# TAXONOMIC AND BIOLOGICAL STUDIES OF CYPRINID GENUS *Poropuntius* IN PENINSULAR MALAYSIA

**MUHAMMAD RASUL BIN ABDULLAH HALIM**

**UNIVERSITI SAINS MALAYSIA**

**2022**

# TAXONOMIC AND BIOLOGICAL STUDIES OF CYPRINID GENUS *Poropuntius* IN PENINSULAR MALAYSIA

**by**

**MUHAMMAD RASUL BIN ABDULLAH HALIM**

**Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy**

**March 2022**

## ACKNOWLEDGEMENT

I am profoundly grateful to my supervisors, Dr. Khaironizam Md Zain and Profesor Dr. Rosli Ramli, for guidance and encouragement, supervision and constructive criticisms on the preparation of this thesis.

I am thankful to Mr. Marisi and the staff from ISB, University of Malaya for their company and field assistance. Special thanks to Mr Mohamad Faizal Ismail for field assistance. For access to institutional specimens, we thank Mr. Kelvin K.P. Lim (ZRC), Dr. Amirrudin Ahmad (Universiti Malaysia Terengganu), Dr. Chaiwut Grudpan and Dr. Jarungjit Grudpan (Ubon Ratchathani University), Dr. Sontaya Koolkalya (Rambhai Barni Rajabhat University), Dr. Zainal, A. Muchlisin and Dr. Agung Setia Batubara (Syiah Kuala University), Dr. Mohammad Saiful Mansor (Universiti Kebangsaan Malaysia), and the National Museum of Natural History, Smithsonian Institution, Washington DC, for the online collection data base.

I wish to express my gratitude to the boards and staff of Jabatan Perhutanan Semenanjung Malaysia, and Perbadanan Taman Negara Johor kindly facilitated surveys and issued permissions. This study was funded by the Ministry of Higher Education of Malaysia under the MyBrain15 programme and FRGS grant 203.PBIOLOGI.6711522.

To all my family members especially to my beloved parents, Abdullah Halim bin Hussin and Kintan binti Kahar for their patient and support throughout my Ph.D. journey.

## TABLE OF CONTENTS

**ACKNOWLEDGEMENT** ........ **ii**

**TABLE OF CONTENTS** ........ **iii**

**LIST OF TABLES** ........ **vii**

**LIST OF FIGURES** ........ **x**

**LIST OF SYMBOLS** ........ **xiv**

**LIST OF ABBREVIATIONS** ........ **xv**

**LIST OF APPENDICES** ........ **xvi**

**ABSTRAK** ........ **xvii**

**ABSTRACT** ........ **xix**

**CHAPTER 1 INTRODUCTION** ........ **1**

1.1 General background ........ 1

1.2 Problem statement ........ 4

1.3 Objectives ........ 6

**CHAPTER 2 LITERATURE REVIEW** ........ **7**

2.1 Taxonomy order ........ 7

2.2 Classification and systematics ........ 7

2.3 Paleo river system of Sundaland ........ 9

2.4 Diversity of fishes in Peninsular Malaysia ........ 14

2.5 Freshwater fishes studies in Peninsular Malaysia ........ 16

2.6 Cyiprinidae fishes in Peninsular Malaysia ........ 19

2.7 Fishes of genus *Poropuntius*. ........ 21

2.8 *Poropuntius* studies in Malaysia. ........ 26

2.9 Molecular and DNA barcoding. ........ 27

3.0 Some biological aspect studies of *Poropuntius* ........ 29

**CHAPTER 3 TAXONOMY AND DISTRIBUTIONAL PATTERN OF *Poropuntius* IN PENINSULAR MALAYSIA ................................ 34**

3.1 Introduction ........................................................................................ 34

3.2 Material and Methods.......................................................................... 36

3.2.1 Study area.......................................................................... 36

3.2.2 Museum collection examination ..................................................... 38

3.2.3 Measurements and counts .................... …………………………..38

3.2.4 Statistical analysis......... ………………………………………….39

3.3 Result..................................................................................................... 48

3.3.1 Morphology......................................................................... 48

3.3.2 Distributional pattern ..................................................................... 50

3.3.3 *Poropuntius smedleyi* (de Beaufort, 1933) ................................... 62

3.3.4 *Poropuntius* cf. *normani* ................................................................ 68

3.3.5 *Poropuntius normani* Smith, 1931................................................ 70

3.3.6 *Poropuntius tawarensis* (Weber & de Beaufort, 1916) ................. 75

3.4 Discussion………………………… ......... …………………………………81

3.4.1 Morphology, morphometric and meristic comparisons ................. 81

3.4.2 The distributional pattern of *Poropuntius* in Peninsular Malaysia 84

3.4.3 Interspecific interaction................................................................. 87

3.4.4 The identity of *Poropuntius tawarensis* ....................................... 88

3.5 Conclusion…………….......... ……………………………………………...89

**CHAPTER 4 MOLECULAR PHYLOGENETICS OF *Poropuntius* INFERRED FROM MITOCHONDRIAL DNA SEQUENCE .......................... 90**

4.1 Introduction ........................................................................................ 90

4.2 Material and Method ........................................................................... 93

4.2.1 Samples collections........................................................................ 93

4.2.2 DNA extraction ........ 94
4.2.3 PCR amplification ........ 99
4.2.4 Gel electrophoresis and sequences of PCR product ........ .99
4.2.5 Data analysis… ........ 100
4.3 Result ........ 103
4.3.1 Phylogenetic analyses ........ 106
4.3.2 Haplotype network… ........ 107
4.4 Discussion ........ 112
4.5 Conclusion ........ 116
**CHAPTER 5 SOME BIOLOGICAL ASPECTS OF *Poropuntius normani*.... 117**
5.1 Introduction ........ 117
5.2 Material and Methods ........ 120
5.2.1 Study area ........ 120
5.2.2 Sex ratio ........ 121
5.2.3 Length-weight relationship (LWR) ........ 121
5.2.4 Condition factor ........ 122
5.2.5 Length at first maturity ........ 122
5.2.6 Gonadosomatic index (GSI) ........ 123
5.2.7 Fecundity ........ 124
5.3 Result ........ ..127
5.3.1 Rainfall ........ 127
5.3.2 Sex ratio ........ 127
5.3.3 Size class frequency ........ 127
5.3.4 Length-weight relationship (LWR) ........ 131
5.3.5 Relative condition factor (K) ........ 131
5.3.6 The size of immature and mature fish ........ 135
5.3.7 Gonadosomatic index (GSI) ........ 135

5.3.8 Fecundity....................................... ................................136

5.4 Discussion…………………………………………………………… ........ 142

5.5 Conclusion………………………………………………………………… 151

**CHAPTER 6 GENERAL DISCUSSION............................................................ 152**

**CHAPTER 7 CONCLUSION................................................................................... 168**

**REFERENCES……………………………… ……………………………...171**

**APPENDICES**

**LIST OF PUBLICATIONS**

# LIST OF TABLES


**Page**

Table 3.1 The characters, abbreviation and description of the measurements used in the taxonomical analysis ........................................................ 41

Table 3.2 The characters, abbreviations and descriptions of the meristic count used in the taxonomical analysis. The number of soft spine and rays is shown by lower case of Roman and Arabic numerals, respectively. A gill raker straddled at the angle of the arch is included in the count of total gill raker but excluded from the count of upper or lower arms ........................................................ 45

Table 3.3 Morphometry measurements of *Poropuntius* spp. in Peninsular Malaysia ........................................................ 54

Table 3.4 Meristic data of *Poropuntius* spp. in Peninsular Malaysia ................ 56

Table 3.5 Component loadings from Principal Component Analysis (PCA) of morphometric data from *Poropuntius smedleyi* (N=69), *Poropuntius* cf. *normani* (N=32), and *Poropuntius normani* (N=180). The three highest loadings for each component are highlighted in boldface ........................................................ 58

Table 3.6 Component loadings from Linear Discriminant Analysis (LDA) of morphometric data from *Poropuntius smedleyi* (N=69), *Poropuntius* cf. *normani* (N=32), and *Poropuntius normani* (N=180). Characters of greater contribution in each discriminant function are in bold. ........................................................ 59

Table 3.7 Percentage of Poropuntius spp. specimens from Peninsular Malaysia correctly classified into their group. Rows is a given groups, column is a predicted groups. ................................................ 60

Table 3.8 Frequency of lateral line scales of *Poropuntius* spp. Number of lateral line scales indicate in bold. .................................................... 66

Table 3.9 Frequency of circumferential scales of *Poropuntius* spp. Number of circumferential scales indicate in bold........................................66

