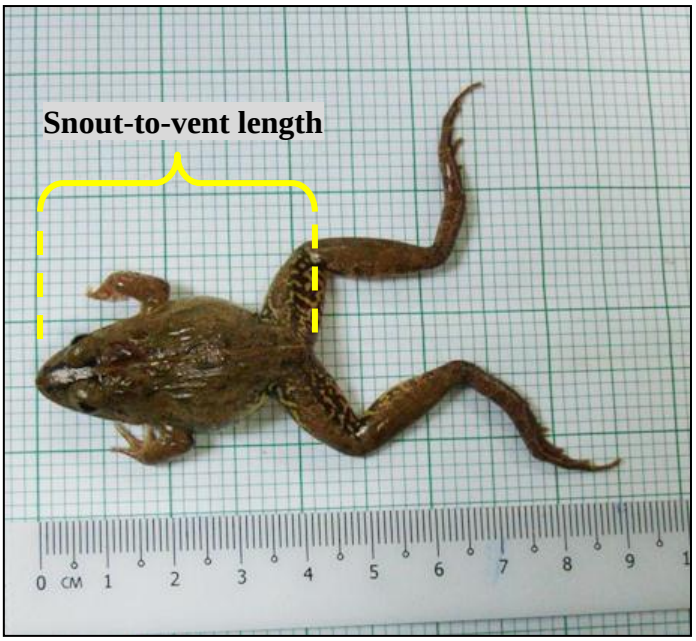
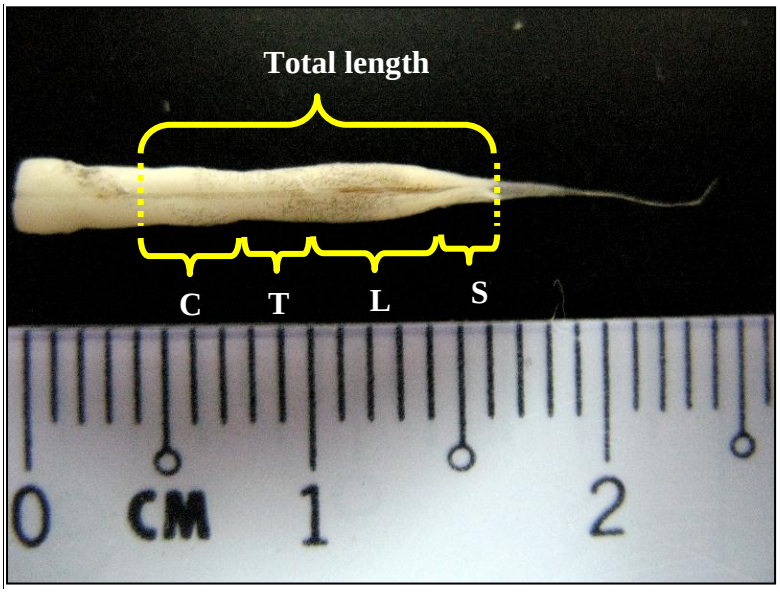
APPENDICES

Appendix A – Morphometric analysis guideline of frog and its spinal cord



Dorsal view of *Fejervarya limnocharis*



Ventral view of frog spinal cord (C: length of cervical level, T: length of thoracic level, L: length of lumbar level, S: length of sacral level)

Appendix B – Preparation of chemical solutions for animal perfusion and histology

General

1) Tricaine methanesulfonate (MS-222)

Tricaine methanesulfonate	350mg
Distilled water	1000ml

2) 0.6% frog physiological saline

Sodium chloride (NaCl)	0.6g
Distilled water	100ml

3) 10% neutral buffered formalin

Formaldehyde	100ml
Distilled water	900ml
Sodium phosphate, monobasic, monohydrate (NaH_2PO_4)	4g
Sodium phosphate, dibasic, anyhydrous (Na_2HPO_4)	6.5g

4) 70% alcohol solution

95% alcohol solution	700ml
Distilled water	250ml

5) 85% alcohol solution

95% alcohol solution	850ml
Distilled water	100ml

6) Mayer's albumin

Egg white	5g
Acetone	2ml
Distilled water	2ml

Hematoxylin and Eosin (H&E) Staining Method

1) Eosin stain

Eosin Y solution (water-soluble)	0.2ml
95% alcohol	99.8ml

2) Harris' alum hematoxylin stain

Hematoxylin crystals	1g
Absolute alcohol (99.5%)	10ml
Aqueous aluminum ammonium sulphate	1ml

Note: Hematoxylin crystals are dissolved in alcohol and then, aluminum ammonium sulphate is added and brought to boil. 0.5 gram of mercuric oxide is slowly added to oxidize the staining solution. The solution is left to cool and then filtered.

Nissl Staining Method

1) Buffer solution pH 3.5

0.1M acetic acid (6ml/1,000ml water)	94ml
0.1M sodium acetate (13.6g/1,000ml water)	6ml

2) Cresyl violet (can only be used twice)

Cresyl violet acetate	0.2g
Distilled water	150ml

3) Working solution

Buffer solution	100ml
Cresyl violet	10ml

Lillie's Variant of the Weil-Weigert Method (Lillie, 1954)

1) 1% acetic acid

Acetic acid	1ml
Distilled water	100ml

2) 1% alcoholic hematoxylin

Hematoxylin crystals	1g
Absolute alcohol	100ml

3) 10% alcoholic hematoxylin

Hematoxylin crystals	10g
Absolute alcohol	100ml

4) **0.5% iron alum**

Iron alum	0.5g
Distilled water	100ml

5) **4% iron alum**

Ferric ammonium sulphate	4g
Distilled water	100ml

6) **2.5% potassium ferricyanide solution**

Potassium ferricyanide	2.5g
Distilled water	100ml

7) **Safranin O**

Safranin	0.1g
1% acetic acid	100ml

Modification of Golgi Method

1) 3% potassium bichromate

Potassium bichromate	3g
Distilled water	100ml

2) 2% silver nitrate

Silver nitrate	2g
Distilled water	100ml

Thionin Staining Method (Gurr, 1956)

