TAXONOMIC AND ECOLOGICAL STUDIES OF STONEFLIES (INSECTA: PLECOPTERA) IN SELECTED RIVERS AT ROYAL BELUM STATE PARK, PERAK, MALAYSIA

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By

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LIST OF SYMBOLS AND ABBREVIATIONS

a.s.l	=	Above Sea Level
CCA	=	Canonical Correspondence Analysis
COD	=	Chemical Oxygen Demand
DO	=	Dissolved Oxygen
ETOH	=	Ethanol
KOH	=	Potassium Hydroxide
m	=	Metre
NST	=	News Straits Times
PCA	=	Principal Component Analysis
pH	=	Hydrogenise ion
RBSP	=	Royal Belum State Park
SE	=	Standard Error
SPSS	=	Statistical Package for Social Science
TDS	=	Total Dissolved Oxygen
TNB	=	Tenaga Nasional Berhad
TSS	=	Total Suspended Solid
WQI	=	Water Quality Index

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LIST OF PUBLICATIONS, PROCEEDING ORAL AND POSTER PRESENTATIONS

International Journal

1. Wan Nur Asiah, W. M. A., Che Salmah, M. R., & Sivec, I. (2009). Description of *Etrocorema belumensis* sp.n. from Royal Belum State Park, Perak,Malaysia. *Illiesia* **5(17):**182-187.

Oral and Poster Presentations

1. Wan Nur Asiah, W. M. A., Che Salmah, M. R., Abu Hassan, A., Zhafarina, R., & Ahmad Najmi, N. H. (2010). Diversity of stoneflies (Insecta: Plecoptera) in selected streams of Royal Belum State Park, Gerik, Perak, Malaysia. Proceedings The 11th Symposium Of The Malaysian Society Applied Biology.Hotel Grand Riverview Kota Bharu Kelantan, Malaysia. 13-15 June 2010.

2. Wan Nur Asiah, W. M. A., Che Salmah, M. R., Abu Hassan A., & Zhafarina, R. (2010). The life cycles and growth pattern of two *Kamimuria* species (Plecoptera: Perlidae) in the Mes Stream of Royal Belum State Park, Gerik, Perak, Malaysia. Proceedings of The IMT-GT Uninet and the 3rd Joint International PSU-UNS Conferences. Prince of Songkla University, Hat Yai, Songkla, Thailand. 7-8 October 2010.

3. Wan Nur Asiah, W. M. A., Che Salmah M. R., Abu Hassan, A., & Siti Mariam Zhafarina, R. (2011). Distribution of Plecopteran nymphs in selected rivers of Royal Belum State Park, Gerik, Perak: Influence of environmental factors. Proceedings Taxonomist and Ecologist Conference 2011. Auditorium CAIS Universiti Malaysia Sarawak, Samarahan, Sarawak, Malaysia. 19-20 April 2011.

KAJIAN TAKSONOMI DAN EKOLOGI LALAT BATU (INSECTA: PLECOPTERA) DI BEBERAPA SUNGAI TERPILIH DI TAMAN NEGERI ROYAL BELUM, PERAK, MALAYSIA.

ABSTRAK

Kajian taksonomi lalat batu (Insecta: Plecoptera) telah dijalankan secara memeriksa variasi genitalia ♂ dan ♀, morfologi nimfa, saiz dan bentuk telur serta struktur pada korion telur. Spesimen dipungut secara 'bucket trapping' (dewasa) dan teknik 'kick sampling' (nimfa) dari sungai di Taman Negeri Di Raja Belum (RSBP); Kejar, Mes, Tan Hain dan Ruok. Sejumlah of 98 (54♂, 44♀) dewasa dan kebanyakkan dari 8132 nimfa telah dikenalpasti ke tahap spesies. Satu spesies baru, *Etrocorema belumensis* telah ditemui dan dinamakan sempena RBSP. Beberapa spesies lain seperti *Etrocorema nigrogeniculatum, Kamimuria trang, Neoperla fallax, Neoperla hamata*, *Neoperla asperata*, *Phanoperla malayana, Cryptoperla fraterna* adalah rekod baru di kawasan tadahan ini. Satu kekunci bergambar nimfa Plecoptera dari RSBP telah dibuat supaya menjadi rujukan yang berguna dalam kajian lalat batu di Malaysia pada masa hadapan.

Nilai parameter fizik-kimia mengkatogerikan semua sungai sebagai mempunyai kualiti air yang terbaik. Keterdapatan nimfa lalat batu sepanjang tahun penyampelan juga menunjukkan sungai-sungai tersebut menampung kelimpahan dan diversiti yang tinggi bagi order serangga ini. Perlidae adalah famili yang paling dominan (88%) antara tiga famili yang telah dikumpul diikuti oleh Peltoperlidae (12%). Perlid *Etrocorema, Neoperla, Kamimuria* dan *Phanoperla* bertabur secara meluas di semua sungai. Nimfa Plecoptera terdiri daripada pemangsa (88%) dan

pengoyak (12%) dengan kelimpahan komuniti yang tinggi dijumpai pada longgokan daun diikuti pada kerikil besar, kerikil, 'boulder' dan di dalam lubuk.