Table 3.10 Frequency of circumpeduncular scales of *Poropuntius* spp. Number of circumpeduncular scales indicate in bold. ......................67

Table 3.11 Frequency of predorsal scales of *Poropuntius* spp. Number of predorsal scales indicate in bold. ....................................................67

Table 3.12 Morphometry of *Poropuntius tawarensis*. SD, standard deviation, n indicates number of sample used ..................................................78

Table 3.13 Meristic of *Poropuntius tawarensis*. n indicates number of samples used ..........................................................................................79

Table 4.1 GenBank accession numbers and associated information for samples of *Poropuntius,*spp. and additional group of fishes included in the phylogenetic analysis ...............................................96

Table 4.2 Interspecific and intraspecific genetic distances in seven presumed species of *Poropuntius* used in this study ......................................104

Table 4.3 Number of samples, K2P values of maximum intraspecific, mean intraspecific and nearest neighbour distances as well as nearest neighbour species of 7 presumed *Poropuntius* species...................105

Table 4.4 Comparison of the percentage (%) of nucleotide composition, haplotype diversity and nucleotide diversity among the 7 forms of *Poropuntius* studied ........................................................................111

Table 5.1 Characteristics of gonadal maturity stages of *Poropuntius normani* from Gombak River .......................................................................126

Table 5.2 The number (n), percentage and sex ratio of male and female *Poropuntius normani* sampled in Gombak River from January 2017 to December 2017 ..................................................................130

Table 5.3 Length-weight relationship and condition factor of *Poropuntius normani* from Gombak River males, females and combination of both sexes.........................................................................................132

Table 5.4 Range, mean and standard deviation of relative condition factor (*K*) of male and female individuals of *Poropuntius normani* sampled in Gombak River from January to December 2017. Student's t-test no significant difference between sexes ($p > 0.05$). 134

Table 5.5 Monthly variation of mean and standard deviation of gonadosomatic index (GSI) of *Poropuntius normani* from the Gombak River .................................................................... 139

Table 5.6 Mean, standard deviation (±) and range of absolute fecundity of the combined maturing and gravid ovarian stages of *Poropuntius normani* from Gombak River. ......................................................... 140

Table 6.1 Meristic data of *Poropuntius normani*. Data includes specimens from the type locality in Thailand and Peninsular Malaysia............ 156

Table 6.2 Range, mean, and standard deviation (SD) of the morphometric data of *Poropuntius normani.* Data for all specimens includes specimens from the type locality in Thailand and Peninsular Malaysia ........................................................................... 157

# LIST OF FIGURES

**Page**


Figure 2.1 The geological time scale. Figure adapted from Locker & Hine (2020). .......... 11

Figure 2.2 A paleo-drainage map of the region. Map adapted from Voris (2000). .......... 11

Figure 2.3 Map distribution of type locality of fish genus *Poropuntius* based on Kottelat (2013). .......... 23

Figure 3.1 The sampling locations of Poropuntius spp. used in this study. Blue circle indicates sampling locations. Details of sampling localities are listed in Appendix A .......... 37

Figure 3.2 Diagrammatic illustration showing the measurements used in the study. Abbreviations to the measurement of the characters as listed in Table 3.1 .......... 40

Figure 3.3 Distribution of *Poropuntius* spp. in Peninsular Malaysia. *Poropuntius smedleyi* (black circle), *P*. *normani* (red triangle) and *P*. cf. *normani* (blue rectangular) .......... 53

Figure 3.4 Scatterplots with 95% confidence interval ellipses of the sheared second principal component (PC2) and sheared third principal component (PC3) of morphometric data on *Poropuntius* from Peninsular Malaysia and Thailand. *Poropuntius smedleyi* (black dots), *P*. *normani* (red +, and red dot indicates specimens from type locality) and *P*. cf. *normani* (blue triangle). .......... 57

Figure 3.5 Scatterplots with 95% confidence interval ellipses of the discriminant function analysis (LDA) of morphometric data on *Poropuntius* from Peninsular Malaysia and Thailand. *Poropuntius smedleyi* (black dots), *P*. *normani* (red +, and red dot indicates specimens from type locality) and *P*. cf. *normani* (blue triangle). .......... 61

Figure 3.6 *Poropuntius smedleyi*, from near type locality, UMKL 12006, 109.85 mm SL, Johor, Malaysia. A) Preserved specimens; B) Live coloration of *Poropuntius smedleyi*; C) Radiograph image of vertebrae .......... 65

Figure 3.7 *Poropuntius normani*; A) Specimen from topotype UMKL 12287, 109.75 mm of SL; B) Holotype, USNM 90297, Chanthaburi, Thailand. Photo by Sandra J. Raredon, National Museum of natural History, Smithsonian Institution; C) Radiograph of *Poropuntius normani* UMKL 12287 .......... 73

Figure 3.8 Tuberculation on male and female fish of *Poropuntius normani*. Anterior, dorsal and lateral view of head part. A1, B1 and C1 referring to male; A2, B2, and C2 referring to female .......... 74

Figure 3.9 *Poropuntius tawarensis*; A) Fresh specimen; B) Radiograph image; C) Last unbranched dorsal-fin ray thick and strongly serrated posteriorly .......... 80

Figure 3.10 Figure 3.10 Ventral view of head A) *Acrossocheilus* sp. adapted from Yuan & Zhang, 2010. B) *Poropuntius normani* UMKL 12234 .......... 84

Figure 4.1 Maximum intraspecific divergence (% K2P) plotted against nearest neighbour distance (% K2P) for the 7 *Poropuntius* species used in this study. Points above the line indicate species with a barcode gap .......... 105

Figure 4.2 The number of genetically distinct OTUs according to the prior intraspecific divergence value generated by ABGD based on K2P distance .......... 106

Figure 4.3 Neighbor-joining (NJ) phylogenetic relationships (K2P) among *Poropuntius* species sequences (the present study) and *Poropuntius* spp. sequences retrieved from GenBank. *Notopterus notopterus* from GenBank were used to root the tree as outgroup .. 109

Figure 4.4 Maximum likelihood (ML) COI gene phylogenetic tree among *Poropuntius* species sequences (the present study) and

*Poropuntius* spp. sequences retrieved from GenBank generated through HKY + G. Value at nodes represents the bootstrap support. *Notopterus notopterus* was employed as an outgroup .................... 110

Figure 4.5 Median-joining haplotype network of the *Poropuntius* species from this study, constructed based on 574 COI sequences. Black dots represent hypothetical intermediate haplotypes ...................... 111

Figure 5.1 Map of study area in the Gombak Field Study Center Universiti Malaya, located in the upper part of Gombak River, Selangor........ 125

Figure 5.2 Monthly rainfalls at the study area in Ulu Gombak Forest Reserve, Selangor from January until December 2017 (Malaysian Department of Drainage and Irrigation station (DID)) .................... 128

Figure 5.3 Frequency distribution per total length class (mm) for *Poropuntius normani* sampled in Gombak River from January 2017 to December 2017 male and female .................................................... 129

Figure 5.4 Length-weight relationship of A) male, B) female and C) combination of male and female of *Poropuntius normani* from Gombak River .................................................................................. 133

Figure 5.5 Occurrence of gonad maturity A) male, B) female of *Poropuntius normani* in relation to total length size classes from Gombak River ......................................................................................... 137

Figure 5.6 $Lm_{50}$ of *Poropuntius normani* from Gombak River. A) male; B) female of *Poropuntius normani*. The dotted line indicates 50% of this figure which is taken as the average length at maturity ........... 138

Figure 5.7 Monthly rainfalls and gonadosomatic index (GSI) of male and female of *Poropuntius normani* in the Gombak River from January 2017 until December 2017 ............................................................. 139

Figure 5.8 Relationship between A) fecundity and total length, B) fecundity and body weight, and C) fecundity and ovary weight of the combined maturing and gravid ovarian stages of *Poropuntius normani* from Gombak River.......................................................... 141

Figure 5.9 Gonad conditions of *Poropuntius normani*. A) Immature; B) Female mature; C) Male mature ......................................................146

# LIST OF SYMBOLS

| | |
|---|---|
| n | Samples size |
| p | P-value |
| SD | Standard deviation |
| mm | Millimetre |
| cm | Centimetre |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| BC | Before century |
| ISC | Indian subcontinent |
| LGM | Last glacial maximum |
| MYA | Million years ago |
| BP | Before the present |
| ICZN | International Commission on Zoological Nomenclature |
| DNA | Deoxyribonucleic acid |
| RNA | Ribonucleic acid |
| tRNA | Transfer ribonucleic acid |
| rRNA | Ribosomal ribonucleic acid |
| COI | Cytochrome c oxidase subunit 1 |
| CBOL | Consortium for the barcode of life |
| BOLD | Barcode of life data system |
| LWR | Length-weight relationship |
| K | Condition factor |
| GSI | Gonadosomatic index |
| UMKL | Zoological Museum of the University Malaya Kuala Lumpur |
| UMT | Universiti Malaysia Terengganu |
| ZRC | Lee Kong Chian Natural History Museum of Singapore |
| USNM | National Museum of Natural History, Washington, United State of America |
| MNHN | Muséum National d'Histoire Naturelle, Paris, France |
| KIZ | Kunming Institute of Zoology, Chinese Academy of Science, China |
| KIZZLP | Kunming Institute of Zoology, Chinese Academy of Science, China |
| ihb | Institute of Hydrobiology, Chinese Academy of Science, China |
| PCA | Principal component analysis |
| AIS | Alien invasive species |
| PCR | Polymerase chain reaction |
| UV | Ultraviolet |
| ML | Maximum likelihood |
| NJ | Neighbour-joining |
| vs | In contrast to |

## LIST OF APPENDICES


APPENDIX A Sampling locations of *Poropuntius* spp. collected in the study.