1) 0.5% thionin in 10% formalin

Thionin	0.5g
10% neutral buffered formalin	100ml

Appendix C - Histological staining procedures

H&E Staining Method

Step	Duration
Deparaffinization	
1) Xylene (I)	3 minutes or more
2) Xylene (II)	3 minutes or more
Hydration	
1) 95 % alcohol (I)	3 minutes or more
2) 95 % alcohol (II)	3 minutes or more
3) 70 % alcohol	3 minutes or more
4) Distilled water (I)	Rinse
5) Distilled water (II)	Rinse
Staining	
1) Harris's Alum Hematoxylin *(Note: filter before use)*	22 seconds
2) Running tap water	3 minutes
3) 0.2% hydrochloric acid (HCl)	3 seconds
4) Running tap water	3 minutes
5) 0.2% sodium bicarbonate (NaH_3CO_2) **Slides were examined to avoid overstaining*	1 minute
6) Running tap water	3 minutes
7) Distilled water (III)	Rinse
8) Eosin	1 minute
Dehydration	
1) 95 % alcohol (III)	1 – 2 seconds
2) 95 % alcohol (IV)	1 – 2 seconds
3) Absolute alcohol (I)	3 minutes
4) Absolute alcohol (II)	3 minutes
Clearing	
1) Xylene (III)	3 minutes
2) Xylene (IV)	3 minutes or more
Mount in Canada Balsam	

Nissl Staining Method

Step	Duration
Deparaffinization	
1) Xylene (I)	3 minutes or more
2) Xylene (II)	3 minutes or more
Hydration	
1) 95% alcohol (I)	3 minutes or more
2) 95% alcohol (II)	3 minutes or more
3) Coat with 1% celloidin	
4) 70% alcohol	3 minutes or more
5) Distilled water (I)	Rinse
6) Distilled water (II)	Rinse
Staining	
1) Cresyl violet	20 minutes
2) 70% alcohol **Slides were examined to avoid overstaining*	Rinse
Dehydration	
1) Tertiary Butyl Alcohol – TBA (I)	2 – 3 minutes
2) Tertiary Butyl Alcohol – TBA (II)	2 – 3 minutes
3) Tertiary Butyl Alcohol – TBA (III)	2 – 3 minutes
Clearing sections from dehydrant	
1) Xylene (III)	3 minutes
2) Xylene (IV)	3 minutes or more
Mount in DPX	

Lillie's variant of the Weil-Weigert Staining Method (Lillie, 1954)

Step	Duration
Deparaffinization	
1) Xylene (I)	3 minutes or more
2) Xylene (II)	3 minutes or more
Hydration	
1) 95% alcohol	3 minutes or more
2) 85% alcohol	3 minutes or more
3) 70& alcohol	3 minutes or more
Staining	
1) A mixture of equal volumes of 4% iron alum and 1% alcoholic hematoxylin solution (ripen for 1-5 days only) *Note: Stain in paraffin oven at 55-60°C*	40 minutes
Decolourizing	
1) 0.5% iron alum	30 – 40 minutes
2) Distilled water (I)	Rinse
Bluing	
1) 1% Borax + 2.5% potassium ferricyanide solution	10 minutes
2) Distilled water (II)	Rinse
Counterstain	
Safranin O in 1% acetic acid	5 minutes
Dehydration and clearing sections from dehydrant	
1) Acetone	3 minutes
2) Acetone + Xylene (1:1)	3 minutes
3) Xylene	3 minutes
Mount in DPX	

Modification of Golgi Staining Method

Step	Duration
Initial staining	
1) 3% potassium bichromate	3 – 4 days
2) 2% silver nitrate	1 – 3 days
Dehydration in graded alcohol *(refer to Chapter 2.3.4)*	
Clearing in toluene *(refer to Chapter 2.3.4)*	
Infiltration and embedding in paraffin *(refer to Chapter 2.3.4)*	
Sectioning and mounting of tissue sections *(refer to Chapter 2.3.4)*	
Deparaffinization	
3) Xylene (I)	3 minutes or more
4) Xylene (II)	3 minutes or more
Mount in DPX	

Thionin (Gurr, 1956)

Step	Duration
Initial staining	
1) 0.5% thionin in 10% formalin	5 – 7 days
Dehydration in graded alcohol *(refer to Chapter 2.3.4)*	
Clearing in toluene *(refer to Chapter 2.3.4)*	
Infiltration and embedding in paraffin *(refer to Chapter 2.3.4)*	
Sectioning and mounting of tissue sections *(refer to Chapter 2.3.4)*	
Deparaffinization	
2) Xylene (I)	3 minutes or more
3) Xylene (II)	3 minutes or more
Mount in DPX	

Appendix D – Preparation of chemical solutions for immunohistochemistry

1) **2% silane adhesive solution in acetone**

Silane	10ml
Acetone	500ml

2) **TBS-Tween 20 wash buffer**

Tris-buffered saline (TBS)	1000 ml
Tween-20	0.5 ml

3) **10% normal goat serum**

Normal goat serum	1000 µl
TBS	100 µl

4) **0.2% ammonia water solution (Bluing)**

Ammonium hydroxide (concentrated)	2 ml
Distilled water	1000 ml

Appendix E – Immunohistochemistry procedure

Step	Duration
Pre-warm slides at 50°C	20 minutes
Deparaffinization	
1) Xylene	20 minutes
Hydration	
1) Absolute alcohol	10 minutes
2) 95% alcohol	3 minutes
3) 70% alcohol	3 minutes
4) 50% alcohol	3 minutes
5) Distilled water	2 minutes
Epitope retrieval	
1) Tris/EDTA pH 9.0 buffer solution	20 – 30 minutes
2) Leave to cool at room temperature	20 minutes
3) Wash buffer (TBS-Tween 20)	Rinse
Endogenous peroxidase blocking	
1) Peroxidase-blocking solution (H_2O_2)	30 minutes
2) Wash in TBS-Tween 20	5 minutes
Normal serum blocking	
1) 10% normal goat serum	30 minutes
Primary antibody incubation (either one)	
1) Enkephalin (1:500), or	30 minutes
Substance P (1:500), or	30 minutes
Serotonin (1:200)	40 minutes
2) Wash in TBS-Tween 20 *(Repeat twice)*	5 minutes
Secondary antibody incubation	
1) Secondary antibody (Dako REAL™ Envision™ Detection System)	60 minutes
2) Wash in TBS-Tween 20 *(Repeat thrice)*	5 minutes
Incubation with chromogen	
1) DAB (Dako REAL™ Envision™ Detection System)	5 minutes
2) Distilled water	Rinse
Counterstaining	
1) Mayer's Hematoxylin	30 – 60 seconds
2) Distilled water	Rinse
3) 0.2% ammonia (NH_4OH) for bluing	10 seconds
4) Distilled water	Rinse
Mount in Glycergel	

Appendix F – Statistical analysis on neuronal somas occurrence using SPSS

Test 1

Objective : One-way ANOVA was conducted to compare the differences in the number of recorded somas among five somatal shapes (i.e. spindle, triangular, polygonal, tear and others).

