROWTH OF WALKING CATFISH <i>CLARIAS BATRACHUS</i> (LINNAEUS)	14
Introduction	14
Materials and Methods	15
Results	18



Discussion	22
IV EFFECTIVENESS OF SODIUM CHLORIDE AS A CHEMOTHERAPEUTIC AGENT FOR <i>QUADRIACANTHUS</i> <i>KOBIENSIS</i> HA KY, 1968 IN <i>CLARIAS</i> <i>BATRACHUS</i> (LINNAEUS)	27
Introduction	27
Materials and Methods	29
Toxicity Test of Sodium Chloride	30
The Effectiveness of Sodium Chloride against <i>Q. kobiensis</i>	31
Results	32
Discussion	37
V HISTOPATHOLOGY OF <i>QUADRIACANTHUS</i> <i>KOBIENSIS</i> HA KY, 1968 INFECTION IN <i>CLARIAS BATRACHUS</i> (LINNAEUS)	42
Introduction	42
Materials and Methods	44
Results	45
Discussion	52
VI CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK	56
REFERENCES	59
APPENDIX	66
BIOGRAPHICAL SKETCH	82



LIST OF TABLES

Table	Page
1. Comparison of <i>Quadriacanthus kobeiensis</i> with the Study of Gussev (1976)	10
2. Specific Growth Rate (SGR) (%/day) of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobeiensis</i> after Three Months	18
3. Mean Food Conversion of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobeiensis</i> after Three Months	19
4. Mean Mortality of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobeiensis</i> after Three Months	19
5. Mean Intensity of <i>Q. kobeiensis</i> after Three Months Postinfection	20
6. Mean Concentration of Ammonia in The Experimental Tanks	21
7. Mean Concentration of Dissolved Oxygen in The Experimental Tanks	21
8. Mean pH in The Experimental Tanks	22
9. Concentration of Sodium Chloride, Time Exposure and Number of Replicates	32
10. Lethal Concentration of Sodium Chloride for Catfish Fingerling	33
11. Mean Intensity of <i>Q. kobeiensis</i> after Treatment with Sodium Chloride at Different Time Exposure	34
12. Mean Prevalence of <i>Q. kobeiensis</i> after Treatment with Sodium Chloride	35
13. Mean Intensity of <i>Q. kobeiensis</i> at Different Time Exposures	36
14. Mean Intensity of <i>Q. kobeiensis</i> at Different Concentrations	37



15.	Specific Growth Rate (%/day) of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobiensis</i> after Three Months	66
16.	Analysis of Variance of Specific Growth Rate of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobiensis</i> after Three Months	66
17.	Least Significant Differences of Specific Growth Rate of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobiensis</i> after Three Months	67
18.	Food Conversion of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobiensis</i> after Three Months	67
19.	Analysis of Variance of Food Conversion of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobiensis</i> after Three Months	68
20.	Mean Intensity of <i>Quadriacanthus kobiensis</i> on <i>Clarias batrachus</i> after Three Months Infection	68
21.	Analysis of Variance of Mean Intensity of <i>Quadriacanthus kobiensis</i> on <i>Clarias batrachus</i> after Three Months Infections	69
22.	Mortality of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobiensis</i> after Three Months	69
23.	Analysis of Variance of Mortality of <i>Clarias batrachus</i> Infected with <i>Quadriacanthus kobiensis</i> after Three Months	70
24.	Concentration of Ammonia in The Water in The Experimental Tanks	70
25.	Analysis of Variance of Concentration of Ammonia in The Water in The Experimental Tanks	71
26.	Condition of pH in The Water in The Experimental Tanks	71
27.	Analysis of Variance of pH in The Water in The Experimental Tanks	71
28.	Dissolved Oxygen in The Water in The Experimental Tanks	72