Antara berbagai parameter fizik-kimia air yang telah diukur, kandungan oksigen terlarut (DO), jumlah pepejal terampai (TSS), pH dan kelebaran sungai (ρ DO = 0.152; ρ TSS = -0.153; ρ pH = 0.218; ρ width = 0.083, $p < 0.05$) sangat lemah mempengaruhi kelimpahan dan diversiti nimfa lalat batu. Tiada perbezaan musim pada kelimpahan nimfa yang dipungut ketika musim hujan dan kering berdasarkan nilai Ujian U Mann Whitney ($z=-2.014$, $p =0.44$). Ordinasi PCA menunjukkan pemilihan mikrohabitat yang tinggi oleh nimfa Plecoptera terhadap kerikil besar, kerikil dan longgokan daun.

Plot taburan panjang badan nimfa memisahkan dua spesies *Kamimuria* yang diperolehi dari Sungai Mes kepada sekurang-kurangnya 15 peringkat instar. *Kamimuria trang* adalah lebih besar daripada *Kamimuria* spesies A. Kajian ini telah menunjukkan pertumbuhan yang cepat bagi *Kamimuria trang* dan *Kamimuria* spesies A yang hanya mengambil masa tiga hingga empat minggu bagi melengkapkan satu kitar hidup pada suhu air suam (21^oC – 25^oC) di sebuah sungai tropika. Oleh itu kitaran hidup *Kamimuria* spp di Sungai Mes adalah multivoltin berkadaran dengan jangka masa kitar hidup mereka dan taburan instar nimfa pada kesemua bulan penyampelan.

TAXONOMIC AND ECOLOGICAL STUDIES OF STONEFLIES (INSECTA: PLECOPTERA) IN SELECTED RIVERS AT ROYAL BELUM STATE PARK, PERAK, MALAYSIA.

ABSTRACT

Taxonomic study of stoneflies (Insecta:Plecoptera) was carried out by examining variations of ♂ and ♀ genitalia, morphology of their nymphs, size and shape of eggs and structures on egg chorion. Specimens were collected by bucket trapping (adult) and kick sampling technique (nymph) from rivers of Royal Belum State Park (RSBP); Kejar, Mes, Tan Hain and Ruok. A total of 98 (54♂, 44♀) adults and most of the 8132 nymphs were identified to the species level. A new species, *Etrocorema belumensis* was discovered and named after the RBSP. Several other species such as *Etrocorema nigrogeniculatum*, *Kamimuria trang*, *Neoperla fallax*, *Neoperla hamata*, *Neoperla asperata*, *Phanoperla malayana*, *Cryptoperla fraterna* were new records for this catchment. A pictorial key for stonefly nymphs from RBSP was developed to provide a useful reference for future study of stoneflies in Malaysia.

The values of physico-chemical parameters categorized all rivers as having excellent water quality. The occurrence of Plecoptera nymphs through out a year of sampling also showed that the rivers supported high diversity and abundance of this insect order. Perlidae was the most dominant (88%) among three families collected followed by Peltoperlidae (12%). Perlid *Etrocorema, Neoperla, Kamimuria and Phanoperla*, were widely distributed in all rivers. Plecopteran nymphs consisted of

predators (88%) and shredders (12%) with high abundance of this community encountered in leaf litter followed by on cobble, gravel, boulder and in pool.

Among various physico-chemical parameters of the water measured, dissolved oxygen (DO) content, total suspended solid (TSS), pH and river width ($\rho_{DO} = 0.152$; $\rho_{TSS} = -1.53$; $\rho_{pH} = 0.218$; $\rho_{width} = 0.083$, $p < 0.05$) very weakly influenced the abundance and diversity of stonefly nymphs. There was no seasonal difference in abundance of the nymphs collected in wet and dry periods as indicated by the scores of Mann Whitney U test ($z = -2.014$, $p = 0.44$). The PCA ordination identified high microhabitat preferrence of the plecopteran nymphs for cobble, gravel and leaf packs.

Nymphal body length scatter plots separated two *Kamimuria* species collected from Mes River into at least 15 instars. *Kamimuria trang* was slightly bigger than *Kamimuria* species A. This study had shown rapid growth of *Kamimuria trang* and *Kamimuria* species A that took only 3 to 4 weeks to complete their life cycles in warm water (21^oC – 25^oC) of a tropical river. Therefore the life history of *Kamimuria* spp. in Mes River was multivoltine corresponding with their life cycle durations and distributions of nymphal instars in all sampling months.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Insects are the dominant group of animals on earth today and occur practically everywhere. Over one million insect species described, with approximately 30 000 species are aquatic, living in freshwater (Bonada et al., 2006). Although there are 30-31 insect orders, only 13 orders have aquatic representatives (Merrit and Cummins, 1984; Merrit et al., 2008) and majority of them live in freshwater ecosystems. The work on aquatic insect as indicator of water quality started in the years of '70s and '80s and became dominant forms used in freshwater investigation (Barnes and Minshall, 1983) until nowadays (Chessman, 1995; Throne and Williams, 1997; Kay et al., 2001; Clements and Newman, 2002; Metzeling et al., 2003; Davy-Bowker et al., 2005; Hicham and Lotfi, 2007; Rohasliney and Jackson, 2008; Canobbio et al., 2009; Song et al., 2007; Friberg et al., 2010).