APPENDIX B Material examined.

APPENDIX C One-way analysis variance to determine the differences in the measurement characters of three types of *Poropuntius* spp. from Peninsular Malaysia. Asterisk indicates the significantly different at $p < 0.05$.

APPENDIX D Tukey-Kramer test to determine the differences among the mean of measurement of three types of *Poropuntius spp.* sampled from Peninsular Malaysia. Asterisk indicates the effects of the analysis that are significantly different at $p < 0.05$. Q-table 0.05, 3.31.

APPENDIX E One-way analysis variance to determine the differences in the meristic characters of three types of *Poropuntius* spp. from Peninsular Malaysia. Asterisk indicates the significantly different at $p < 0.05$.

APPENDIX F Tukey-Kramer test to determine the differences among the mean of meristic of three types of *Poropuntius spp.* sampled from Peninsular Malaysia. Asterisk indicates the effects of the analysis that are significantly different at $p < 0.05$. Q-table 0.05, 3.31.

**KAJIAN TAKSONOMI DAN BIOLOGI IKAN CYPRINIDAE GENUS *Poropuntius* DI SEMENANJUNG MALAYSIA**

## ABSTRAK

Ikan cyprinidae dari genus *Poropuntius* merupakan ikan yang bersaiz kecil dan sederhana, ikan dewasa memiliki panjang standard antara 10 ke 20 cm, taburanya meluas di tanah besar Asia Tenggara dan kebiasaanya mendiami sungai-sungai di kaki bukit yang berketinggian sederhana. Oleh kerana status taksonominya yang mengelirukan, terdapat beberapa nama spesis yang telah digunakan di dalam kajian yang lepas di Semenanjung Malaysia antaranya ialah *Poropuntius deauratus*, *Poropuntius normani* dan *Poropuntius smedleyi*. Kajian ini bertujuan untuk mengenalpasti status taksonomi, corak taburan dan aspek biologi terutamanya corak pembiakan ikan *Poropuntius* di Semenanjung Malaysia. Kajian awal morfologi terhadap 281 spesimen dari berberapa lokasi mendapati terdapat tiga jenis *Poropuntius* di Semenanjung Malaysia, iaitu *Poropuntius smedleyi*, *Poropuntius* cf. *normani*, dan *Poropuntius normani*. Ketiga-tiga spesis ini dibezakan dengan memiliki bilangan sisik di sekeliling pangkal ekor 12, 13 dan 14. Namun, analisis PCA dan LDA gagal membezakan spesis ini kerana memiliki ciri yang bertindih samada dari segi pengukuran ataupun meristik. Ikan genus *Poropuntius* telah direkodkan di semua aliran sungai utama di Semenanjung Malaysia, kecuali di kawasan barat laut (Penang dan Kedah). Kajian molekular hubungan filogenetik telah dijalankan menggunakan jujukan DNA mitikondria sitokrom oksidase subunit I (COI). Semua spesimen *Poropuntius* dari Semenanjung Malaysia dan *Poropuntius normani* dari type lokaliti (Chanthaburi, Thailand) memiliki maklumat genetik yang sama, dan telah menyokong bahawa semua specimen adalah satu spesis. Nama *Poropuntius normani* telah dikekalkan kerana *Poropuntius normani* Smith, 1931 merupakan nama tertua, dan *Poropuntius smedleyi* (de Beaufort, 1933) menjadi sinonim junior kepada *Poropuntius normani*. Kajian ekologi *Poropuntius normani* telah dijalankan di Sungai Gombak. Lebih banyak ikan jantan berbanding ikan betina telah direkodkan dengan nisbah jantan kepada betina 11:5. Ikan jantan (74 mm) matang pada saiz yang lebih kecil berbanding ikan betina (112 mm). Analisa hubungan panjang-berat menunjukkan corak tumbesaran alometrik negatif, menjadikan dimensi badan ikan memanjang apabila saiz bertambah. *Poropuntius normani* di Sungai Gombak memiliki kondisi badan yang sama diantara ikan jantan dan betina. Nilai indeks gonadosomatik *Poropuntius normani* tertinggi pada bulan Julai dan Disember, selari dengan jumlah hujan yang tinggi. Kehadiran individu ikan matang sepanjang tahun menunjukkan ikan *Poropuntius normani* di Sungai Gombak bertelur sepanjang tahun. Fekunditi mutlak *Poropuntius normani* rendah, diantara 100 ke 1680 setiap ovari dan ianya berkolerasi dengan panjang total ikan berbanding berat badan dan berat ovari. Kajian taksonomi bersepadu dengan menggunakan kaedah morfologi dan molekular sangat membantu untuk menyelesaikan masalah taksonomi ikan genera *Poropuntius* di Semenanjung Malaysia. Tambahan, maklumat corak taburan dan aspek biologi ikan *Poropuntius normani* telah dikenalpasti dan penting untuk merancang pengurusan konservasi spesis ini.

**TAXONOMIC AND BIOLOGICAL STUDIES OF CYPRINID GENUS *Poropuntius* IN PENINSULAR MALAYSIA**

## ABSTRACT

The cyprinid fishes of the genus *Poropuntius* consisted of small to medium size, range 10 to 20 cm of standard length as adults are widely distributed mainly in the mainland of Southeast Asia and inhabit foothill streams at moderate elevations. Due to the confusing taxonomic, several species names have been used in the old literature in Peninsular Malaysia, namely, *Poropuntius deauratus*, *Poropuntius normani*, and *Poropuntius smedleyi*. This study aims to determine the taxonomical status, distributional pattern, and some biological aspects of the reproductive pattern of *Poropuntius* in Peninsular Malaysia. Morphology studies of 281 specimens from different localities indicated three types of *Poropuntius* species found in Peninsular Malaysia: *Poropuntius smedleyi*, *Poropuntius* cf. *normani*, and *Poropuntius normani*. These species can be distinguished by the character of circumpeduncular scale counts 12, 13 and 14 respectively. However, PCA and LDA analyses failed to separate among species groups as those species show substantial overlap in both proportional measurements and meristic counts. Fish genus *Poropuntius* has been recorded from all major drainages in Peninsular Malaysia, however absent in the northwest areas (Penang and Kedah). The molecular phylogenetic relationships were investigated using mitochondrial cytochrome oxidase subunit I (COI) DNA sequence. All specimens of *Poropuntius* from Peninsular Malaysia and those from the type locality of *Poropuntius normani* (Chanthaburi, Thailand) are genetically similar, supporting the conclusion that all specimens examined are conspecific. *Poropuntius normani* name is retained here since *Poropuntius normani* Smith, 1931 is the oldest available name, and *Poropuntius smedleyi* (de Beaufort, 1933) has become a junior synonym of *Poropuntius normani*. Some of the ecological aspects of *Poropuntius normani* in the Gombak River were studied. More male fish have been recorded than female fish, with the sex ratio of male to female being 11:5. The males (74 mm) sexually matured at a smaller size than females (112 mm). Analysis of the length-weight relationship showed a negative allometric growth pattern, which means body dimension has become slender as its length increases. *Poropuntius normani* in the Gombak River has similar body conditions in both male and female fish. The gonadosomatic index values of *Poropuntius normani* peak in July and December, coinciding with the high rainfall. Throughout the year, the constant presence of mature individuals indicates *Poropuntius normani* in Gombak River is multiple spawners. The absolute fecundity is relatively low, ranging from 100 to 1680 per ovary, and it is highly correlated to the total length of the fish than bodyweight and ovary weight. An integrated taxonomic study using morphological and molecular methods is useful to solve the taxonomic problem of fish genera *Poropuntius* in Peninsular Malaysia. Additionally, information on distribution patterns and some biological aspects of *Poropuntius normani* have been identified and important for the conservation management of this species.