DESCRIPTIVES

NO

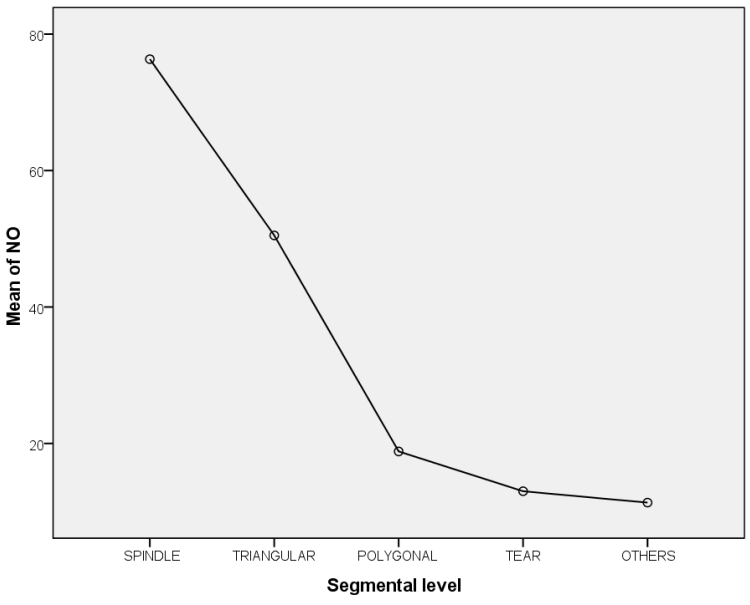
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
SPINDLE	6	76.33	18.970	7.745	56.43	96.24
TRIANGULAR	6	50.50	12.178	4.972	37.72	63.28
POLYGONAL	6	18.83	9.283	3.790	9.09	28.57
TEAR	6	13.00	3.795	1.549	9.02	16.98
OTHERS	6	11.33	7.005	2.860	3.98	18.68
Total	30	34.00	28.030	5.117	23.53	44.47

ANOVA

NO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19495.000	4	4873.750	37.046	.000
Within Groups	3289.000	25	131.560		
Total	22784.000	29			

MEAN PLOT



Conclusion : It was indicated that there was a significant difference in the numbers of recorded somas among the five somatal shapes [$F(4, 25) = 37.046$, $p<0.05$].

Test 2

Objective : One-way ANOVA was conducted to compare the differences in the number of neuronal somas among cervical, thoracic, lumbar and sacral regions.

DESCRIPTIVES

NO

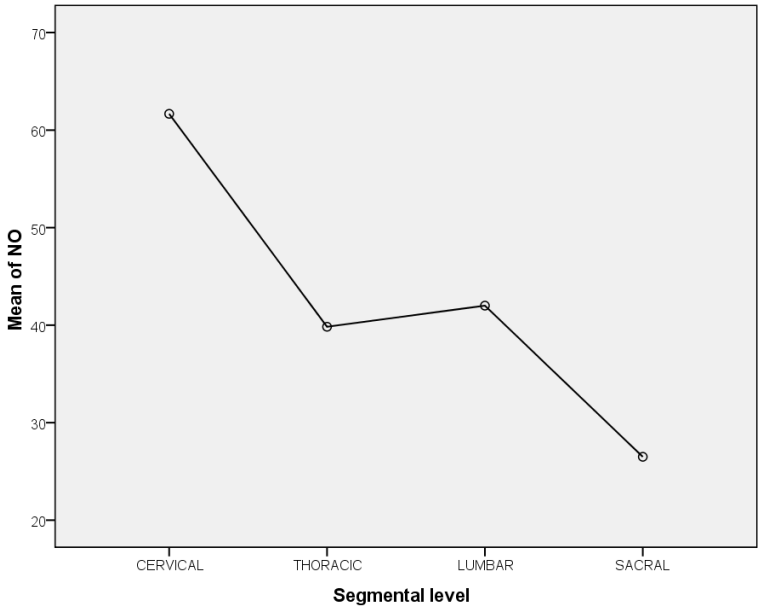
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CERVICAL	6	61.67	15.769	6.438	45.12	78.22	47	87
THORACIC	6	39.83	14.703	6.002	24.40	55.26	25	65
LUMBAR	6	42.00	11.524	4.705	29.91	54.09	30	62
SACRAL	6	26.50	8.871	3.622	17.19	35.81	16	42
Total	24	42.50	17.651	3.603	35.05	49.95	16	87

ANOVA

NO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3784.333	3	1261.444	7.460	.002
Within Groups	3381.667	20	169.083		
Total	7166.000	23			

MEAN PLOT



Conclusion : It was indicated that there was a significant difference in the numbers of neuronal somas among the four segmental levels [$F(3, 20) = 7.46$, $p<0.05$].

Test 3

Objective : One-way between subjects ANOVA was conducted to compare the differences in the number of recorded somas among seven fields of the spinal grey, i.e. dorsal field (DF), lateral field (LF), central field (CF), lateral motor field (LMF), medial motor field (MMF), ventrolateral field (VLF) and ventromedial field (VMF).