In the last few decades there has been an upsurge of interested in assessment techniques for the biological monitoring of water quality in several developed countries (Chessman, 1995; Throne and Williams, 1997; Kay et al., 2001; Clements and Newman, 2002; Metzeling et al., 2003; Davy-Bowker et al., 2005; Hicham and Lotfi, 2007; Song et al., 2007; Rohasliney and Jackson, 2008; Canobbio et al., 2009; Friberg et al., 2010). These methods emphasized on a low cost approach, achieved by reduced sampling and more effective data analysis (Resh et al., 1995; Throne and Williams, 1997). The low cost of such approaches make them attractive for use in developing countries (Resh et al., 1995; Che Salmah et al., 1999; Mustow, 2002; Azrina et al., 2006; Arimoro, 2009; Boonsoong et al., 2009), and others features enhance this suitability. Up to date, there are a very few studies concerning the macroinvertebrate as a biological indicator in Malaysian aquatic ecosystem (Che Salmah et al., 1999; Wahizatul Afzan, 2004; Azrina et al., 2006).

As an important component of aquatic ecosystem community (Zwick, 2004), Plecoptera have been proven to be useful biological indicators because of their response to physical and chemical changes in aquatic ecosystems (Harper, 1994). Plecoptera are frequently associated with cold and clean streams (Williams and Feltmate, 1992). In the tropics, many taxa are limited to hilly and forested areas. The Plecoptera nymphs are common in fast, clean and cold waters with stone bottoms (Macan, 1962; Bachmann, 1995; Harper and Stewart, 1996). Together with Ephemeroptera and Trichoptera, Plecoptera is generally considered to be intolerant of pollution (Rosenberg and Resh, 1993; Wiggins, 1996). Several factors influence the growth of Plecoptera in its habitats. These factors include the size of water bodies, habitat heterogeneity, adaptations to species niches, ecological requirements, competition, predation, historic events, temperature and latitude, amongst others (Gaston, 2000).

Although tropical Asian river research has increased over the last two decades, great attention has been paid to the loss of biodiversity in the tropical rain forests because of the recent increase of anthropogenic influences in the region (Dudgeon, 2000). There is still a huge gap in the knowledge of fauna and ecological understanding of tropical rivers compared with that of temperate river (Jackson and Sweeney, 1995; Ometo et al., 2000; Morse et al., 2007; Dudgeon, 2006; 2008). For example, the taxonomy of tropical benthic fauna is poorly known in the tropical rivers (Cranston, 1995; 2007; Jacobsen et al., 2008).

The diversity of Plecoptera declines rapidly from temperate Asian latitudes (nine families) to tropical latitudes (four or fewer families) (Jacobsen and Encalada, 1997). The only diverse stonefly family in the Malaysian region is the Perlidae. Comparative to their temperate counterparts, tropical stoneflies are incompletely understood (Sheldon and Theischinger, 2009) although these regions have the highest diversity (Zwick, 2000). Stoneflies diversity in Asia is much greater than that of Europe or North America but the knowledge of the enduring Asiatic species is extremely poor except for Japan (Fochetti and Tierno de Figuera, 2008). In Malaysia, there has been some efforts to undertake systematic studies on aquatic insects (Jackson and Sweeney, 1995; Ometo et al., 2000; Dudgeon, 2006; Morse et al., 2007; Wan Nur Asiah et al., 2009). Sivec and Yang (2001) estimated that there are approximately 350 Plecoptera species in countries forming the Oriental Region except for Southern China. One reason for the scantiness of ecological studies of tropical river invertebrates in the international literature is the fact that identification of tropical species is difficult. Many lower taxa (especially genera and species) have received limited study and relevant literature is scarce (Boyero, 2002). Therefore the number of species cannot be estimated accurately in Malaysia.

This study is focused on the taxonomic and ecological studies of Plecoptera within the aquatic insect community in Royal Belum State Park (RBSP), Perak, Malaysia. The taxonomic study is important (such as keys) for future identification of members this family. Apart from that, this study also can help to increase knowledge on the reliability of using Plecoptera as an indicator of water quality assessment.

1.2 Objectives

The objectives of this study are:

1. To study the taxonomy of Plecoptera nymph in RBSP area and provide a checklist of Plecoptera in Malaysian region.

2. To examine the diversity of Plecoptera in various microhabitat in running water ecosystem in headwater streams of RBSP.