# CHAPTER 1

# INTRODUCTION

## 1.1 General background

The extent of diversity in the world of the living is astonishing. More than 1 million animal species have been identified, with estimates ranging from 3 million to 10 million living species yet to be discovered (Myers *et al*., 2000; Sodhi *et al*., 2004; Sodhi & Brook, 2006). Each species may exist in diverse body shapes and morphs, behaviours, physiology, life history, habitats and seasonal. This massive diversity would be challenging to deal with if it did not arrange and classified. Therefore, a systematic study is needed to arrange the animals' rich diversity globally and develop methods and principles to make this task possible.

Taxonomy is an essential and precise science that lays the foundation for understanding biodiversity. For centuries, taxonomy has established universal naming and classification systems for biodiversity, and continues effectively to accommodate new knowledge (Thomson *et al*., 2018). Through taxonomic research, our knowledge of biodiversity and classifications of living organisms will continue to develop. The species name is the reference to all available information of the species. An essential for all fundamental and applied biological research is the correct knowledge of identifying the species. All theories and strategies for the future conservation and sustainable use of our biodiversity can only be efficient when species are identified correctly. Meanwhile, the term classification refers to placing objects in groups so that the members of the groups bear a closer relationship to other members of the same group (Mayr & Ashlock, 1991).

Systematic is known as the study of the biodiversity of living organisms and relationships among the taxa (Eldredge, 1992). The term systematics is derived from Latinized Greek word '*systema*'. Systematics is the empirical study of organism forms and diversity, and all relationships between them. Systematics involves taxonomy, identification, classification and nomenclature and all other aspects of dealing with various types of organisms. The approach generally used in systematics is based on morphological and biological species concepts (Mayr & Ashlock, 1991), whereas the information needed for these concepts are taken from morphological, physiological, molecular, behaviour, ecological and geographical information. Consequently, the lack of basic information is a major obstacle to understanding the biology and the aquaculture potential of economic species to improve the production and growth performance.

Species richness within vertebrate groups is high in Sundaland (Myers *et al*., 2000), including freshwater fishes. Geography, Sundaland comprises the Malay Peninsula, Borneo, Sumatra, Java and Palawan, which were once an exposed mainland during the Pleistocene's low sea level (Bird *et al*., 2005). This biodiversity hotspot is among the most important reservoirs of diversity and endemism (Sodhi *et al*., 2004), and this pattern was heavily influenced by a series of ongoing plate tectonic events, dynamic river systems, climatic oscillations, fluctuating land and sea levels, mainly during Cenozoic (Wooddruff, 2010). Cenozoic geological process and Quaternary climatic change played a significant role in the spatiotemporal evolution of aquatic taxa across South East Asia (de Bruyn *et al*., 2013).

Cyprininae is the largest subfamily comprised of more than 120 genera in over 1300 freshwater species, including almost 4% of bony fish diversity, and subfamily Cyprininae has the most polyploid species (~400) than any other group of fishes (Yang *et al*., 2015). Species of this subfamily are found in waters of Africa to southern Eurasia. There are found in all types of freshwater ecosystems, and some of them can tolerate brackish water (Nelson, 2006). Some are well known, such as the common carp and Goldfish. The presence of one to three rows of pharyngeal teeth, or the 'teeth' arrangement in the throat, distinguishes cyprinids from other fishes. Systematically, the fish family of Cyprinidae are divided into 11 subfamilies, namely Labeoninae, Probarbinae, Torinae, Smiliogastrinae, Cyprininae, Acrossocheilinae, Spinibarbinae, Schizothoracinae, Schizopygopsinae, Barbinae, and Poropuntiinae (Tan & Armbruster, 2018). These subfamilies divided into subgroups were based on morphological characteristics, including varying levels of ploidy, serration of fin rays, scale patterns, and morphology of the lips (Yang *et al*., 2015).

Despite the vast amount of literature on Malaysian cyprinid fishes, the systematics and taxonomy of Peninsular Malaysian cyprinid fishes were poorly understood until recently (Ismail, 1989; Lim & Tan, 2002). For decades, the lists of fishes recorded from Peninsular Malaysia, especially cyprinids, have referred to the taxonomic works of literature available at the time. As a result, the validity of many species is debatable. Lim *et al*. (1993) listed some cyprinid genera that need to be revised, namely *Hypsibarbus*, *Labiobarbus*, *Neolissochilus*, *Poropuntius*, *Puntioplites*, *Puntius*, *Rasbora* and *Tor*. Thus, these species' validity and accurate numbers in Peninsular Malaysia are still unknown.

The genus *Poropuntius* fish consisted of small to medium, range 10 to 20 cm of standard length as adult freshwater fish. The cyprinid fishes of the genus

*Poropuntius* are widely distributed in the river systems of Ganges-Brahmaputra in India to Southeast Asia and Xi Jiang in Southern China. This genus mainly inhabits high gradient foothill streams at moderate elevations (usually under 1000 m). A single species was reported from the Ganges-Brahmaputra basin of India and Sumatra of Indonesia. Several species occur in Myanmar, the Indo-China peninsula and Yunnan of China (Rainboth, 1981; Roberts, 1998). While in Peninsular Malaysia, there are probably more than one species that occur in the river systems (Roberts, 1998).

Revision study of the genera made by Roberts (1998) has listed 29 nominal species of *Poropuntius,* including new species and three new subspecies described from the Salween, Tenasserim, Maeklong and Mekong basins. Furthermore, Kottelat (2013) has listed a total of 34 nominal species of *Poropuntius* where he has upgraded the subspecies sensu Roberts (1998) to the full species. Although the cyprinid genus *Poropuntius* is readily distinguished from other cyprinids based on the existence of the well-developed barbells, tubercles on the snout, and serrated dorsal-fin spine (Roberts, 1998), the conservative morphology displayed within the group has confounded taxonomy at both generic and specific levels. Rainboth (1996) reported that most of the porupuntids as well as other barbin fishes have a high degree of polymorphism or ecophenotypic variation.

## 1.2 Problem statements

In spite of its commercial interest and the declining status of *Poropuntius* globally, little is known about the population and demographic structures of wild populations, and, to my knowledge, no documented reports or research has been done in Malaysia to analyse the inter or intraspecific patterns of genetic variability, population structure, as well as barcoding assignment of *Poropuntius* using molecular genetic markers. Thus, this research is deemed necessary in providing the reappraisal of taxonomy and systematics of *Poropuntius* spp. to resolve this group fish's complexity by using the combination of morphology and molecular genetic marker. It is also an important aspect that may be crucial for effective conservation (i.e., conservation genetics) of the species concerned. The genetic information obtained would help delineate proper sustainable management strategies and predict the effects of proposed management alternatives on a species' viability.

The current study is focused on the taxonomy and phylogenetic relationships of *Poropuntius* spp. collected from wild populations in different localities. Considering the morphological similarities among the *Poropuntius* species, an integrative taxonomy approach using morphology and molecular data was used to identify the identity of *Poropuntius* species in Peninsular Malaysia. Other than that, the biological aspects of the fish are very important for use in management and conservation. This study also explores the distribution pattern of *Poropuntius* in Peninsular Malaysia as well as some biological aspects of *Poropuntius* in a specific locality.

## 1.3 Objectives

The confusing state about their taxonomy and lack of biological information are two main reasons this study was undertaken. Therefore, to address the issues described above, this study is aimed to achieve the following objectives:

1. to examine the morphological study and distribution of the fish genus *Poropuntius* in Peninsular Malaysia using traditional morphometrics and meristic counts technique.
2. to conduct a molecular taxonomic study of the *Poropuntius* in Peninsular Malaysia based on a molecular approach.
3. to provide insight into the biological aspects and reproductive biology of *Poropuntius* in the Gombak River, Selangor, Malaysia.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Taxonomy order

| | |
|---|---|
| KINGDOM: | Animalia |
| PHYLUM: | Chordata |
| CLASS: | Actinopterygii |
| INFRACLASS: | Teleostei |
| ORDER: | Cypriniformes |
| FAMILY: | Cyprinidae |
| TRIBE: | Poropuntiinae |
| GENUS: | *Poropuntius* Smith, 1931 |

## 2.2 Classification and systematics

The term taxonomy comes from the Greek words' '*taxis*', meaning arrangement or structure, and '*nomos*', meaning law or method. It is best to describe it as follows, as this aligns with current thinking: Taxonomy is the science and practice of classifying organisms (Mayr, 1969). In biology, taxonomy is the science of naming and categorizing biological organisms' classes based on common characteristics. Classification is the practice of organizing items into groups or categories. The objective in making a classification of a group of organisms is to build a hierarchical system of the various taxa relationships (Nelson, 2006). Taxa (singular taxon) is a category of organisms recognized in the classification and given biological names (e.g., Cypriniformes, Cyprinidae, *Poropuntius*, *Poropuntius normani*). Taxonomy is part of the theory and practice of describing diversity and establishing classifications systematically (Nelson, 2006).