29.	Analysis of Variance of Dissolved Oxygen in The Water in The Experimental Tanks	72
30.	Statistical Analysis for Data on LC ₅₀ for 24 hours	73
31.	Statistical Analysis for Data on LC ₅₀ for 48 hours	74
32.	Statistical Analysis for Data on LC ₅₀ for 96 hours	75
33.	Mean Intensity of <i>Q. kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure	76
34.	Analysis of Variance of Mean Intensity of <i>Q. kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure	76
35.	Duncan's Multiple Range Test of Mean Intensity of <i>Q. kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure	77
36.	Mean Intensity of <i>Q. kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure without Control	78
37.	Analysis of Variance of Mean Intensity of <i>Q. kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure without Control	78
38.	Duncan's Multiple Range Test for Concentration Group	79
39.	Duncan's Multiple Range Test for Time Exposure Group	79
40.	Prevalence of <i>Quadriacanthus kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure	80
41.	Analysis of Variance of Prevalence of <i>Quadriacanthus kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure	80
42.	Duncan's Multiple Range Test of Prevalence of <i>Quadriacanthus kobiensis</i> after Treatment with Sodium Chloride at Different Time Exposure	81



LIST OF FIGURES

Figure		Page
1.	<i>Q. kobiensis</i> , 1.Dorsal Anchor, 2. Ventral Anchor, 3. Hook, 4. Copulatory Complex . . .	9



LIST OF PLATES

Plate		Page
1.	Anchor of The Parasite Embedded amongst the Hyperplastic Epithelial Cells. Note The Compression of Cells at The Base of The Anchor (arrow)	47
2.	Penetration of the Anchor through the Secondary Lamellae	47
3.	Lamellae Epithelial Hyperplasia. Note The Presence of Mitotic Cells (arrow)	48
4.	Clubbing of The Secondary Lamellae due to Presence of Hyperplastic Cells	48
5.	Red Blood Cells Interspersed between The Lamellar Hyperplastic Cells	49
6.	Large Vacuolation at The Base of Secondary Lamellae. Note The Remaining Red Blood Cells within Vacuole (arrow)	49
7.	Lamellae Telangiectasis (t) with Severe Hyperplasia. Note The Interspersed Red Blood Cell (r)	50
8.	High Magnification of Lamellar Telangiectasis. Note The Mitotic Cells (arrow)	50
9.	Haemorrhage due to Rupture Telangiectasis . .	51
10.	Haemorrhage due to Rupture of Dilated Vessel of Primary Filament. Note The Presence of Red Blood Cells Undergoing Degenerative Changes (arrow)	51



ABSTRACT

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in partial fulfillment of the requirements for the degree of Master of Science.

SOME ASPECTS OF THE BIOLOGY AND CONTROL OF *QUADRIACANTHUS KOBIIENSIS* HA KY, 1968 (MONOGENEA: DACTYLOGYRIDAE) FROM *CLARIAS BATRACHUS* (LINNAEUS) IN INDONESIA

By

Oman Komarudin

October, 1989

Supervisor : Associate Prof. Dr. Mohamed Shariff

Co-supervisor : Dr. Faizah Shaharom

Faculty : Fisheries and Marine Science

This study covered some aspects of the biology and control of monogenetic trematode from walking catfish *Clarias batrachus* (Linnaeus).

The monogenetic trematode from walking catfish at Wiryana Fish Farm, Depok, Bogor, Indonesia was identified as *Quadriacanthus kobeiensis* Ha Ky, 1968.

Three levels of infection with *Q. kobeiensis* were established in *C. batrachus* to study its effect on growth rate. The mean number of parasite for the three treatments were: low 12.2, medium 12.8 and high 187.3. Results of the effect of



this parasite on walking catfish *Clarias batrachus* showed that the specific growth rate at all levels of infection: low, medium and high were significantly lower than the control. This parasite can reduce the specific growth rate of walking catfish by 52.9% as compared to the control. There was no significant difference of the specific growth rates amongst fish with high, medium and low levels of infection. No significant difference was seen in the mortality rate at these levels of infection when compared with the control.