3. To investigate the influence of water quality on the distribution and diversity of Plecoptera nymphs.

4. To study the life histories of selected Plecoptera species in Mes River of RBSP.

CHAPTER 2

LITERATURE REVIEW

2.1 Headwater Ecosystem

Headwater is the most sensitive aquatic ecosystem, the crucial point of mountain landscape, sustaining unique plant and animal communities and influences the quality and quantity of lowland rivers (Lowe and Likens, 2005). Higher level of habitat diversity, among and within streams, creates niches for different organisms, including headwater-specialist species of aquatic invertebrates, amphibians and fishes (Lowe and Likens, 2005). Integrity of freshwater ecosystem is becoming a priority demand (Leopold et al., 1964, Brittain and Milner, 2001). The rate of biodiversity decline is five time faster in freshwater ecosystems than in terrestrial and marines ones (Ricciardi and Rasmussen, 1989), this is due to over exploitation and pollution of water bodies, which consequently accelerating loss of species, populations and natural habitat such as tropical rainforest and wetland (Olomukoro and Ezemonye, 2007).

In the last decade, growing attention has been demonstrated by the scientific community in developed countries to freshwater ecosystems, arising out the knowledge for a better preservation of their functionality (Vescovi et al., 2009). However, in tropical area of developing countries, the knowledge of freshwater ecology is fragmentary (Dudgeon, 1999). Although tropical rivers support very diverse aquatic ecosystem (Dudgeon, 2000), and leading diverse aquatic fauna compared to that of temperate rivers (Helson et al., 2006). There is still huge gap in the knowledge of tropical rivers compared with that of temperate rivers (Jackson and Sweeney, 1995, Dudgeon, 2000). For instance, the taxonomy of tropical aquatic insects including stonefly is poorly known compared to that of temperate regions

(Yule and Yong, 2004). More research is required for better understanding of tropical rivers ecosystem and to reduce the huge gap of knowledge between tropical and temperate rivers.

2.2 Aquatic Insects Including Plecoptera as Bio-indicator

The aquatic insect had been used for monitoring water pollution around the world (Holt and Miller, 2011). Several families of aquatic fauna act as major groups of indicator for water quality assessment such as dipterans, ephemeropterans, plecopterans and trichopterans (Holt and Miller, 2011). The significance of these four families as indicators in trophic status for freshwater ecosystem has been documented (Paterson and Fernando, 1970, Ogbeibu, 2001). The ephemeropterans, plecopterans and trichopterans are restricted to cool, clean streams and rivers with high dissolved oxygen content; dipterans like chironomids usually have high tolerance to pollution.

Plecoptera is a very interesting group to study when assessing biotic responses to predicted climate changes especially global warming (Tierno de Figueroa et al., 2010) because they are they are very sensitive to environmental alterations when used as bio-indicator (Rosenberg and Resh, 1993). Generally, Plecoptera is included in an endangered species list, with populations that have decreased mainly as a consequence of habitat alteration. Plecopterans also could suffer from the effects of climate change such as increase continental run-off, increased of water temperature, increased in the intensity of extreme precipitation events over many regions and changes in seasonality of river flows (EEA, 2008). Knowledge of Plecoptera such as taxonomy and their ecology is fundamental and is needed for better preservation of freshwater ecosystems. By concentrating on a single aquatic insect order, a relatively complete taxonomic list can be obtained at the species level, eliminating the uncertainties of incomplete identifications.

2.3 Stonefly (Insecta: Plecoptera)

2.3.1 Morphology with Descriptions of Male, Female and Egg

Plecoptera is a hemimetabolous insect (Beer-stiller and Zwick, 1995) having three life stages with adults resembling winged nymphs (Sivec and Yule, 2004). Stoneflies are generally poor fliers and are seldom found far from water (Sivec and Yule, 2004). The adult is mostly medium sized or small, somewhat flattened and soft bodied, mandibulate with filiform antennae, bulging compound eyes, and two or three ocelli (Stewart and Stark, 1988) (Figure 2.1 a). The thoracic segments are sub-equal and the fore and hind wings are membranous and similar (except the hind wings are broader), with the folded wings partly wrapping the abdomen and extending beyond the abdominal apex (Gullan and Cranston, 2005). The legs are unspecialized, and the tarsi comprise three segments (Gullan and Cranston, 2005). The abdomen is soft and has 10 obvious segments plus an 11^{th} and 12^{th} are that serve as paraparocts, cerci, and epiproct, a combination of which serve as male accessory copulatory structures, sometimes in conjunction with the abdominal sclerites of segments 9 and 10 (Gullan and Cranston, 2005).

The hemitergites 10 of male Perlinae are modified into anteriorly curved hooks (Stark and Szczytko, 1984). Determination of Perlidae male is characterised by the reduced epiproct. Males mostly lack a hammer and have unmodified paraprocts and have hair brushes on mesal areas of abdominal sternites (Stark and Szczytko, 1988). The penis is eversible, complex sclerites, teeth, or trichomes adorn a more or less sclerotized basal tube of the penis or an eversible sac in it; especially in Neoperlini examples of lock-and-key fit with female vaginal or receptacle structures is apparent (Zwick, 2000).

Typical stonefly nymphs are elongated, flattened insects with long antennae and long cerci, and often with branched gills on the thorax and on the bases of the legs (Stewart and Stark, 1988) (Figure 2.1 b). Typical stonefly nymphs have two tarsal claws and their gills are always finger like either simple or branched, and only occur ventrally (Sivec and Yule, 2004). This study focused more on Perlidae nymphs based on their distribution in Oriental region.

