UNIVERSITI PUTRA MALAYSIA

BEHAVIOUR AND PHYSIOLOGY ASSESSMENT OF FALSE GHARIAL [Tomistoma schlegelii (Müller, 1838)] IN CAPTIVITY IN PENINSULAR MALAYSIA

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

October 2017

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DEDICATIONS

This thesis is dedicated specially to my beloved parents, Ab Latip Mohd Aref and Normala Kadir. A lot of thanks also goes to my siblings, lecturers and friends for all the tremendous support, prayers and encouragement during my entire postgraduate life.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of the requirement for the Degree of Master of Science

BEHAVIOUR AND PHYSIOLOGY ASSESSMENT OF FALSE GHARIAL [*Tomistoma schlegelii* (Müller, 1838)] IN CAPTIVITY IN PENINSULAR MALAYSIA

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Chairman : Tengku Rinalfi Putra Tengku Azizan, PhD
Faculty : Veterinary Medicine

Tomistoma schlegelii or the "False Gharial" is one of the crocodile species in Malaysia. It is one of the largest extant crocodilians, restricted to Southeast Asia. Investigation of old localities show that the species may still be present, but the basic ecology and behaviour of this species still remain undocumented. Captive False Gharial of 2 cohorts, adult and juvenile, were restrained and sampled from the Semi wild captive environment, National zoo and Taiping zoo. The research has 3 objectives. (i) to identify the bacterial flora in cloacal cavities of captive and semi-wild False Gharial in Peninsular Malaysia, (ii) to determine the blood profile of captive and semi-wild False Gharial in Peninsular Malaysia and (iii) to determine nesting behaviour and ecology of False Gharial in natural pond. Bacterial cultures were done from cloacal swabs collected from 10 samples of captive False Gharial. 13 species of bacteria were cultured from 42 isolates. Each individual crocodiles yielded 3 to 5 bacterial species and no crocodiles were found to yield a single species. The most commonly isolated bacteria were *Proteus vulgaris* and *Salmonella* spp. and all the bacteria were gram negative. Most of the genera belong to the family Enterobacteriaceae. Blood was collected from the lateral coccygeal vein and used for identification of the wide range of haematological and biochemical parameters as a preliminary study on this species. The values were compared between the sexes, ages and location of False Gharial. Comparison was also done with other species in the same family *Gavialis gangeticus*, Indian Gharial, False Gharial in the previous study and *Crocodylus porosus*, Salt-water Crocodile. Peripheral blood cells of False Gharial showed erythrocyte cell which is an oval in shape and with centrally located prominent round or oval nucleus. Erythrocyte and haemoglobin count in False Gharial that placed at zoos had significantly lower mean than False Gharial at semi wild captive environment. The mean of white blood cell was significantly lower in Flase Gharial that kept at zoos than semi wild captive environment. The average most abundant leukocyte type in False Gharial was heterophils (56.90%), followed by lymphocytes (17.60%), monocytes (10.60%), eosinophils (9.70%), basophils (5.40%) and thrombocytes (3.70%). Serum biochemistry showed total protein, albumin, potassium, bilirubin, creatine kinase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

were higher in Flase Gharial at zoos than False Gharial at semi wild captive environment. For the nesting behavioural ecology of captive False Gharials, observation were conducted in semi wild captive environment because the natural substrate of the captive pond simulates the wild environment. Throughout the study, one female individual laid eggs twice, 34 eggs and 28 eggs per clutch, respectively, which is in June 2013 and June 2014. Unfortunately all the eggs were infertile. This species build the nest mounds from sand, soil, dried leaves and twigs. The composition ratio of vegetation matters to soil is 60:40, while the temperature range that was recorded is between 29°C – 32°C.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENILAIAN KELAKUAN DAN FISIOLOGI BUAYA JENJULUNG [*Tomistoma schlegelii* (Müller, 1838)] DALAM KURUNGAN DI SEMENANJUNG MALAYSIA