DESCRIPTIVES

NO

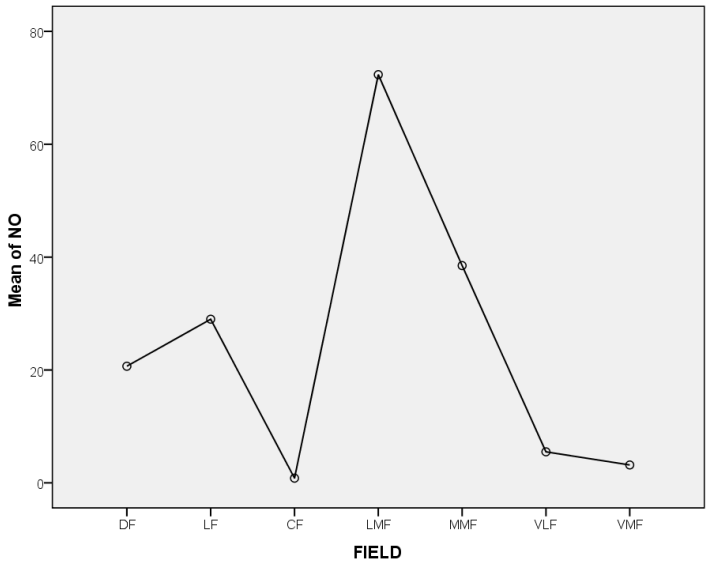
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
DF	6	20.67	5.465	2.231	14.93	26.40	15	30
LF	6	29.00	7.127	2.910	21.52	36.48	23	41
CF	6	.83	.753	.307	.04	1.62	0	2
LMF	6	72.33	20.412	8.333	50.91	93.75	57	113
MMF	6	38.50	11.077	4.522	26.88	50.12	23	56
VLF	6	5.50	2.811	1.147	2.55	8.45	3	9
VMF	6	3.17	1.329	.543	1.77	4.56	1	5
Total	42	24.29	25.433	3.924	16.36	32.21	0	113

ANOVA

NO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23369.238	6	3894.873	43.258	.000
Within Groups	3151.333	35	90.038		
Total	26520.571	41			

MEAN PLOT



Conclusion : It was indicated that there was a significant difference in the numbers of recorded somas among the seven fields of the spinal grey [$F(6, 35) = 47.258$, $p<0.05$].

Appendix G – Morphological profiles (in shape and size) of neuronal somas in different fields of spinal cord. The randomly-recorded number of somas measured is given in parentheses (n).

Field	Somatal shape	Somatal size in width × length, *μm*	(n)
Dorsal	Spindle	4–15 × 12–44	(29)
	Triangular	8–19 × 8–34	(18)
Lateral	Spindle	6–15 × 15–42	(23)
	Triangular	5–23 × 8–27	(16)
	Polygonal	6–14 × 6–14	(6)
	Tear	7–12 × 12–21	(11)
Central	Spindle	3–5 × 10–12	(6)
Ventrolateral	Spindle	8–20 × 9–35	(10)
	Triangular	8–20 × 12–20	(5)
Ventromedial	Spindle	8–17 × 14–34	(6)
	Triangular	10–17 × 16–26	(3)
Lateral motor	Spindle	6–29 × 16–82	(42)
	Triangular	9–32 × 12–64	(30)
	Polygonal	9–30 × 10–64	(20)
	Tear	8–25 × 15–56	(22)
Medial motor	Spindle	7–22 × 15–49	(41)
	Triangular	7–24 × 12–29	(15)
	Polygonal	6–29 × 11–39	(14)
	Tear	7–17 × 12–37	(11)

Appendix H – List of publications and presentations

PUBLICATIONS

Published Journal

a) ISI-Cited Publication

Tang, J.M.Y., and Durriyyah S. H. A. (2012). Histological characterization of spinal cord cytoarchitecture in Rice-Paddy Frog (*Fejervarya limnocharis*). *(Submitted to Sains Malaysiana)*

b) SCOPUS-Cited Publication

Tang, J.M.Y., Durriyyah S. H. A. and Belabut, D. (2010). Histological development of selected neural structures of Dark-sided Chorus Frog, *Microhyla heymonsi* (Amphibia: Anura). *Malaysian Journal of Science, 29*(1): 30-36.

Conference Proceeding

Tang, J. M. Y., and Durriyyah S. H. A. (2010). Histological Characterization of Frog (*Fejervarya limnocharis*) Spinal Cord Tissue Architecture. *In Proceedings of the International Anatomical Sciences and Cell Biology Conference* held on 26 May – 29 May 2011 at the Department of Anatomy, National University of Singapore, Singapore (pp. 116). Singapore: National University of Singapore

Chapter in Book

Tang, J. M. Y., Kwong, S. C., Mamat, M., and Durriyyah S. H. A. (2010). Diversity of a Central Nervous System Structure: Spinal Cord of a Terrestrial Frog in Comparison to an Aquatic Fish. In Hee et al. (Eds.). *Harnessing the Potential of Biodiversity* (pp. 300-303). Kuala Lumpur: Universiti Putra Malaysia. (ISBN 978-983-2519-03-4)

PRESENTATIONS

10 – 11 Oct 2011		Oral Presentation in Women in World Neurosciences Conference 2011, Universiti Brunei Darussalam, Brunei *Presentation Title: Histological Characterization of Fejervarya limnocharis (Grass Frog)Spinal Grey Cytoarchitecture]*
15 – 17 Dec 2010	:	Poster Presentation in the 15th Biological Sciences Graduate Congress (BSGC) 2010, University of Malaya *Poster Title: Morphology and Distribution of Neurons in the Spinal Cord Upper Half Grey Matter of Grass Frog (Fejervarya limnocharis)*
26 – 29 May 2010	:	Poster Presentation in International Anatomical Sciences and Cell Biology Conference (IASCBC) 2010, National University of Singapore *Poster Title: Histological Characterization of Frog (Fejervarya limnocharis) Spinal Cord Tissue Architecture.*
14 April 2010	:	Candidature Defence in Institute of Biological Sciences, Faculty of Science, University of Malaya *Presentation Title: Spinal Cord Cytoarchitectonic Organization of Fejerverya limnocharis in Relation to Nociceptive System.*
1 – 3 April 2010	:	Poster Presentation in Innovation and Creativity Expo University of Malaya 2010, University of Malaya Poster Title: Diversity of a Central Nervous System Structure: Spinal Cord of a Terrestrial Frog in Comparison to an Aquatic Fish.

17 – 18 Nov 2009 : Poster Presentation in *Simposium Biologi Malaysia 2009*, Universiti Putra Malaysia.

Poster Title: Diversity of a Central Nervous System Structure: Spinal Cord of a Terrestrial Frog in Comparison to an Aquatic Fish

1 – 2 Jul 2009 : Oral Presentation in Postgraduate Research Seminar Biohealth Science Programme 2009, Institute of Biological Sciences, Faculty of Science, University of Malaya.

Presentation Title: Spinal Cord Cytoarchitectonic Organization of *Fejerverya limnocharis* in Relation to Nociceptive System.

13 – 15 Jan 2009 : Poster Presentation in *Eskpo Penyelidikan, Rekacipta & Inovasi 2009*, University of Malaya.