According to Stark and Szcytzko (1988), stonefly's egg look likes an ovoid structure with polar arrangements of micropyles, eclosion lines and attachment structure and the chorionic surface typically bears some reticular manifestation of the follicular epithelium.

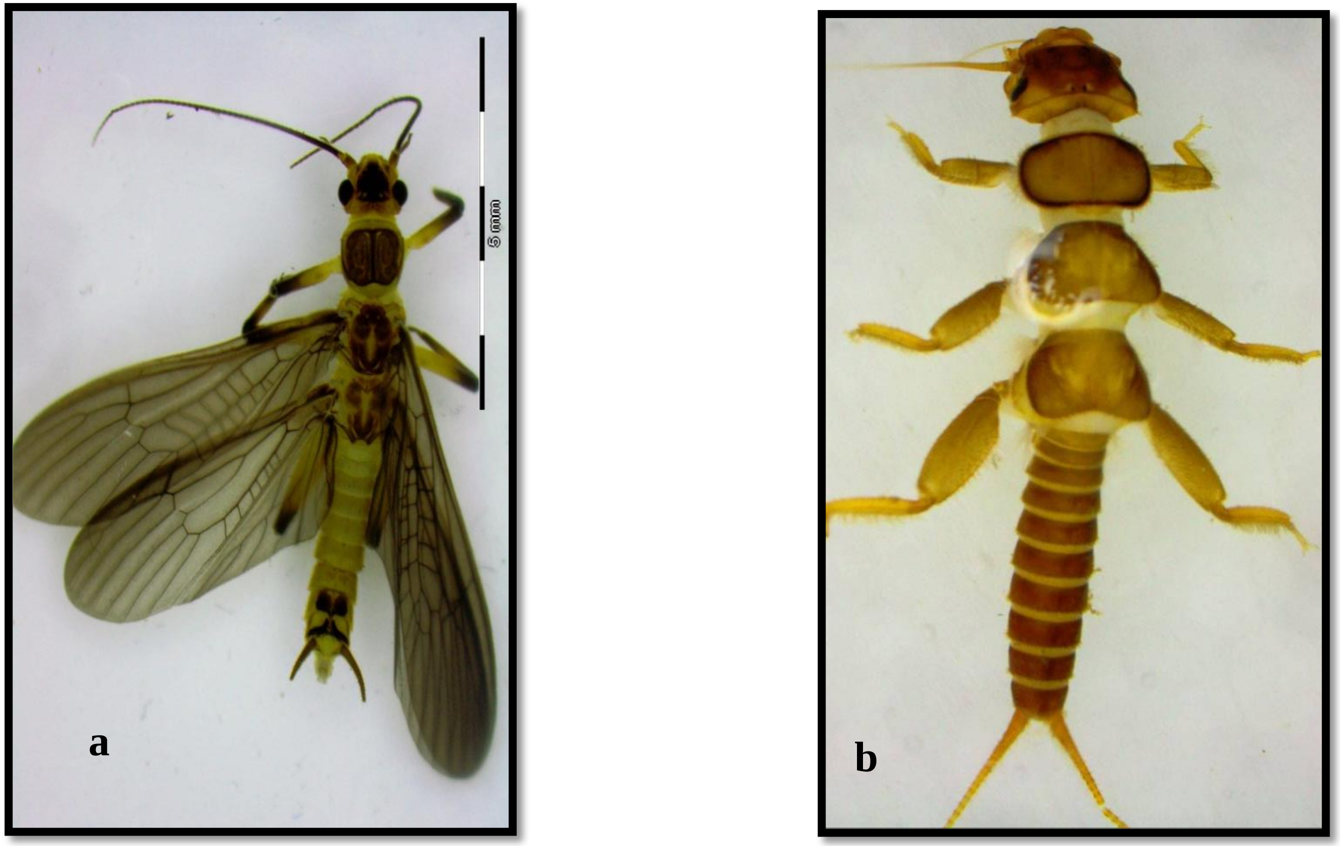

Figure 2.1: *Etrocorema belumensis*: **a.** Adult **b**. Nymph

2.3.2 Classification of Plecoptera

Plecoptera, are neoptera Pterygota insects (Hexapoda). It is a small order of hemimetabolous insects, closely related to other orders of Exopterygota (Zwick, 2000). Plecopterans have retained many primitive characters that confirm their ancestral position and the basal role with respect to the rest of Neoptera. They are possibly the sister group of the remaining Exopterygota, and they could have had an evolutionary link with the Ephemeroptera (Henning, 1981; Kristensen, 1991; Beutel and Gorb, 2001).

The order includes 16 families of which relationships and biogeography have been studied by several authors (Henning, 1981; Kristensen, 1991; Zwick, 2000; Beutel and Gorb, 2001). Here the taxonomy of Plecoptera followed the more recent and widely accepted classification by Zwick (2000) (Figure 2.2). He recognizes two large suborders: Antarctoperlaria, present only in the Southern Hemisphere, and Arctoperlaria, distributed mostly in the Northern Hemisphere. The first taxon includes 4 families; the latter includes 12 families belonging to 2 superfamilies; Systellognatha (predaceous stonefly) and Euholognatha (herbivour and detrivores stonefly) each with 6 families.