The toxicity of sodium chloride (NaCl) on the fingerling of walking catfish was tested to determine the dose range for sodium chloride as chemotherapeutic agent for the control of *Quadriacanthus kobiensis*. The values of LC₅₀ of sodium chloride for 24 and 48 hours were 14.49 ppt and 14.04 ppt respectively. The value of LC₅₀ for 96 hours was the same as of LC₅₀ for 48 hours. Sodium chloride at a concentration of 12.45 ppt or 13.00 ppt at 24 or 36 hours was 100% effective in eradicating *Quadriacanthus kobiensis*.

Histological study on catfish infected with *Quadriacanthus kobiensis* revealed lamellar epithelial hyperplasia, lamellar telangiectasis, haemorrhages and necrosis of the hyperplastic cells.



ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia, sebagai memenuhi sebahagian daripada keperluan untuk mendapat Ijazah Master Sains.

**BEBERAPA ASPEK BIOLOGI DAN RAWATAN *QUADRIACANTHUS*
KOBIENSIS HA KY, 1968 (MONOGENEA: DACTYLOGYRIDAE) DARIPADA
IKAN KELI *CLARIAS BATRACHUS* (LINNAEUS) DI INDONESIA**

oleh

Oman Komarudin

Oktober, 1989

Penyelia Bersama: Prof. Madya Dr. Mohamed Shariff

Penyelia : Dr. Faizah Shahrarom

Fakulti : Perikanan dan Sains Samudera

Satu kajian telah dijalankan mengenai aspek biologi dan rawatan monogenetic trematode, dari ikan keli *Clarias batrachus* (Linnaeus).

Sampel ikan keli diambil dari Kolam Ternakan Wirya Mina, Depok, Bogor, Indonesia dan monogenetic trematode yang didapati dikenalpasti sebagai *Quadriacanthus kobiensis* Ha Ky, 1968.

Tiga peringkat serangan dengan *Q. kobiensis* telah dikaji pada *C. batrachus* untuk mengetahui kesan parasit ini terhadap kadar pertumbuhan. Kandungan parasit pada ketiga peringkat serangan adalah: rendah 12.2, pertengahan 12.8 dan tinggi



187.3. Hasil kajian kesan parasit ini terhadap kadar pertumbuhan ikan keli *C. batrachus* mendapati bahawa kadar pertumbuhan pada semua peringkat serangan (rendah, pertengahan dan tinggi) adalah lebih rendah berbanding dengan ikan tanpa parasit (ikan sihat sebagai ikan kawalan). Parasit ini boleh menyebabkan pengurangan kadar pertumbuhan sehingga 52.9% apabila dibanding dengan ikan tanpa parasit. Walaupun begitu tiada perbezaan bererti di antara kadar pertumbuhan pada semua peringkat serangan. Perbezaan kadar kematian ikan tidak bererti pada semua peringkat serangan apabila dibanding dengan ikan tanpa parasit.

Kadar keracunan Natrium klorida terhadap anak ikan keli telah diuji untuk mendapatkan julat dose Natrium klorida untuk merawat *Quadriacanthus kobiensis*. Kadar LC₅₀ untuk Natrium klorida bagi tempoh 24 dan 48 jam adalah 14.49 ppt dan 14.04 ppt. Sementara itu nilai LC₅₀ untuk 96 jam adalah sama dengan tempoh 48 jam. Natrium klorida pada kepekatan 12.45 ppt atau 13.00 ppt dengan masa pendedahan samada 24 atau 36 jam adalah 100% berkesan untuk merawat *Q. kobiensis*.

Kerosakan insang pada ikan keli yang dijangkiti oleh *Q. kobiensis* adalah hiperplasia lamela epithelium, telangiektasis lamela, haemorrhages dan nekrosis pada sel hiperplasia.