Historically, naming and classifying existed as early as 3000 BC in China by Shen Nung, Emperor of China who is the Father of Chinese medicine. The pharmacopoeia *Divine Husbandmans Materia Medica* which included 365 medicines obtained from animals, plants, and minerals (Zhao *et al*., 2018). Meanwhile, in Egypt, the illustration of medicinal plants on wall painting is believed to have existed in 1500 BC. This painting gives knowledge about medicinal plants in old Egypt and their names (Manktelow, 2010).

Among the earliest figures in classifying animals is the Greek philosopher, Aristotle 384-322 BC. He divided animals with blood and without blood that we recognized today as vertebrates and invertebrates, respectively. Later, the Roman naturalist and natural philosopher, Plinius in his work *Naturalis Historia* described several plants and gave them Latin names. Since then, Latin was later kept for botanical science and known as the Father of Botanical Latin (Manktelow, 2010).

Carolus Linnaeus is referred to as the Father of Taxonomy for his science's outstanding contribution. Given the fact that Linnaeus was not the first to use binomial nomenclature, he was the one to use it consistently, and it is now the scientific standard internationally. He published his classification scheme in his famous book *Systema Naturae* in 1753 and introduced the principles of naming an organism by two words known as the "binomial system of nomenclature". Later, this work of Linnaeus has become the foundation of systematics (Maguire, 2012).

No scientific ecological survey can be carried out without the most basic information, the identification of all the species of ecological significance. Without the knowledge of systematics, the discovery of new species of organisms is not possible.

Furthermore, due to the rapid habitat loss, there is an urgency to document our planet's biological and make appropriate conservation efforts before they disappear forever (Wheeler *et al*., 2012).

**2.3 Paleo river system of Sundaland**

Southeast Asia is a subregion of Asia made up of the regions that include east of the Indian subcontinent, south of China, and north-west of Australia. In the current description, Southeast Asia contains two geographic areas: first is Mainland Southeast Asia, historically also known as Indochina, comprising Myanmar, Vietnam, Thailand, Laos, Cambodia and Peninsular Malaysia, and second is Maritime Southeast Asia, also called historically as the Malay Archipelago, covering the Andaman and Nicobar Island (India), Cartier Island, Ashmore, Cocos Island, and Chrismas Island (Australia), East Malaysia, Indonesia, Brunei, Philippines, and Singapore. The region includes Borneo and Sumatra (world's third and sixth largest islands, respectively). Southeast Asia covers about 4.5 million $km^2$, which is 10.5% of Asia or about 3% of the earth's total land area (Frederick & Leinbach, 2020).

Southeast Asia is among the most important reservoirs of species richness and exceptional endemism (Sodhi & Brook, 2006; Woodruff, 2010). In fact, Southeast Asia entirely contains four of the earth's 34 biodiversity hotspots, namely (Indo-Burma, Sundaland, Philippines, and Wallacea) (Myers *et al*., 2000). However, the growth of the human population has put pressure on the existing habitat. Understanding the pattern and mechanism in the dynamic of evolutionary novelty is very important, especially in areas containing rich biodiversity, to plan a good conservation strategy (Sodhi & Brook, 2006; Lohman *et al*., 2011). South-East Asia has a deforestation rate among the highest globally besides South America;

additionally, it has the highest mining rate in the tropics and the most significant number of hydropower dams under construction, becoming a threat to biodiversity (Hughes, 2017). Moreover, many natural habitat areas have been converted to other land uses, including oil palm plantations (Shevade & Loboda, 2019). As a result, it is the most biologically threatened globally, and many species in the region may be driven to extinction while still undescribed (Schipper *et al*., 2008).

Southeast Asia is situated at the meeting point of several converging, major tectonic plates; the Australian, Philippine, Indian, and Eurasian Plates (Simons *et al*., 2007). The most important biotic exchange between the Indian subcontinent (ISC) and Eurasia happened when the tectonic collision between the ISC and the Eurasian landmass during the Early Cenozoic (Figure 2.1). The collision was widely recognized as a critical occasion that caused important geologic, and climatic changes that indirectly influenced the composition of biota presently (Harrison *et al*., 1992; Solovyeva *et al*., 2018). Past climatic fluctuations, such as the Quaternary glacial cycles, are seen as primary determinants of biodiversity distribution (Hewitt, 2000). The changes of sea levels are believed to have influenced the composition of biodiversity especially in Southeast Asia (Bohlen *et al*., 2020a; 2020b). The last glacial maximum (LGM) that occurred around 17 000 years before present has resulted in low sea level as much as 120 meters below the present level (Figure 2.1). This has resulted in the exposure of the Sunda and Sahul shelves (Figure 2.2) (Voris, 2000). The exposure of the Sunda Shelf resulting the Sumatra, Malay Peninsula, Java and Borneo are connected and formed a region known today as "Sundaland" (Voris, 2000; Lohman *et al*., 2011). Meanwhile, on the east, the exposure of the Sahul Shelf mainly linked Australia and New Guinea and surrounded the Aru Islands (Figure 2.2) (Voris, 2000).

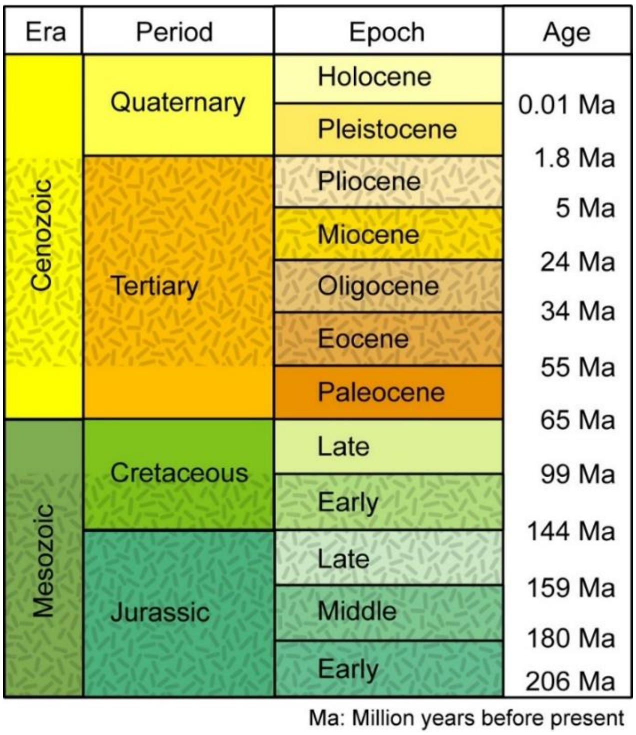


Figure 2.1 The geological time scale. Figure adapted from Locker & Hine (2020).

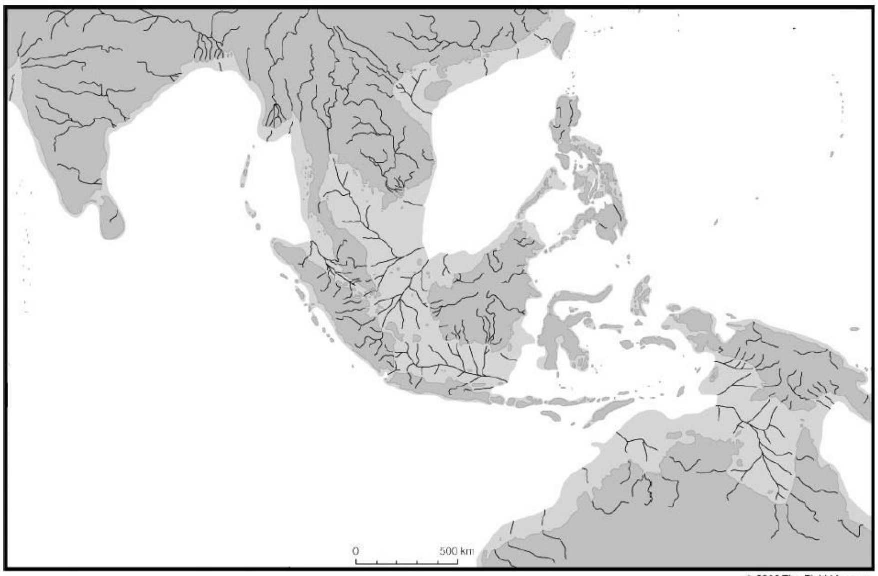


Figure 2.2 A paleo-drainage map of the region. Map adapted from Voris (2000).