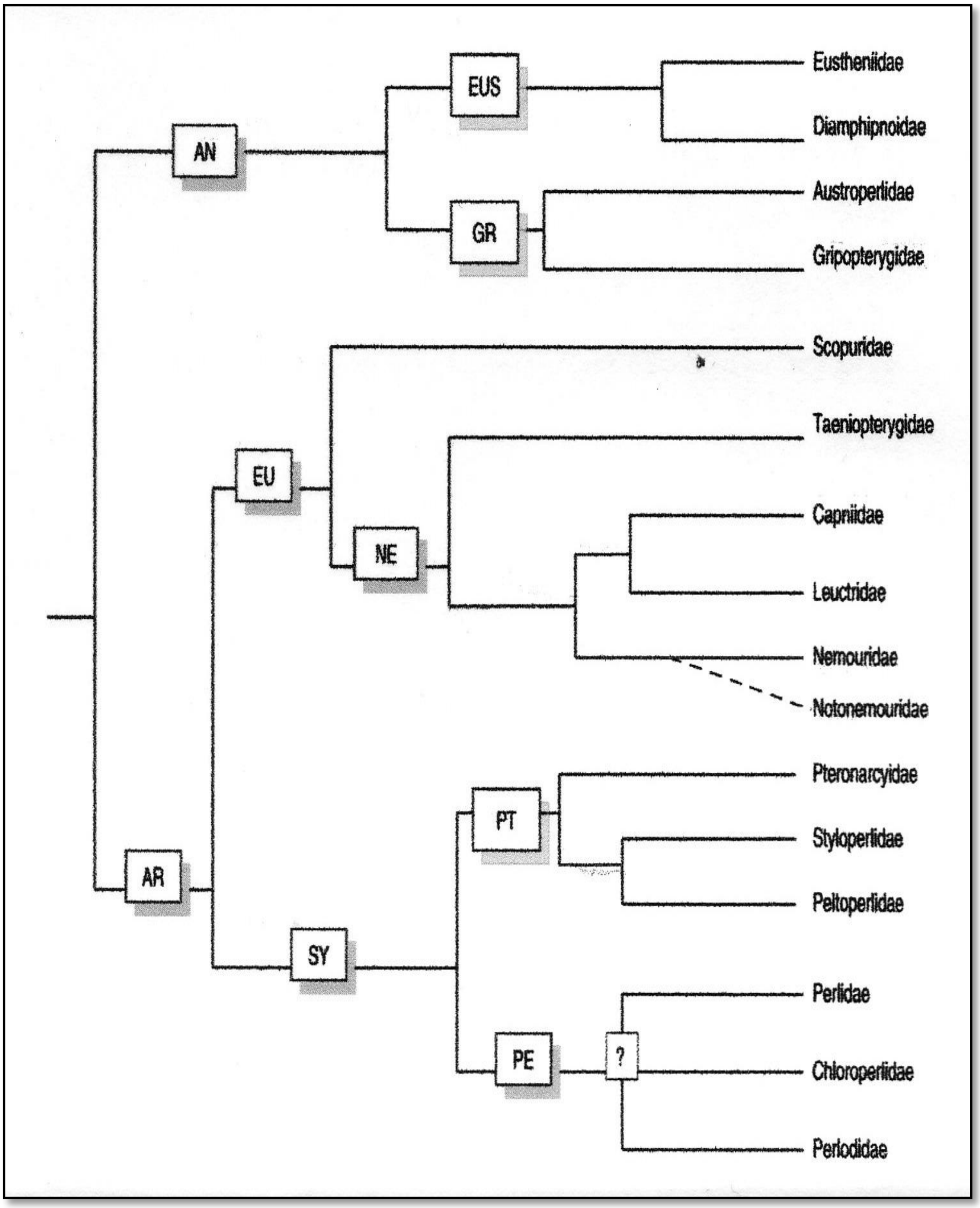

Figure 2.2: Plecoptera phylogeny according to Zwick (2000). AN-Antarctoperlaria; AR-Arctoperlaria; EUS-Eusthenidae; GR- Gripopterygidae; EU-Euholognatha; NE-Nemouroidea; SY-Systellognatha;PT-Pteronarcyoidea;PE-Perloidea (from Fochetti and Tierno de Figuera, 2008).

2.3.3 The Oriental Plecoptera

Asia lies within Paleartic and Oriental geographic regions. With the largest continental area, Asia contains the greatest number of stonefly species of 11 families and minimum of 1050 species (Table 2.1) (Levanidova and Zhiltzova 1979, Shimizu 2001, Sivec at al., 1988, Stark 1989, Teslensko 2003, Zhiltzova, 2003 and Yang et al., 2004). The Oriental realm has the richest stonefly fauna in the world (Sivec and Yang, 2001), unfortunately very few people are studying the region to estimate the number of species present. China alone could have between 500 to 1000 plecopteran species (Sivec and Yang, 2001). The Perlidae and Nemouridae families contain almost 70% of the species known in Asia and Scopuridae and Styloperlidae only occur in small region of the Orient (Uchida and Maruyama, 1987, Uchida and Isobe, 1989).