Oleh

MOHD QAYYUM BIN AB LATIP

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Tomistoma schlegelii atau "Buaya Jenjulung" adalah salah satu spesies buaya di Malaysia. Ia adalah salah satu buaya paling besar, yang hanya terdapat di kawasan Asia Tenggara. Penyiasatan terdahulu menunjukkan bahawa spesies masih lagi wujud, tetapi kajian asas tentang ekologi dan kelakuan spesies ini masih banyak yang belum didokumentasikan. Buaya Jenjulung di dalam kawasan kurungan dari 2 kumpulan, dewasa dan hamper matang, di kawasan kurungan separa liar, Zoo Negara dan Zoo Taiping telah digunakan sebagai sampel dalam kajian ini, Kajian ini mempunyai 3 objektif. (i) untuk mengenal pasti bakteria flora dalam rongga cloacal daripada Buaya Jenjulung di dalam kawasan kurungan dan kawasan kurungan separa liar di Semenanjung Malaysia, (ii) untuk menganalisis profil darah dari Buaya Jenjulung di dalam kawasan kurungan dan kawasan kurungan separa liar di Semenanjung Malaysia dan (iii) mengkaji kelakuan Buaya Jenjulung semasa bersarang dan ekologi sarang di dalam habitat semula jadi. Kultur bakteria telah dilakukan dengan menggunakan sampel swab cloaca dari 10 ekor Buaya Jenjulung di dalam kawasan kurungan. 13 spesis bakteria telah dikultur daripada 42 pengasingan. Setiap individu menghasilkan 3 ke 5 spesis bakteria dan tiada individu yang menghasilkan 1 spesis bakteria. Pengasingan bakteria yang paling tinggi adalah *Proteus vulgaris* dan *Salmonella spp.* dan semua bakteria adalah dari gram negatif. Kebanyakkan genera bakteria yang diperolehi adalah dari keluarga Enterobacteriaceae. Darah telah diambil daripada vena coccygeal lateral dan digunakan untuk pengenalpastian julat bagi parameter hematology dan biokimia sebagai kajian awal mengenai spesies ini. Nilai yang diperoleh kemudian dibandingkan diantara jantina, peringkat umur dan lokasi bagi semua Buaya Jenjulung dalam kajian ini. Perbandingan juga dilakukan dengan spesis buaya lain yang tergolong dalam keluarga yang sama iaitu *Gavialis gangeticus*, Indian Gharial, buaya Jenjulung daripada kajian lepas dan *Crocodylus porosus*, buaya Tembaga. Sel-sel darah periferal Buaya Jenjulung menunjukkan sel darah merah yang bentuknya bujur dan dengan pusingan menonjol ditengah-tengahnya atau nucleus berbentuk bujur. Sel darah merah dan hemoglobin dalam buaya yang diletakkan di zoos mempunyai min yang lebih rendah berbanding buaya di kawasan kurungan separa liar. Jumlah min bagi sel darah putih adalah lebih

rendah pada buaya yang diletakkan di zoo berbanding Pusat Pemuliharaan Hidupan Liar. Purata sel darah putih paling banyak adalah heterofil (56.90 %), diikuti dengan limfosit (17.60 %), monosit (10.60 %), eosinofil (9.70 %), basofil (5.40 %) dan trombosit (3.70%). Bacaan biokimia serum menunjukkan jumlah protein, potassium, bilirubin, creatine kinase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) dalam haiwan jantan lebih tinggi berbanding adalah lebih tinggi pada buaya di zoo berbanding buaya di kawasan kurungan separa liar Sungai Dusun. Untuk. Untuk kajian ekologi kelakuan bersarang bagi Buaya Jenjulung di dalam kawasan kurungan, pemerhatian telah dijalankan di kawasan kurungan separa liar kerana substrat semula jadi yang terdapat dalam kawasan kurungan tersebut menyamai persekitaran liar bagi sepsis ini. Sepanjang kajian dijalankan, seekor haiwan betina telah bersarang dan bertelur sebanyak dua kali, 34 biji telur dan 32 biji telur dalam setiap sarang masing-masig pada bulan Jun 2013 dan bulan Jun 2014. Malangnya tiada persenyawaan yang berlaku pada kesemua telur tersebut. Spesis ini membuat sarang jenis busut. Bahan-bahan dari persekitaran yang digunakan untuk membina sarang seperti pasir, tanah, daun-daun kering dan ranting-ranting kecil. Nisbah komposisi bahan-bahan dari tumbuhan kepada tanah adalah 60:40, manakala julat suhu yang telah dicatatkan adalah antara 29°C - 32°C.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the Degree of Master of Science. The members of the Supervisor Committee were as follows:

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LIST OF ABBREVIATIONS

A:G	Albumin : Globulin
Alb	Albumin
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
Ca^{2+}	Calcium
CITES	Conventional of International Trade of Endangered
CK	Creatine Kinase
Cl^-	Chloride
cm	Centimetre
°C	Degree Celsius
DNA	Deoxyribonucleic Acid
DWNP	Department of Wildlife and National Parks
EDTA	Ethylenediaminetetraacetic acid
EMB Agar	Eosin Methylene Blue Agar
G	Gauge
g	Gram
g/dL	Grams Per Decilitre
GGT	Gamma-glutamyltransferase
Hb	Haemoglobin
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
ind./km^2	Individual per Kilometre Square
IU	International Unit
IUCN	International Union for Conservation of Nature
K^+	Kalium / Potassium
kg	Kilogram
km^2	Kilometre Square
L	Litre
m	Meter
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Haemoglobin Concentration
mm^3	Millimetre Cubic
mL	Millilitre
MR	Methyl Red
mtDNA	Mitochondrial DNA
N	Number of Sample

Na^+	Natrium / Sodium
PCV	Packed Cell Volume
PERHILITAN	Perlindungan Hidupan Liar dan Taman Negara
PO_4^{2-}	Phosphate
PP	Plasma Protein
RBC	Red Blood Cell
rpm	Revolutions per minute
RV	Rappaport Vassiliadis
S.I. unit	Standard International unit
Spp.	Species
SS Agar	Salmonella-Shigella Agar
TB	Total Bilirubin
TP	Total Protein
UPM	University Putra Malaysia
VP	Voges-Proskauer
WBC	White Blood Cell
XLD	Xylose Lactose Sodium Deoxycholate
μm	Micrometre
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Introduction

The False Gharial (*Tomistoma schlegelii*) or also known as Malayan Gharial is one of the species in the family, Gavialidae. Gavialidae is a family of reptiles, in the order of Crocodilia. Gavialidae have typically consisted of only one surviving species, the Indian Gharial (*Gavialis gangeticus*), which originate from India. The False Gharial was previously classified in the family Crocodylidae based on several features and characters including skull morphology. After deliberation, biologist suggested it to be classified as a Gavialidae base on common similarity in morphology and behaviour. Molecular studies have consistently indicated that the two species have a very close relationship, supporting the argument that they are in the same family. Currently, False Gharial is classified as a Gavialidae under the subfamily Tomistominae and Genus *Tomistoma* (Mader, 2006). False Gharial is a freshwater crocodilian with a distinctively narrow and long snout. Previous study reported that the male False Gharial can grow up to 4 to 5 meter maximum (Staniewicz and Behler, 2011).

False Gharial is endemic to Indonesia and Malaysia (Bezuijen *et al.*, 2001; Auliya *et al.*, 2006). Currently there is no definitive population estimate for the False Gharial, and their distribution is limited to Indonesia (Kalimantan, West Java, and East Sumatra), Malaysia (Peninsular Malaysia, Sarawak) and Brunei. This species was reported to be extinct in Thailand where the last sightings were made in 1970 (Steubing *et al.*, 2006). The previous survey studies have all shown a gradual decline of False Gharial sightings in Sumatra and Kalimantan from the 1990s to 2000s (Bezuijen *et al.*, 1998; Bezuijen *et al.*, 2001; Auliya *et al.*, 2006) while in Malaysia sightings have been rarely conducted since the late 1980s (Cox and Gombek, 1985; Sebastian, 1993; Simpson *et al.*, 1998).