Poster Title: Histological Development of Selected Neural Structures of Dark-sided Chorus Frog, *Microhyla heymonsi* (Amphibia: Anura)

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Tuan/Puan,

Manuskrip: "Histological Characterization of Spinal Cord Cytoarchitecture in Rice-Paddy Frog (*Fejervarya limnocharis*)"

Dengan hormatnya merujuk perkara di atas, sukacita dimaklumkan bahawa manuskrip tersebut telah pun diterima dan pihak kami akan menghubungi tuan/puan setelah manuskrip tersebut dibuat penilaian.

Terima kasih kerana menghantar sumbangan kepada Sains Malaysiana.

Yang benar,

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Histological Characterization of Spinal Cord Cytoarchitecture in Rice-Paddy Frog (*Fejervarya limnocharis*)

(Pencirian Histologi Sitoarkitektur Korda Spina Katak Sawah Padi, *Fejervarya limnocharis*)

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ABSTRACT

Indisputably, most neurohistological researches to date were largely done on the mammalian system. Therefore, this study served as part of initial efforts in establishing a non-mammalian species as one of the alternative model systems to counter the overemphasis of mammalian model in neurobiology. Using a locally available Amuran, *Fejervarya limnocharis* or rice-paddy frog as experimental animal, this study described the anatomy of the frog spinal cord in terms of its general organization and the cytoarchitectonic characteristics by employing neurohistological method in combination with light microscopy. Stained transverse sections of the frog spinal cord revealed a classical organization as commonly seen in higher vertebrates, with the composition of inner grey and outer white matter layers, surrounding the central canal. From the cytoarchitectonic aspect, poor differentiation of the frog spinal grey resulted in the failure of employing the conventional Rexed's laminae. This study instead, adapted the Ebbeson's system that demarcated this area into seven fields with no clear-cut margins: dorsal field, lateral field, central field, ventrolateral field, ventromedial field, lateral motor field and medial motor field. The grey matter appeared to be morphologically heterogenous, exhibiting different profiles of the neuronal soma morphology. The frog spinal grey cytoarchitectonic subdivision in accordance

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Vol 29 • No. 1 • April 2010

CONTENTS

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Malaysian Journal of Science **29** (1): 30-36 (2010)

Histological Development of Selected Neural Structures of Dark-Sided Chorus Frog, *Microhyla heymonsi* (Amphibia: Anura)

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Received on 2nd February 2010, accepted in revised form 18th March 2010.

ABSTRACT The development of selected neural structures of Dark-sided Chorus Frog, *Microhyla heymonsi*, from tadpole to adult was characterized from the histological aspect. Six developmental stages studied were the early tadpole, pre-metamorphosis, pro-metamorphosis, metamorphic climax, froglet and the adult. For *M. heymonsi*, metamorphosis is part of a normal developmental process, during which it undergoes transition from an aquatic tadpole into a terrestrial frog. The four neural structures focused on were: (a) cerebral hemispheres of the forebrain, (b) optic lobe of the midbrain, (c) medulla oblongata of the hindbrain, and (d) spinal cord. The specimens were processed for visualization according to H&E histological staining technique. The observed histological changes and the increase in size of each neural structure were in turn correlated to the external morphology and behaviour of different stages of the developing *M. heymonsi*. Changes in the cellular distribution, especially in the area surrounding the ventricle and other specific changes of the selected neural structures are reported. Apart from that, results also showed changes in the ventricular shape and/or size of each structure. All the apparent changes detected, possibly, represented the maturation of the neural structures in correlation to the metamorphosis process. Results from the light microscopy observations are presented in the form of photomicrographs and sketches with highlights of changes of the selected neural structures.

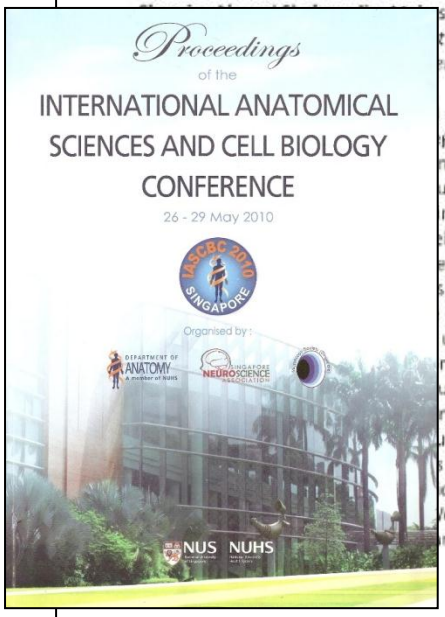
ABSTRAK Perkembangan struktur-struktur saraf tertentu bagi Katak Padi Rusuk-hitam, *Microhyla heymonsi* telah dicirikan dari aspek histologi. Enam peringkat perkembangan yang telah dikaji meliputi peringkat berudu awal, pra-metamorfosis, pro-metamorfosis, metamorfosis klimaks, katak kecil dan katak dewasa. Untuk *M. heymonsi*, metamorphosis merupakan sebahagian proses perkembangan dalam kitar hidupnya yang melibatkan peralihan peringkat berudu yang bersifat akuatik ke peringkat katak yang hidup di daratan. Empat struktur yang diberikan tumpuan adalah: (a) bahagian serebrum yang dijumpai di otak hadapan, (b) lobus optik yang berada di otak tengah, (c) medula oblongata yang merupakan sebahagian daripada otak belakang dan (d) bahagian korda spina. Spesimen tersebut diproses untuk tujuan visualisasi menerusi teknik pewarnaan H&E. Perubahan dalam ciri-ciri histologi dan peningkatan saiz struktur-struktur saraf tertentu telah dihubungkaitkan dengan morfologi luaran dan tabiat pada peringkat berbeza. Perubahan dari segi taburan sel-sel khususnya di kawasan sekitar ventrikel dan perubahan spesifik yang lain juga dilaporkan. Selain itu, keputusan juga menunjukkan perubahan dari segi bentuk ventrikel dan/atau saiz bagi setiap struktur. Perubahan nyata yang dapat diperhatikan berkemungkinan mewakili struktur saraf yang matang. Keputusan daripada pemerhatian mikroskopik cahaya dibentangkan dalam bentuk fotomikrograf dan lakaran.

(Keywords: Dark-sided Chorus Frog, *Microhyla heymonsi*, neural structures, histological development)

INTRODUCTION

Information regarding studies done on the nervous tissue of non-mammalians is relatively scarce, owing to the intricacy of processing such tissue in view of the fact that this tissue does not render itself well to histological techniques. Thus, established histological reports involving such tissues are not as abundant as for the diverse reports on mammalian neural tissue. The situation is more critical for the local amphibians, although Malaysia is endowed with rich fauna diversity.