Table 2.1: Summary of Oriental Stonefly

Family	Number of genera	Number of species
Capniidae	~9	~50
Chloroperlidae	~9	~30
Leuctridae	4	~50
Nemouridae	7	`~350
Peltoperlidae	5	~50
Perlidae	~26	~380
Perlodidae	~20	~100
Pteronarcyidae	1	2
Scopuridae	1	5
Styloperlidae	2	10
Taeniopterygidae	~7	~20

According to Fochetti and Tierno de Figueroa (2008), 784 species of Plecoptera have been reported from Southeast Asia. They are divided according to continents where the fauna is studied as the following: Taiwan 31 species, Korea 32 species, Philipines 25 species, Borneo 39 species, Indonesia 36 species, Malaysia 17 species, Thailand 29 species, Vietnam 20 species, Laos 2 species, Myanmar 3 species, Bhutan 28 species, Bangladesh 2 species, Sri Lanka 10 species, India 112 species, Nepal 63 species, Pakistan 20 species, Afghanistan 9 species and Japan 306 species.

Significant progress has been made in recent year in the understanding of tropical fauna with most subjects receiving at least some attention at one or more tropical location (Maloney and Ferminella, 2005). Thus, additional new species are also recorded and increase the number of Plecoptera in Asian region especially for Malaysia. According to Fochetti and Tierno de Figueroa (2008), there are 17 species of stoneflies recorded from Malaysia but recent statistic shows that 39 species of stoneflies occurred in this country (Table 2.2).

Table 2.2: Checklist of Plecoptera in Asian region.

No	Species	Family	Distributions	Sources
1	*Amphinemura minuta* Kawai	Nemouridae	Malaysia (Borneo)	Sivec and Yule, 2004
2	*Chinorperla fascipennis* (Banks)	Perlidae	Thailand, Malaysia	Zwick 1982c
3	*Etrocorema nigrogeniculatum* (Enderlein)	Perlidae	Thailand, Malaysia, Indonesia (Sumatera)	Kawai 1968; Zwick 1982b; Suhaila and Che Salmah 2011
4	*Etrocorema belumensis* Wan Nur Asiah and Che Salmah	Perlidae	Malaysia	Wan Nur Asiah et al., 2009; Suhaila and Che Salmah 2011
5	*Chinorperla borneensis* Sivec and Zwick	Perlidae	Malaysia (Borneo)	Sivec and Yule, 2004; Suhaila and Che Salmah 2011
6	*Cryptoperla fraterna* Banks	Peltoperlidae	Malaysia (Borneo)	Sivec and Yule, 2004; Suhaila and Che Salmah 2011
7	*Indonemura jacobsoni* (Klapalek)	Nemouridae	Malaysia (Borneo)	Sivec and Yule, 2004
8	*Kamimuria kelantonica* Klapalek	Perlidae	Malaysia	Sivec and Stark, 2008
9	*Kamimuria trang* Sivec and Stark	Perlidae	Malaysia, Thailand	Sivec and Stark, 2008; Suhaila and Che Salmah 2011
10	*Kamimuria jariyae* Sivec and Stark	Perlidae	Malaysia, Thailand	Sivec and Stark, 2008; Suhaila and Che Salmah 2011
11	*Kamimuria curriei* Sivec and Stark	Perlidae	Malaysia, Thailand	Sivec and Stark, 2008; Suhaila and Che Salmah 2011
12	*Neoperla asperata* Zwick	Perlidae	Thailand, Malaysia	Zwick 1988; Suhaila and Che Salmah 2011
13	*Neoperla fallax Klapalek*	Perlidae	Malaysia	Stark and Sivec, 2007 a; Suhaila and Che Salmah 2011
14	*Neoperla banksi* (Illies)	Perlidae	Malaysia, Thailand	Zwick 1988
15	*Neoperla cameronis* Zwick	Perlidae	Malaysia	Zwick 1988
16	*Neoperla divergens* Zwick	Perlidae	Malaysia (Borneo)	Stark and Sheldon, 2009