CHAPTER ONE
GENERAL INTRODUCTION

Background

Walking catfish, *Clarias batrachus* L. is a commercial food fish in Southeast Asian countries and it fetches a high price in the market. In Indonesia it is the second commonly cultured freshwater fish after common carp. The aquaculture industry, in Indonesia accounted for 12.7% of the total fish production for 1985 and catfish production was 694 tons, which is equivalent to 1,093 million rupiahs (Rahardjo, 1987).

It is well known that fish culture is important for a populous country and is the main source of protein. According to Leong (1978) fish is the cheapest source of protein available to a large proportion of the population in many parts of the world. In addition, fish culture is becoming more important because available wild stocks are being overexploited and the demand for fish protein is increasing with the increase in population. Aquaculture is also the only source of income for many fish farmers. In Indonesia, freshwater aquaculture was expected to provide employment for 362,700 for five years period i.e. 1984/85 to 1988/89 (Indonesia, 1983a).



The production of aquaculture would have to increase to meet the demand for continuous population growth and to achieve the target of fish production at 5.6 million tons in the year 2000 (Indonesia, 1988). The popular method which could be initiated to increase production from aquaculture is by practicing intensive culture. In intensive culture, stocking densities are always higher than the carrying capacity of the pond, therefore, exposing the fish to stress and thus increasing the susceptibility of the fish to parasitic infection. High stocking density is also favourable in the spread of diseases (Patcher, 1982). In addition, overcrowding also permits easy transmission of fish parasites by close contact (Brown and Gratzek, 1980).

Disease Problems in Cultured Fish

Disease has been implicated to be one of the main causes of severe mortality in cultured fish. According to Needham and Wootten (1978) parasites often cause serious outbreaks of diseases in cultured fish. In Indonesia there were many instances of disease outbreaks causing severe losses particularly to the freshwater fish, and the causative agents were parasites. The first outbreak of disease in cultured fish was caused by *Ichthyophthirius multifiliis* Fouquet, 1876 recorded in 1932 (Sachlan, 1952). During the period of 1970-



1971 *Lernaea cyprinacea* Linnaeus, 1958 reduced fry production of common carp *Cyprinus carpio* Linnaeus and Java carp, *Puntius javanicus* Bleeker by 30%, causing an estimated loss of 1.48 billion fry equivalent to 7.4 billion rupiahs (Koesoemadinata, 1977). Other parasites that have also been implicated as causal agent of mortalities were myxosporians, causing losses as high as 60-90% of common carp's fry (Djajadiredja *et al.*, 1983). The latest outbreak of ulcerative syndrome in 1980 also caused great damage to fish production and losses of common carp's broodstock were estimated at two million U.S. dollars (Dana, 1987).

In Indonesia the presence of monogenetic trematode (*Dactylogyrus sp.*) was first recorded from walking catfish in 1952 (Sachlan, 1952). During the first incidence of its occurrence, *Dactylogyrus* did not cause any problem. However, culture of walking catfish is now becoming popular and many fish farmers practice intensive culture. Since 1980 disease of walking catfish caused by *Aeromonas* has become serious (Dana, 1987). Supriyadi (1986) also recorded that walking catfish appeared to be highly susceptible to bacterial infection. According to Sopa (1978) disease caused by *Aeromonas* in walking catfish was considered as secondary infection resulting from stress due to external parasite and high stocking density. According to Noble and Noble (1964) parasitic infection reduces the resistance of the host to bacterial infection. Preliminary



study by the author also indicated that monogenean was commonly found on walking catfish with prevalence ranging from 80 to 100%. Supriyadi *et al.* (1986) also recorded monogenetic trematode from walking catfish. According to Brown and Gratzek (1980), Cusack *et al.* (1988), Cusack and Cone (1985 and 1986) monogenean can transmit bacterial infection. Therefore, monogenean could be suspected to be involved as one of the primary agents in causing the occurrence of bacterial disease.