Being one of the smallest local frog species from the Microhylidae family, *Microhyla heymonsi* (Vogt, 1911) or commonly known as the Dark-sided Chorus Frog [1] can be easily distinguished due to its distinctive black lateral bands at the sides of the head



28-006

Presentation slot: 28 May, 10:00-10:30

HISTOLOGICAL CHARACTERIZATION OF CAUDALLY DIRECTED CEREBELLUM OF *OREOCHROMIS SP.* (TILAPIA) AND *LEPTOBARBUS HOEVENI* (JELAWAT)

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metencephalic structure of the rhombencephalon. As in other vertebrates, gulating the control of fish movements; related to maintenance of posture ments. Past studies revealed existence of either rostrally or caudally directed udally oriented cerebellum of two Malaysian fish species were looked at as ng Malaysian freshwater fish central nervous system database. The external ebellum of *Oreochromis sp.* (Tilapia) and *Leptobarbus hoeveni* (Jelawat) were ellum of both fishes were histologically shown to be made up of mostly cor- l, the architectonic of cerebellar cortex in both fishes appeared similar, *i.e.* outermost, followed by Purkinje and granular neuronal layers towards the up the inner region of densely packed stained neuronal somas in cerebellum n was not intensely stained. Transverse and longitudinal sections of Jelawat's unique structure of the innermost area. Transverse sections of Jelawat's cer- n of the innermost area. In addition, at its dorsal middle portion, a triangular urface of cerebellum was observed. In contrast, a 'dome like' pattern of in- s observed in Tilapia's cerebellum transverse sections. Significance of these ological functions of the cerebellum should be further studied. Longitudinal Veil's myelin staining showed fibers in the white matter, which existed within rea. These fibers could be mossy and climbing fibers as had been reported in

28-007

Presentation slot: 28 May, 13:30-14:00

HISTOLOGICAL CHARACTERIZATION OF FROG (*FEJERVARYA LIMNOCHARIS*) SPINAL CORD TISSUE ARCHITECTURE

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The intricacy of processing the non-mammalian tissues, particularly the nervous tissue, due to its nature that scarcely renders itself well to the histological methods, has brought around the lack of established histological reports regarding such tissue at present. This study attempts to delineate the cytoarchitectural organization of the frog spinal cord from the histological approach. *Fejervarya limnocharis* or the Grass Frog was selected as the experimental model whereby its spinal cord was abstracted and processed according to three respective neurohistological stainings, *i.e.* the H&E, Nissl and Lillie's variant of the Weil-Weigert staining techniques. Each of them provides a different comparative emphasis of cellular structures within the spinal cord sections. Supported with photomicrographs, the analysis focused on the representative sections of four spinal cord segmental levels namely cervical, thoracic, lumbar and sacral. The stained transverse sections of the frog spinal cord revealed a classical organization as commonly seen in higher vertebrates, with the composition of inner grey and outer white matter layers, surrounding the central canal. The cytoarchitecture of frog spinal cord exhibits distinctive anatomical and histological characteristics at different levels in terms of size or diameter of a cross section, presence of enlargement, proportion of grey and white matter and the concentration of neuronal parcellation. The findings of this study showed that structural and histological similarities in the spinal cord exist between the species being investigated and those of other Anurans from past researches.

Diversity of a Central Nervous System Structure: Spinal Cord of a Terrestrial Frog in Comparison to an Aquatic Fish

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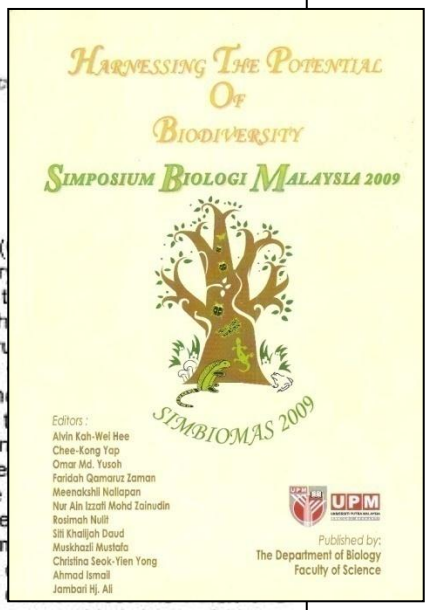

Abstract

Besides the brain, spinal cord is another central nervous system (significant role in a vertebrate's daily activities. The control of voluntar body is directly related to the spinal cord. The nature of the nervous t cord, reflects the possible movements which formed the animals' beh conducted to highlight the anatomical differences of the spinal cord stru with different living behaviour. The experimental models being studied Grass Frog (*Fejervarya limnocharis*) and Catfish (*Clarias* sp.). The form inhabits cleared as well as disturbed land areas while the latter adopts most of its life in shallow, open waters. Gross morphological analysis ar H&E and Nissl staining methods were employed. Comparatively, the fe spinal tissues of both species differed in terms of shape, the presence levels along the spinal cord, as well as the distribution patterns of the gre these dissimilarities could be associated with their distinctive mode of m to the different natural habitats they live in, for instance, the execution frog on firm grounds and body undulation of the catfish with the help overall organization of spinal cord between both species nevertheless resembled those of higher vertebrates.

Introduction

The spinal cord is no less essential than the major controlling centre, i.e. the brain in any living vertebrates especially when it comes to mediating locomotory movement and reflex control of the body (Oksche and Ueck, 1976). Two experimental models with each demonstrating different modes of locomotion, *Fejervarya limnocharis* (Grass Frog or 'Katak Sawah') and *Clarias* sp. (the Catfish or 'Keli'), were put under scrutiny in this anatomical study of the spinal cord structure from both the morphological and histological aspects. The terrestrial frog spends most of its life on land as opposed to the catfish that adopts the aquatic lifestyle in shallow, open waters. Originating from such distinctive habitats, these two animals display differences in their own means of movement. With the presence of fore- and hind limbs, the frog is capable of executing saltatory movements on the firm ground, e.g. jumping, leaping and hopping. Conversely, the fish maneuvers its limbless body in the water to move by body undulation together with the help of fins (Bond, 1979).