Voris (2000) proposed four major river systems to have existed during low sea levels of Sundaland indirectly interconnecting many of existing landmasses. The formation of the Paleo river system of South East Asia was most significant in the time frame of 17,000 years BP (before the present). Paleo river systems were formed during that period, namely Malacca Straits River, Siam River, North Sunda River, and East Sunda River systems (Figure 2.2). The Malacca Straits River system was flowing between the west coast of Malay Peninsula (including Perak River, Bernam River, Muar River and Lenek River) and east coast of Sumatra (including Simpang Kanan River, Panai River, Rokan River and Siak River) flowing north-west to the Andaman Sea. Meanwhile, The Siam River system included Sumatra's Kampar River flowed northwards through the Singapore Straits, was joined by the Johor River and ran north joined branches from the Gulf of Thailand. These rivers formed a network that includes several rivers from the east coast of Peninsular Malaysia rivers namely Endau River, Pahang River, Terengganu River and Kelantan River, and Chao Phraya River from Thailand.

The North Sunda River system is the largest Sunda Shelf rivers, also known as Molengraaff River. This river flowage ran from Sumatra's north-east coast (Batanghari River, Musi River, Indragiri River) to join the Kapuas River from Borneo and then flowed to north-east of Natuna Island. This historical connection is believed to significantly influence the dispersal of freshwater fishes of Borneo and Sumatra (Inger & Chin, 1962). The East Sunda River system includes south-east Sumatra, north Java and south Borneo flowed east across the Java Sea to enter Bali's sea (Voris, 2000). The colonization and diversification of freshwater fish between island and mainland of Southeast Asia can be dated as early as late Eocene (about 35 MYA) observed in Asian and Australian Arowana, *Scleropages* spp. (Figure 2.1) (Cioffi *et al*., 2019).

The available data also suggest late Miocene to Pliocene is an important time in the evolution of many freshwater fish genera, e.g., *Mastacembelus* (Jamaluddin *et al*., 2019), *Paracanthocobitis* (Bohlen *et al*., 2020a), and halfbeaks (de Bruyn *et al*., 2013).

The fish distribution patterns were influenced by climatic processes that result in sea-level change, and tectonic processes that result in shifting and movements of part of the earth's crust (Rainboth *et al*., 2012). The changing sea levels repeatedly in the past have long been considered important to understanding the distribution of both terrestrial and aquatic organisms (Molengraaff & Weber, 1919; Darlington, 1957). The sea-level changes during the Pleistocene generally deduce sea levels from either geological mapping of retreating shoreline margin, e.g., the use of coral reef terraces on tropical coasts (Bloom & Yonekura, 1990) or the marine oxygen isotope record, e.g., deviations from the standard of the $^{18}O/^{16}O$ ratios in the foraminiferal test from deep-sea cores (Fairbanks, 1989). The great naturalist, Alfred Russell Wallace set a baseline of biogeography in the Malay Archipelago islands. Through his study comparing the biota, he concluded that the Malay Archipelago islands must have been connected to the Asian mainland in recent times (Wallace, 1869; 1880).

Biogeographical patterns of freshwater fauna are principally unique as their dispersals are restricted to the freshwater environment. In contrast, the terrestrial fauna can disperse across the geographical landscape via land, air or secondary agents (Abell *et al*., 2008). The freshwater ichthyofauna of Southeast Asia is extraordinarily diverse, with species richness and endemicity ranked after Congo and Amazon (Dudgeon *et al*., 2006). At the present time, approximately 3107 valid native species, in 707 valid genera and 137 families have been recorded in this Southeast Asian inland waters (Kottelat, 2013). Estimated 500 species from the Mekong drainage, 328 from the Mae Khlong – Chao Phraya drainages, 253 from the Red River drainage, 151 species from the Salween drainage, 160 from the streams draining the eastern slope of the Annamite range and 221 from the Malay Peninsula (south of the isthmus of Kra) (Kottelat *et al*., 2012). Other observations have reported of 297 species of freshwater fishes from Batang Hari drainage in Central Sumatra (Hui & Kottelat, 2009), 290 species from Kapuas River basin in west Borneo (Roberts, 1989), and 174 species from Mahakam River basin in East Borneo (Kottelat, 1995). Meanwhile, the Rejang River basin in Sarawak Borneo recorded at least 164 freshwater fish species (Parenti & Lim, 2005). Recently, Ng *et al*. (2017) reported a total of 150 native species of freshwater fish from Sabah, North Borneo. Furthermore, about 104 species of freshwater fishes were reported from Brunei, with six species being endemic (Sulaiman *et al*., 2018).

## 2.4 Diversity of fishes in Peninsular Malaysia

Fishes occur in various habitats such as lakes, stream, estuaries and ocean throughout the world. In most species of fishes, all individuals live entirely either in marine water or freshwater. Diadromous is a species frequently living part of their lives in freshwater and part in the marine. Among this, most of the species are anadromous, which means species spawning in freshwater; however, they spend much of their time in the sea, primarily clupeoids, including several shads and salmons (Rochard *et al*., 2009). Meanwhile, catadromous have the opposite behaviour, spawning in the ocean but returning to freshwater, occur in anguillid eels, and mullets (Kadir *et al*., 2017). Other than that, there are many freshwaters, and marine species commonly inhabit brackish-water estuaries, known as amphidromous. The amphidromous is a type of fish spawning in freshwater, and the larvae travel to the sea after hatching before returning to freshwater. The gobies and gudgeons are the major groups of amphidromous fish, which commonly found on many islands of all the major oceans (Milton, 2009). Diadromous fish populations rely on freshwater flows to maintain their connectivity. (Brown *et al*., 2013).

Freshwater fishes can be categorized into several groups. Myers (1951) established an ecological classification of freshwater fishes based on their tolerance to saltwater. Primary division freshwater species comprised species with little salt tolerance and exclusively spending their whole life cycle in freshwater. Meanwhile, secondary division freshwater species is species related to marine but living in fresh or sometimes brackish water.

The diversity of freshwater fish in Peninsular Malaysia is comparatively high due to the variety of available habitats. The inland water bodies' freshwater lotic and lentic habitats in Malaysia consist of riverine, acidic peat swamps, wetlands, lowland forest streams, hillside forest streams, inland human-made lakes, and a total surface area of 45 459 $km^2$ (Yusoff & Gopinanth, 1995). The availability of a large area of the water bodies and varied aquatic habitats offer a good opportunity for fish colonization.

Zakaria-Ismail (1994) proposed the distribution of Southeast Asian freshwater fishes can be divided into several zoogeographic regions and Peninsular Malaysia belong to The Malay Peninsular zoogeographic region which was heavily influenced by the Siamese and Indonesian species. The composition of the Malay Peninsula's fish fauna is complex due to the mixture of the two species elements. The southern part of the peninsula has a strong Sundaic fauna (Sundaland referring to the south of the Malay Peninsula and the Great Sunda Islands (Sumatra, Java and Borneo)). The influence of Sundaic fauna is also observed in Southeastern Thailand (Chanthaburi and Trat provinces) and coastal areas of Cambodia with the presence of genera (e.g., *Barbucca*, *Vaillantella*, and *Silurichthys*). Meanwhile, along the western slope of Malay

Peninsula, several species belong to Ayeyarwaddy-Salween-Tenasserim with the presence of genera (e.g., *Paracanthocobitis* and *Batasio*) and their proportion of the total fauna decreases southwards (Kottelat *et al*., 2012).

Meanwhile, Abell *et al*. (2008) categorized Peninsular Malaysia into two freshwater ecoregions: Malay Peninsular Eastern Slope and North Central Sumatra – West Malaysia. The North Central Sumatra – West Malaysia freshwater ecoregion comprises three countries: Malaysia, Indonesia, and Thailand. The western section lies along the northern coast of Sumatra, and the west sections with the south of Krabi, Thailand forming its northern border, with the Muar River forming its southern border in Malaysia. Some of the large rivers in Peninsular Malaysia such as Muda River, Perak River and Bernam River were included in this ecoregion. All the drainages of this ecoregion flowing into Strait of Malacca.

Meanwhile, the Malay Peninsular Eastern Slope represent the Malay Peninsula's eastern drainages, from Prachuap Khiri Khan, Thailand in the north and Singapore in the south. It also includes Tioman Island and Anambas Island. Among the large rivers in Peninsular Malaysia such as Golok River, Kelantan River, Terengganu River, Pahang River, Endau River and Muar River were included in this ecoregion (Abell *et al*., 2008).