The current status of captive False Gharial held at various facilities around Peninsular Malaysia is approximately 6 animals at the National Zoo, 9 animals at the Wildlife Conservation Centre Sungai Dusun, 4 animals at the Malacca Zoo, 2 animals at the Malacca Crocodile Farm and 2 animals in the Taiping Zoo. Natural breeding of captive False Gharial in Malaysia have only been documented in the National Zoo with four hatchlings in 2003 (Mathew *at el.*, 2011) and the Jong Crocodile Farm, Sarawak with 19 hatchlings produced from 1996-2001 (Steubing *et al.*, 2006).

The main threats to this species were habitat destruction due to land used for development, agricultural activity, illegal logging and forest fires which have been strongly linked with their extinction (Auliya *et al.*, 2006; Stuebing *et al.*, 2006; Bezuijen *et al.*, 2010; Staniewicz and Behler, 2011). False Gharial was previously classified as endangered before being lowered to vulnerable in the International Union for Conservation of Nature (IUCN) Red List (IUCN 2016) due to insufficient data. The species is also listed under Conventional of International Trade of Endangered (CITES)

and protected by the United State Endangered Species Act and Indonesian laws. In Malaysia, False Gharial has been listed as totally protected under the Act 716, Wildlife Protection Act 2010. They are protected within Peninsular Malaysia by the Department of Wildlife and National Park (PERHILITAN).

To date, research in both Malaysia and Indonesia have focused on the census of False Gharial in natural habitats and issues that related to conservation of habitat for this species (Bezuijen *et al.*, 1998; Bezuijen *et al.*, 2001; Bezuijen *et al.*, 2002; Bezuijen *et al.*, 2004; Auliya *et al.*, 2006; Stuebing *et al.*, 2006; Stuebing, 2014).

1.2 Problem statements

False Gharial is not breeding well in captivity in Peninsular Malaysia. In order to prevent this species from extinction, assisted breeding in captivity under semi-wild environment is being carried out in PERHILITAN Wildlife Conservation Centre, Sungai Dusun, as well as a population study in the wild (Bezuijen *et al.*, 2001). Unfortunately health status of captive False Gharial in Peninsular Malaysia is not known because of the lack of studies on the animal. The living conditions of the False Gharial in the wild is not fully documented because they are very elusive, therefore knowledge on how to emulate the captive environment for the captive False Gharial is lacking. Being a reptile, physiological baseline data is not the same as mammals, therefore creating difficulty in referring to the normal range value. A deficient normal flora may be one of the factors predisposing farmed or captive crocodiles to enteritis and usually associated with septicaemia (Lovely and Leslie, 2008). One of the method to monitor and control the health status of the False Gharial is by clinical pathology evaluation. Clinical pathology can be used to monitor the health status of the species, but basic haematology and plasma biochemistry values need to be first established from the False Gharial. Until today, haematology and plasma biochemistry values and also the normal intestinal flora of the False Gharial have not been properly documented in Malaysia. Another significant issue regarding the False Gharial is the breeding season. Physiological wellbeing, the environment and meteorological factors might affect the breeding mechanism. To date, there is no proven information on the breeding season of the species. This animal listed as Totally Protected under the Act 716, Wildlife Protection Act 2010. Being a potentially dangerous animal, a totally protected one and an exotic, it is very difficult to handle, restrain and sedate without issues of safety, welfare and accuracy.

1.3 Objectives

i. To identify the bacterial flora in cloacal cavities of captive and semi-wild False Gharial in Peninsular Malaysia.
ii. To determine the blood profile of captive and semi-wild False Gharial in Peninsular Malaysia.
iii. To determine nesting behaviour and ecology of False Gharial in captive natural pond.

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