Objectives

Studies to determine the effect of monogenean on its host has been conducted by many workers: Sarig (1971), Lester (1972), Lester and Adams (1974), Heggbert and Johnsen (1982), Cone *et al.* (1983) Kamiso and Olson (1986) and Cusack and Cone (1986). However none of them have studied monogenetic trematode from walking catfish, while Sachlan (1952), Gussev (1976) Lim and Furtado (1983) and Supriyadi *et al.* (1986) studied only the taxonomy of monogenetic trematode of walking catfish. The objectives of the present study are as follow:

1. To identify the monogenetic trematodes from walking catfish, *C. batrachus*.
2. To determine the effect of monogenean on the growth of walking catfish, *C. batrachus*.

3. To evaluate the effectiveness of sodium chloride against monogenetic trematode.
4. Histological studies of monogenetic trematode infection in walking catfish.

CHAPTER TWO

IDENTIFICATION OF MONOGENETIC TREMATODE FROM *CLARIAS BATRACHUS* (LINNAEUS)

Introduction

Studies on identification of monogenetic trematodes have been carried out since a long time ago. According to Shaharom (1983) the first study was done by Van Beneden in 1858. Monogeneans are well studied, however, most of these studies were conducted in the temperate zone. Studies on the monogenean on the gill of warm-water cultured fishes were few and far between (Shaharom, 1983). Studies on monogenea from the walking catfish (*Clarias batrachus*) are few: Sachlan (1952), Paperna (1965), Hanek and Furtado (1972) Gussev (1976), Lim and Furtado (1983) and Supriyadi *et al.* (1986). They discussed only taxonomic characteristics.

There are many genera of monogenetic trematode from *Clarias batrachus*. *Gyrodactylus* infects the skin and fins (Hanek and Furtado, 1972) whilst *Quadriacanthus* infects the gills (Gussev, 1976). Hanek and Furtado (1972) studied *Gyrodactylus fernandoi* sp. n. from the skin and fins of *Clarias batrachus* from Sungai Besar, Sabak Bernam, Selangor, Malaysia. Gussev (1976) identified *Quadriacanthus kobiensis* Ha Ky from the gill of *C. batrachus* from the water bodies near Lucknow, India.



In Indonesia Sachlan (1952) found *Dactylogyrus* sp. from the gill of *C. batrachus*, while Supriyadi *et al.* (1986) reported *Dactylogyrus* sp. and *Gyrodactylus* sp.. These however were not identified to species level.

The objective of this study was to determine the species of monogenetic trematode from walking catfish, *C. batrachus*.

Materials and Methods

Fish were obtained from the Wirya Mina fish farm in Depok, Bogor, Indonesia. The fish were kept in the aquarium tanks at the Fish Disease Laboratory, Research Institute for Freshwater Fisheries, Bogor, Indonesia, and subsequently transferred to the Fish Diseases Aquarium, Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia.

The parasite was identified by examining fresh live and semipermanent mounted specimens. The parasites were teased out from the gill filaments using fine needle. Parasites were placed on a glass-slide and covered with a coverslip for examination. The specimens were examined with phasecontrast microscope. Illustrations of the worm were made with a drawing tube. Parasites were also removed from the gills by dipping the infected gills in small petri dish containing 1:4,000

formalin (Putz and Hoffman, 1964). Specimens were then fixed in 10% formalin at room temperature and mounted on glass-slide in ammonium-picrate glycerin (Cone *et al.*, 1983).

Results

The measurements of the monogenetic trematode are shown in Table 1. The terms of reference used in this study followed Gussev (1976). The characteristic of the anchors, hooks and copulatory complex are shown in Figure 1.

Class Monogenea Bychowsky, 1937.

Subclass Polyonchoinea Bychowsky, 1937.

Order Dactylogyridea Bychowsky, 1937.

Suborder Dactylogyrynea Bychowsky, 1937.

Family Dactylogyridae Bychowsky, 1933.

Subfamily Ancyrocephalinae Bychowsky, 1937.

Genus *Quadriacanthus* Paperna, 1961.