## 2.5 Freshwater fish studies in Peninsular Malaysia

The study of Malaysian freshwater fish systematics began over 170 years ago. Cantor (1849) through his publication of "Catalog of Malayan Fishes" lists a few freshwater fishes from Peninsular Malaysia. Among his 12 species of freshwater fishes recorded from Penang Island was *Betta pugnax.* Later, Gunther in his monumental work "Catalog of Fishes in the British Museum" includes the Cantor's specimens

(Gunther, 1864; 1866; 1868a; 1868b). The first substantial contribution to the knowledge of freshwater fishes of the Malay Peninsula is by Georg Duncker (1904) in "Die fische de Malayieschen Halbinsel" describing some 104 species of which 14 were new to science. Furthermore, Maxwell (1921) compiled the Malayan fish with his book entitled "Malayan Fishes" including freshwater and marine fishes.

The book by Fowler (1938) entitled "A list of the fishes known from Malaya" is probably the most extensive record of the fishes from Peninsular Malaysia. He listed about 165 species of fish, among them, are species recorded by Hanitsch (1904) and Maxwell (1921), which were misidentified by them. Then, Herre described 18 new species of fish from the Malay Peninsula and Borneo. He believed fish composition in Malay Peninsula was influenced by Sumatra, Borneo, Siam and little from Burma (Herre, 1936; 1940a; 1940b).

Tweedie (1936; 1940) managed to collect a large number of fish specimens, mainly from Malaya and deposited in the Raffles Museum in Singapore (presently known as Lee Kong Chian Natural History Museum). The specimens are well preserved and accompanied by good field information. He next assembled Malay names of freshwater fishes commonly used by the locals, especially in Pahang (Tweedie, 1952). Later, Eric Ronald Alfred, born in Johor, became the first Malayan-born to be a curator of zoology of the Raffles Museum in 1956. He was actively involved in the research, especially fish collections. One of the most significant works by him was describing new species of loaches from Malaya (Alfred, 1967; 1969). Johnson (1967) on his work of distributional patterns of Malayan freshwater fish proposed the distribution pattern of freshwater fish within Malaya might be influenced by ecological factors such as edaphic (influenced by the soil) and climatic not by geographical factors. Since then, knowledge of freshwater fishes has been enriched by other workers. Mohsin & Ambak (1983) published a book on the taxonomy of freshwater fishes in Peninsular Malaysia gathered all the information species of freshwater fishes.

Check list study of freshwater fishes always confined to single drainage such as Endau drainage (Lim *et al*., 1990; Ng & Tan, 1999; Zakaria-Ismail & Fatimah, 2007; Chow *et al*., 2014); Pahang drainage (Zakaria-Ismail, 1984; Zakaria-Ismail, 1993; Khan *et al*., 1996; Lee & Zakaria-Ismail, 1997; Khaironizam *et al*., 2009; Rashid *et al*., 2015; Rashid *et al*., 2018; Mohd-Azham & Singh, 2019); Terengganu drainage (Cramphorn, 1983; Kottelat *et al*., 1992); Kelantan drainage (Hashim *et al*., 2015; Alias *et al*., 2019); Muda drainage (Sah *et al*., 2012; Lee *et al*., 2013; Khaironizam *et al*., 2021); Perak drainage (Hashim *et al*., 2012; Amirrudin & Zakaria-Ismail, 2014; Ahmad *et al*., 2018; Ng *et al*., 2019); Bernam drainage (Ng *et al*., 1994); Selangor drainage (Zakaria-Ismail & Fatimah, 2002); Klang drainage (Bishop, 1973; Fatinizzati *et al*., 2018); and Muar drainage (Jeffrine *et al*., 2004; Halim *et al*., 2018). Other than that, there are also studies focusing on specific habitats such as lakes (Kamaruddin *et al*., 2011; Shafiq *et al*., 2014; Fahmi-Ahmad *et al*., 2015; Sah *et al*., 2016); acid-water swamp (Mizuno & Furtado, 1982; Ng *et al*., 1994; Shah *et al*., 2006; Amirrudin *et al*., 2011; Ahmad & Samat, 2015; Sule *et al*., 2016; Amal *et al*., 2020); agriculture areas (Aqmal-Naser & Ahmad, 2018); and drainages in islands (Ahmad & Lim, 2006; Azmir & Samat, 2010; Tan *et al*., 2015; Aqmal-Naser *et al*., 2018; Aqmal-Naser *et al*., 2019).

Ichthyological study in Malaysia is still in the discovery and exploratory stage mentioned by many researchers (Lim *et al*., 1993; Zakaria-Ismail, 1994; Ahmad & Khairul-Adha, 2005; Chong *et al*., 2010). Lim & Tan (2002), listed about 278 native species and 24 introduced species present in Peninsular Malaysia. A revision study by Chong *et al*. (2010) has listed 470 species of freshwater fishes in Malaysia (including

Peninsular Malaysia, Sarawak, and Sabah). A total of 16 species have been categorized as highly threatened, and two species have been considered extinct in wild, which are *Balantiocheilos melanopterus* and *Neobarynotus microlepis* (Chong *et al*., 2010). The main factors that cause a threat to freshwater fish species in Malaysia are habitat modification, such as the development of hydroelectric and reservoir dams across the rivers. This cause ecological disruption, especially to migrate species during spawning seasons, such as *Probarbus jullieni* and *Thynnichthys thynnoides* in Perak River. Another factor is overfishing, this happened to the species *Balantiocheilos melanopterus*. This species has a high demand as aquarium fish, and uncontrolled harvest from the wild habitats has resulted this fish becoming extinct in the wild (Chong *et al*., 2010; Zakaria-Ismail *et al*., 2019). Furthermore, the latest comprehensive publication on the taxonomy of freshwater fishes in Peninsular Malaysia has listed a total of 289 native species of freshwater fishes (Zakaria *et al*., 2019). Although many studies have been made, the discovery of new species is still to be discovered recently, such as *Betta omega*, *Betta nulohan*, *Trigonostigma truncata*, *Rasbora marinae*, *Parosphromenus barbarae*, *Barbodes sellifer*, and *Barbodes zakariaismaili* (Hui & Ahmad, 2018; Hui, 2020; Kamal *et al*., 2020; Hui & Grinang, 2020; Hui & Kottelat, 2020; Kottelat & Lim, 2021).

## 2.6 Cyprinidae fishes in Peninsular Malaysia

Fish family Cyprinidae belongs in the order Cypriniformes, the most diverse order of freshwater fishes numbering over 7500 currently recognized species (Fricke *et al*., 2020). Occurring throughout North America, Africa, Europe, and Asia, Cypriniformes are dominant members of a range of freshwater habitats, and some have adapted to extreme habitats such as caves and acidic peat swamp (Nelson, 2006).

The family Cyprinidae is the largest family of freshwater fishes with 1733 valid species (Fricke *et al*., 2020). Within family Cyprinidae, there are eleven recognized subfamilies, which are Acrossocheilinae, Barbinae, Cyprininae, Labeoninae, Poropuntiinae, Probarbinae, Schizothoracinae, Schizopygopsinae, Smiliogastrinae, Spinibarbinae, and Torinae (Tan & Armbruster, 2018). The unique features found in the family of Cyprinidae are; consists of pharyngeal teeth in one to three rows, but never more than eight teeth in any row; the presence of Weberian organ at the beginning of vertebrate column; the lips usually thin; barbels may present or absent; upper jaw usually protrusible; always toothless; adipose fin absent and head almost always scaless. Cyprinidae have range of sizes. The Siamese Giant Carp, *Catlocarpio siamensis* from Thailand is the largest tetraploid species, which is known to reach at least 2.5 to 3 meter of total length, meanwhile, the smallest cyprinid is *Paedocypris* with matured individual measured ~8 mm (Kottelat *et al*., 2006). However, the placement of fish genera *Paedocypris* still uncertain and have led to multiple conflicting hypotheses (Mayden & Chen, 2010; Britz *et al*., 2014; Tan & Armbruster, 2018; Malmstrøm *et al*., 2018). Furthermore, members of family Cyprinidae are important as food fish, recreational fishes, popular in the global ornamental pet trade, and biological research (Orban & Wu, 2008).