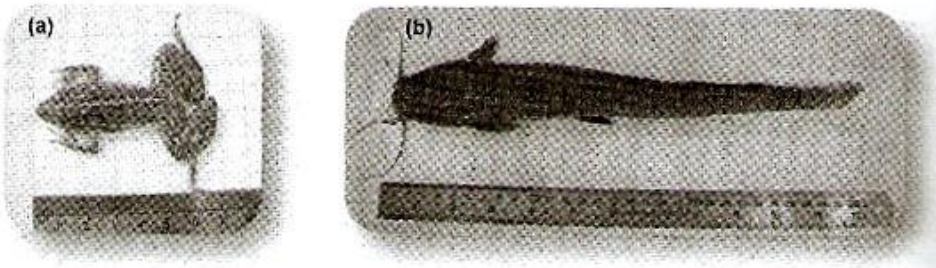

Figure 1: Dorsal view of (a) *Fejervarya limnocharis* (b) *Clarias*

Materials and Methods

Hee et al. (eds.). Harnessing the Potential of Biodiversity, page 300-303

Histological Characterization of *Fejervarya limnocharis* (Grass Frog) Spinal Grey Cytoarchitecture

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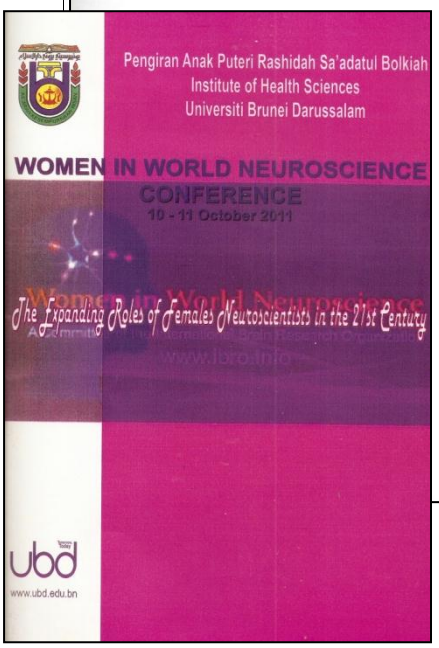
Using *Fejervarya limnocharis* or Grass Frog as experimental model, the spinal cord cytoarchitectonic organization was studied by employing neurohistological methods in combination with light microscopy. Analysis of representative frog spinal cord Nissl- and Golgi-stained transverse sections demonstrated comparable general histological features to the mammalian spinal cord. On cytoarchitectonic level, poor differentiation of the frog spinal grey matter made it impossible to use the conventional mammalian Rexed's laminae nomenclature. Instead, findings of this study adapted the Ebbeson's system that demarcated the spinal grey area into eight fields with no clear-cut margins: dorsal field (DF), lateral field (LF), central field (CF), ventrolateral field (VLF), ventromedial field (VMF), lateral motor field (LMF) and medial motor field (MMF). The grey matter appeared to be morphologically heterogenous, with somas differing in shape and size. DF, or the presumable dorsal horn, failed to reveal any significant differential identification of each Rexed lamina I-IV normally making up the mammalian dorsal horn. Correlating to lamina V-VI, LF showed segregation of lateral and medial zones, with the former having larger somas than those found in the latter. CF, a region with occasional occurrence of neuronal somas surrounding the central canal, was homologously positioned as lamina X. Spinal grey ventral horn constituted the remaining fields. VLF and VMF, comprised of similar sized somas in low densities, were in correspondent to lamina VII and VIII, respectively. Presence of large motor neurons, especially pooled in LMF and MMF of cervical and lumbar spinal enlargements were observed. Both fields were collectively equivalent to Rexed lamina IX. Thus, the frog spinal grey cytoarchitectonic subdivision was fairly comparable to the laminar arrangement but with one or more laminae likely representing a homolog region/field. Further studies are required to reveal additional features, which could assist in more specific identification of the frog spinal grey neuronal population.



PT1- 15

Morphology and Distribution of Neurons in the Spinal Cord Upper Half Grey Matter of Grass Frog (*Fejervarya limnocharis*)

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Frog's spinal grey matter, in general, is poorly d conventional lamination and nuclear group system to was hardly applicable. Thus, following the Ebbeson spinal grey dorsal horn is comparable to the d intermediate zone formed the lateral field (located field) as well as the central field (surrounding However, no clear-cut boundaries could be ascertai division. Spinal cord of *Fejervarya limnocharis* o histologically processed according to two staining te and modification of Golgi methods for visualizati somas. Focusing on representative sections of the ce lumbar spinal segments, general neuronal soma distribution pattern of different somatal cell types v lateral field has presumably higher neuronal popula the loosely packed dorsal field, while central field appeared to be greatly concentrated with supporting glial cells with scant amount or almost none recognizable neuronal somas being observed. The identifiable neuronal somas in these areas were primarily categorized based on its shape: fusiform, pyramidal and multipolar. For cells with indeterminate shapes, they were grouped as unclassified. The overall proportion of the four morphological cell types were 60% fusiform, 19% pyramidal, 9% multipolar and 12% unclassified, with fusiform type having the highest distribution in all fields. Unlike in mammals, there was no specific pattern of cell arrangement in the frog spinal grey to accurately define the investigated fields.

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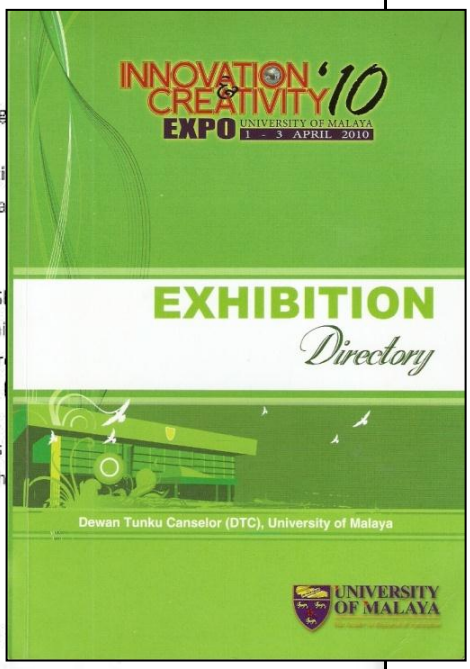
Contact (Off): 03- 79674213
Contact (Email): durriyyah@um.edu.my
Co-Researcher/s: Mahassan Bin Mamat, Joan Tang May Yin, Kwong Soke Chee
Synopsis: Two experimental models, each demonstrating different modes of locomotion, *Fejervarya limnocharis* or (Katak Sawah) and *Clarias* sp. or (Keli), were put under scrutiny in this anatomical study of the spinal cord structure from both the morphological and histological aspects. Comparatively, the features observed between the spinal tissues of both species differed in terms of shape, the presence of enlargements at particular levels along the spinal cord as well as the distribution patterns of the grey and white matters. Some of these dissimilarities could be associated with their distinctive mode of movements in correspondence to the different natural habitats they live in. The overall organization of spinal cord between both species nevertheless resembled those of higher vertebrates.