The description of *Q. kobiensis* was based on 19 specimens and units of measurements were in micrometer. The worms varied in size, the length of the largest worm was 681.1 and the smallest worms was 230.3. The width of the largest worm was 198.0, while the smallest was 73.5. Eye spots were not visible. The pharynx was almost circular with diameter of 25.0 micrometers. The vitellaria was well developed.



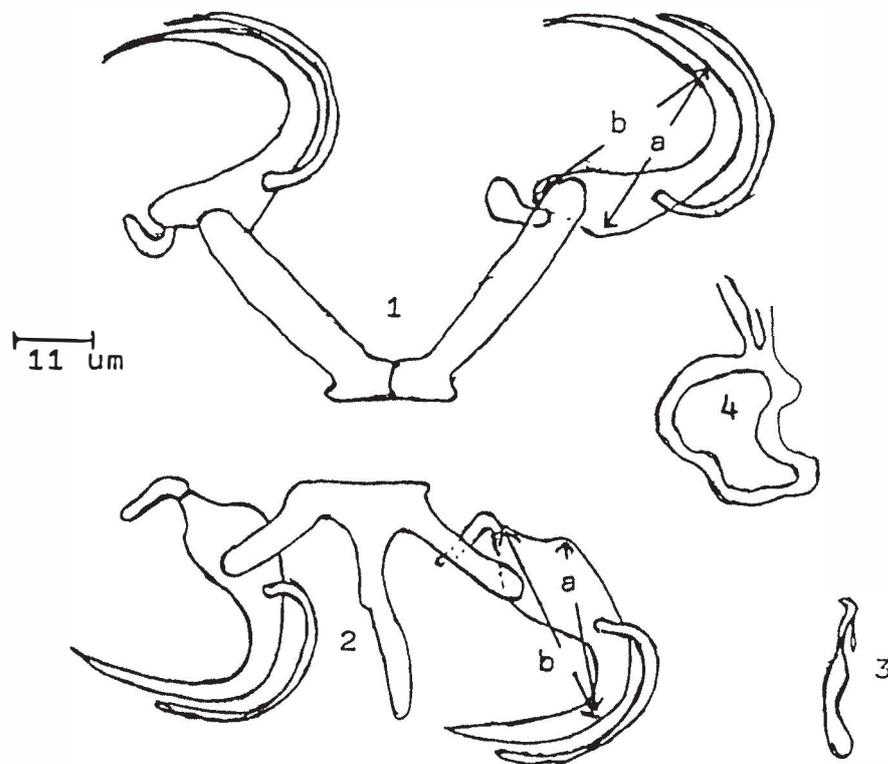


Figure 1. *Q. kobeensis*, 1. Dorsal Anchor, 2. Ventral Anchor, 3. Hook, 4. Copulatory Complex; a. Length between base of point and apex of the root, b. Length between the end of point and outer angle of basal region of the root.

Table 1

Comparison of *Quadriacanthus kobiensis* with the
Study of Gussev (1976)*)

Dimension of body and chitinous parts	<i>Q. kobiensis</i> (Gussev, 1976)	<i>Q. kobiensis</i> (present study)
	------(micrometer)-----	
Body: -length	350	364.6 (230.3-681.1)
-width	150	141.7 (73.5-198.0)
Length of dorsal anchor		
a)	- (24-29)	27.9 (25.6-32.9)
b)	- (30-33)	32.1 (27.7-42.3)
Length of ventral anchor		
a)	-	27.4 (20.9-33.1)
b)	-	28.8 (25.3-33.1)
T-shape dorsal bar		
length	- (29-42)	43.8 (38.0-52.5)
width	-	37.8 (32.1-50.0)
V-shape ventral bar		
length	- (45-52)	39.6 (35.8-44.5)
width	- (8-9)	4.6 (3.1-6.2)
The longest hook	- (22-25)	23.3 (19.3-29.6)
The shortest hook	- (12-15)	13.4 (11.8-15.7)
Number of specimens	10	19

a): Length between base of point and apex of the root.

b): Length between the end of point and outer angle of basal region of root.

*): Value in parenthesis indicate range.