Despite the bulk of literature on Malaysian cyprinid fishes, the systematics and taxonomy of Peninsular Malaysian cyprinid fishes have been poorly understood until recently (Ismail, 1989; Lim & Tan, 2002). For decades, the lists of fishes recorded from Peninsular Malaysia, especially cyprinids, have referred to the taxonomic works of literature available at that time. Therefore, the validity of many species is uncertain. Furthermore, several groups' taxonomic status is still not clear, especially species that contain "cryptic" species. Besides, smaller and less attractive species often attract less scientist attention as compared to more significant and more attractive species such as Asian arowana. Even, the taxonomic status of the several large species such as *Tor*, *Poropuntius*, and *Osteochilus* are still unsettled. Lim *et al*. (1993) have listed some cyprinid genera that need to be revised, namely *Hypsibarbus*, *Labiobarbus*, *Neolissochilus*, *Poropuntius*, *Puntioplites*, *Puntius*, *Rasbora*, and *Tor*. Thus, the validity and actual numbers of these species in Peninsular Malaysia are waiting for confirmation.

### 2.7 Fishes of the genus *Poropuntius*

The cyprinid fishes of the genus *Poropuntius* are widely distributed in the river systems of Ganges-Brahmaputra in India to Southeast Asia and Xi Jiang in Southern China (Roberts, 1998). This genus usually inhabits at high gradient foothill streams at moderate elevations (usually under 1000 m). Currently, the genus consists of 34 nominal species (Kottelat, 2013) in its distributional ranges (Figure 2.3). A single species was reported from Ganges-Brahmaputra basin of India and Sumatra of Indonesia. Meanwhile, several species occur in Myanmar, the Indo-China peninsula and Yunnan of China (Rainboth, 1981; Roberts, 1998). Furthermore, Roberts (1998) suspected there are probably more than one species that occurs in the river systems of Peninsular Malaysia.

Previously, this group of fish was classified under the subfamilies of Barbinae (Rainboth, 1981) and Cyprininae (Rainboth, 1996), but currently, it is classified under the subfamily of Poropuntinae (Tan & Armbruster, 2018). The systematic history and generic placement of this genus are quite complicated as previously it was placed under various genera, such as *Barbus* (Valenciennes, in Cuvier & Valenciennes, 1842), *Puntius* (Weber & de Beaufort, 1916), *Lissochilus* (de Beaufort, 1933), *Cyclocheilichthys* (Chevey, 1934), *Lissochilichthys* (Pellegrin & Chevey, 1934), *Acrossocheilus* (Smith, 1945) and *Barbodes* (Wu *et al*., 1977).

The early taxonomic and classification works on this group of fish was started by the famous French ichthyologist, Achille Valenciennes who work with his mentor Georges Cuvier in Southeast Asia, where both of them recorded *Barbus deauratus* (now is *Poropuntius deauratus*) from Vietnam (Valenciennes, in Cuvier & Valenciennes, 1842). Initially, they classified this species under the genus *Barbus* due to barbels' presence around the mouths. Later, Weber & de Beaufort (1916) followed Hamilton's (1822) work, and the latter split many South Asian and Southeast Asian barbins from European, African and western Asian barbins by placing them under the genus *Puntius*. In the same publication, Weber & de Beaufort (1916) erected new generic name, *Lissochilus* and differentiated it from *Puntius* by the unique characters

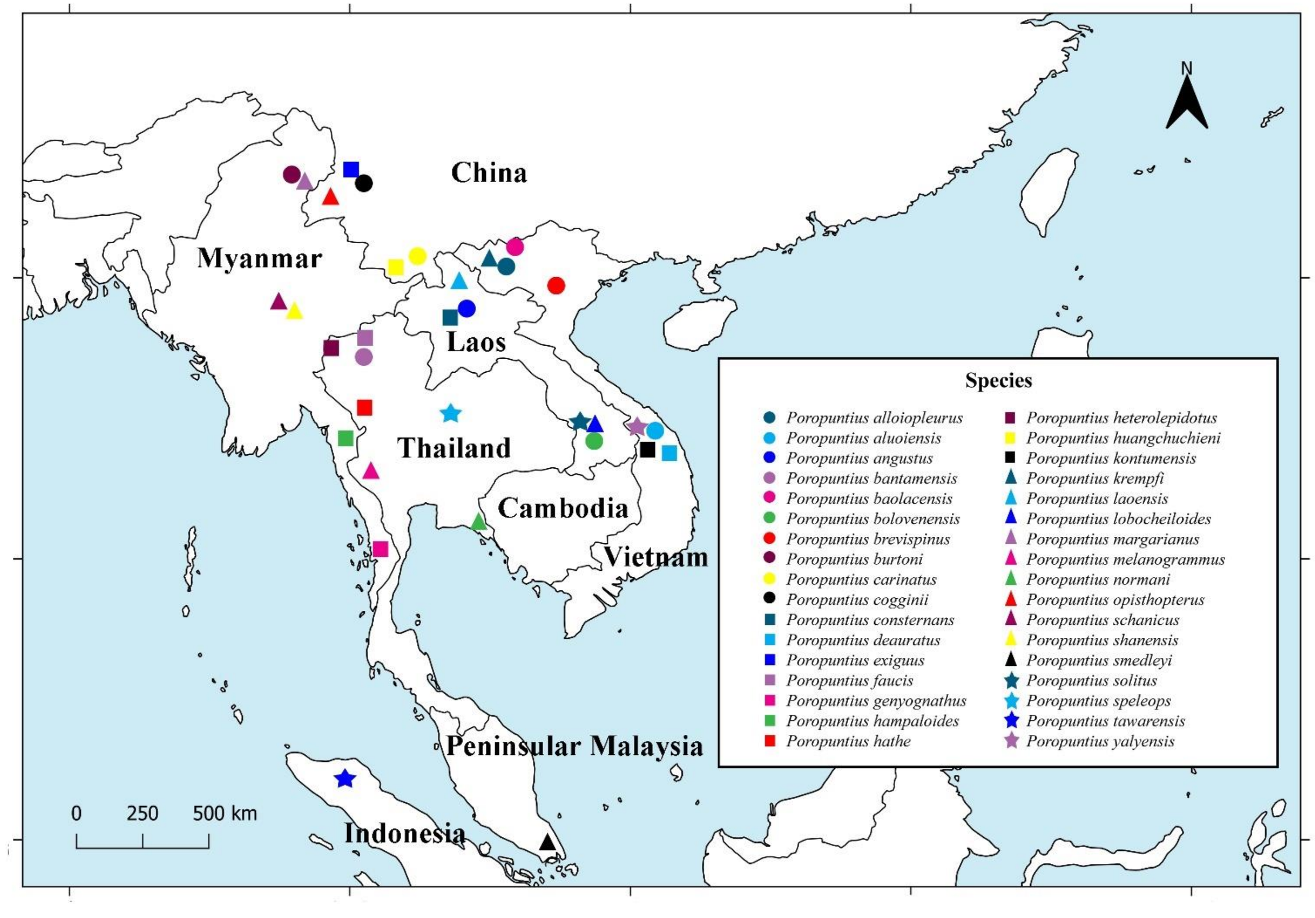


Figure 2.3 Map distribution of type locality of fish genus *Poropuntius* based on Kottelat (2013).

of the oblong and compressed body, convex and bluntly snout, presence of tubercles below the eyes, the horny sheath covering the lower jaw and a deep sulcus separating it from the lower lip, absence of lateral lobe, last simple dorsal ray is weakly ossified and not denticulated. Weber & de Beaufort (1916) have listed two species under the genera *Lissochilus* namely, *Lissochilus dukai* from India and *Lissochilus sumatranus* from Sumatra.

Smith (1931) described a new genus *Poropuntius* from Siam which differs from *Lissochilus* by having a last simple ray strongly denticulated and a rostral groove. However, de Beaufort (1933) disagreed with Smith (1931) that description of *Poropuntius* is separated with *Lissochilius* with only by a character of the denticulated spine, and he did agree with Herre & Myers (1931) that the ossification of the dorsal spine cannot be used as a generic character to spilt up this group. de Beaufort (1933) retain the generic name of *Lissochilus* for the new species *Lissochilus smedleyi* that he described from Johor, Malaysia.

Later, Myers (1941) discovered the generic name *Lissochilus* was occupied by *Lissochilus* (Pethoe) in Zittel (1882), applied to a gastropod fossil and automatically had made it junior homonym and thus not available. Later, he transferred all former species of *Lissochilus* to *Acrossocheilus* sensu Oshima, 1919 (Myers, 1941). However, in the same year as well, Hora and Misra (1941) revised the fish of the genus *Lissochilus* and regarded that most of the species of *Lissochilus* from Southern China, Siam, Burma, the Malay Peninsular and the Archipelago as synonym of *Barbus* (*Lissochilus*) *hexagonolepis*, and they tentatively placed *Lissochilus* (currently *Poropuntius*) as subgenus of *Barbus*, which distribution of this group of species is widely distributed in South-eastern Asia-Assam, Siam and Malay Peninsula.