Project ID: GB05710
Project Title: Diversity of Cerebellum in Relation to Swimming
Fishes.
Principal Researcher: Assoc. Prof. Dr. Durriyyah Sharifah Binti
Address: Institut Sains Biologi, Fakulti Sains, Universiti Malaya
Contact (Off): 03- 79674213
Contact (Email): durriyyah@um.edu.my
Co-Researcher/s: Mahassan Bin Mamat, Shamiza Bt Ahmad S
Synopsis: Just as in other vertebrates, the cerebellum, a h
structure of teleosts is expected to play an important role in r
general function is related to maintenance of posture and
which are very much involved in swimming. Thus, swimming
species should be controlled by cerebellum. The cerebellums
looked at as part of an ongoing study in developing local fresh

Project ID: GB05810
Project Title: The Gross Morphology and Histology of Wound
Treatment: *A Preliminary Study*
Principal Researcher: Assoc. Prof. Dr. Durriyyah Sharifah Binti Haji Hasan Adli
Address: Institut Sains Biologi, Fakulti Sains, Universiti Malaya 50603, W.Persekutuan Kuala Lumpur
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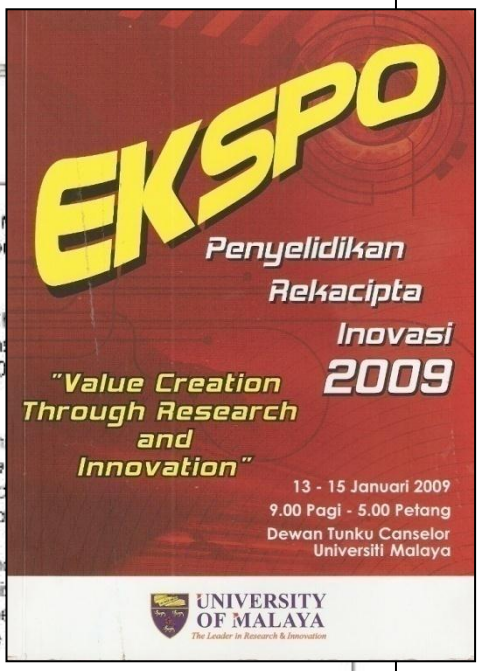
Co-Researcher/s: Tan Mui Koon, Prof. Dr. Kamaruddin Bin Mohd Yusoff
Synopsis: This preliminary study from the gross morphological and histological aspects of the wound healing process was carried out to contribute additional data for the efficacy of Gelam honey, using male *Sprague-Dawley* rats and full-thickness incisional wounds. On the back of each rat, two full



27 **Tajuk Penyelidikan** : Genotypic Characterization of f
Klebsiella pneumoniae, Pseudo
enterica

Ketua Penyelidik : Profesor Dr. Thong Kwai Lin
Pembantu Penyelidik : Lim King Ting / Dr. Yeo Chiew Ch
Jabatan / Fakulti : Institut Sains Biologi, Fakulti Sains
Tel / Fax / Emel : 03-79674437 / 5836 / 03-7967590

Ringkasan Penyelidikan :
The emergence of MDR bacteria poses a serious antim
E. coli (EC), 51 *K. pneumoniae(KP)* and 48 *P. aeruginosa*
resistotypes and genotypes by disk diffusion metho
multiplex PCR for simultaneous detection of integrons a
EC, 31 *KP* and 33 *PA* strains were MDR. Majority of
producers. Integrons were detected in 55%, 39% an
respectively. All the ESBL producers had large transmi
showed that the strains were diverse and heterogene
understanding of the problems of MDR strains as these
on conjugative plasmids.

28 **Tajuk Penyelidikan** : Histological Development of Selected Neural Structures of Dark-sided Chorus Frog, Microhyla heymonsi (Amphibia: Anura)
Ketua Penyelidik : Profesor Madya Dr. Durriyyah Sharifah Binti Haji Hasan Adli
Pembantu Penyelidik : Joan Tang May Yin / Daicus Anak Belabut
Jabatan / Fakulti : Institut Sains Biologi, Fakulti Sains
Tel / Fax / Emel : 03-79674213 / durriyyah@um.edu.my

Ringkasan Penyelidikan :
Nature of non-mammalian neural tissue (e.g. amphibian neural tissue) does not render itself well to histological processing, thus the lack of established reports on it. The study looked at histological development of selected neural structures of a commonly found frog in Malaysia, the Dark-sided Chorus frog or *Microhyla heymonsi*, one of the smallest Microhylid species. Six developmental stages studied, including its metamorphosis from an aquatic tadpole into a terrestrial frog, were early tadpole, pre-metamorphosis, pro-metamorphosis, metamorphic climax, froglet and adult. The four neural structures focused on were cerebrum of forebrain, optic lobe of midbrain, medulla oblongata of hindbrain, and spinal cord. Specimens were processed according to H&E staining technique. Light microscopy observations are presented in the form of photomicrographs with highlights of changes of the selected neural structures.

29 **Tajuk Penyelidikan** : Peptides from plant and bacterial isolates
Ketua Penyelidik : Profesor Madya Dr. Koshy Philip
Pembantu Penyelidik : Saravana Kumar A/L Sinniah /
Profesor Madya Dr. Sekaran A/L Muniandy
Jabatan / Fakulti : Institut Sains Biologi, Fakulti Sains
Tel / Fax / Emel : 03-79675839/ kphil@um.edu.my

Ringkasan Penyelidikan :
This project undertook to screen and characterizes novel peptides from plant and bacterial sources with potential inhibitory properties on Gram positive and negative bacteria. 16s RNA sequencing methods were employed to identify the bacterial sources from which these peptides were isolated. The pepides were fractionated using cationic exchange chromatography and further purified and characterised by HPLC and mass spectrometry.