17	*Neoperla edmunsi Stark*	Perlidae	Malaysia (Borneo)	Stark and Sivec, 2007a
18	*Neoperla grafei* Stark and Sheldon	Perlidae	Malaysia (Borneo)	Stark and Sheldon, 2009
19	*Neoperla hamata* Jewett	Perlidae	Malaysia	Jewett, 1975; Suhaila and Che Salmah 2011
20	*Neoperla lepthophallus* Zwick	Perlidae	Thailand, Malaysia	Zwick 1988
21	*Neoperla malleus* Zwick	Perlidae	Malaysia	Zwick 1988
22	*Neoperla naviculata naviculata* Klapalek	Perlidae	Indonesia (Borneo), Malaysia (Borneo)	Zwick 1986a
23	*Neoperla nova* Zwick	Perlidae	Vietnam, Malaysia	Zwick 1988
24	*Neoperla parva* Banks	Perlidae	Malaysia (Borneo)	Stark and Sheldon, 2009
25	*Neoperla rougemonti* Zwick	Perlidae	Malaysia (Borneo)	Stark and Sheldon, 2009
26	*Neoperla sabah* Zwick	Perlidae	Malaysia (Borneo)	Stark and Sheldon, 2009
27	*Neoperla theobromeo* Zwick	Perlidae	Malaysia (Borneo)	Stark and Sheldon, 2009
28	*Peltoperlopsis concolor* (Banks)	Perlidae	Malaysia (Borneo)	Sivec and Yule, 2004
29	*Phanoperla malayana* Zwick	Perlidae	Vietnam, Thailand, Malaysia	Zwick 1982 a; Stark 1987; Suhaila and Che Salmah 2011
30	*Phanoperla simplex* Zwick	Perlidae	Malaysia	Sivec and Yule, 2004
31	*Rhopalopsole bakeri* Jewett	Leuctridae	Malaysia (Borneo)	Sivec and Yule, 2004
32	*Rhopalopsole belumensis* Sivec and Harper	Leuctridae	Malaysia	Sivec et al., 2008
33	*Rhopalopsole bicornuta* Jewett	Leuctridae	Malaysia	Sivec and Yule, 2004
34	*Rhopalopsole edwarsi* Sivec and Harper	Leuctridae	Malaysia (Borneo)	Sivec et al., 2008
35	*Rhopalopsole mataikan* Stark and Sivec	Leuctridae	Malaysia (Borneo)	Sivec et al., 2008
36	*Rhopalopsole palawana* Jewett	Leuctridae	Malaysia (Borneo)	Sivec and Yule, 2004
37	*Tetropina fulgescens* (Enderlein)	Perlidae	Malaysia (Borneo)	Sivec and Yule, 2004
38	*Tetropina larvata* Klapalek	Perlidae	Malaysia (Borneo)	Sivec and Yule, 2004
39	*Tominemoura trilari* Stark and Sivec	Perlidae	Malaysia (Borneo)	Sivec and Stark, 2009

2.3.4 Taxonomic Study of Plecoptera in Malaysia

The known stonefly fauna of Malaysia includes members of Leuctridae, Nemouridae, Peltoperlidae and Perlidae (Sivec and Yule, 2004). This study only focused on the Perlidae family due to its wide distribution in Peninsular Malaysia. Classification of Plecoptera given below is after Zwick (2000).

Suborder EUHOLOGNATHA

- Leuctridae - rolled-winged stoneflies
- Nemouridae - spring stoneflies
- Peltoperlidae - roachlike stoneflies

Suborder SYSTELLOGNATHA

- Perlidae - common stoneflies

Taxonomic study of stonefly in Malaysia has been conducted as early as 1973. Bishop (1973) reported one species (*Etrocorema nigrogeniculatum*) of the Family Perlidae. In 1975, *Kamimuria kelantonica* was reported by Zwick from Kelantan, Malaysia. Since then no work had been done on stonefly until 1982, when Zwick reported one male and two females of *Phanoperla simplex* from Kuala Lumpur and described one male of *Phanoperla malayana* also from Kuala Lumpur. Later on Zwick and Sivec (1985) described the egg of this species also from the similar locality.

During 1988, Zwick described *Neoperla asperata* as a new species from Klang, Kuala Lumpur and one male of *Neoperla fallax* from Ulu Gombak. Together with *Neoperla malleus*, *Neoperla banksi* were reported by Zwick in the same year from Cameron Highland, Pahang. During his trip to Cameron Highland, he described two males of *Neoperla cameronis*. A year later, Uchida and Yamasaki (1989), described the egg of *Neoperla fallax* from Kuala Lumpur.

Recently in 2009, *Etrocorema belumensis* was described by Wan Nur Asiah et al. as a new species from Belum area catchment. Then, in 2011, Suhaila and Che Salmah reported a number of species from Tupah River, Kedah such as *Neoperla asperata, Neoperla fallax, Neoperla hamata, Kamimuria trang, Kamimuria jariyae, Kamimuria curriei, Etrocorema nigrogeniculatum and Phanoperla malayana.*

2.4 Factors Influencing the Abundance of Plecoptera Population

Lotic insects, including stoneflies, are subject to both natural and man induced disturbance and change (Resh et al., 1988). Natural factors such as temperature, elevation, latitude, substrate type, discharge, and current velocity are primary factors influencing stoneflies distribution (Benke et al., 1984; Hellawell, 1986; Hall and Ide., 1987; Ward, 1992; Morse et al., 1993; Quinn and Hickey 1994; Malmqvist, 1999; Doisy and Rabeni, 2001). This suggests that the interactions of environmental factors causing aggregation of any benthic species are in balance. Any successful attempt to delineate these factors in one sampling site may not have any applicability to the aggregation produced in other areas (Patersen and Fernando, 1970). The variation in aquatic insect diversity across habitats should be considered while designing biodiversity and biomonitoring studies in aquatic ecosystems (Subramaniam and Sivaramakrishnan, 2005).

2.4.1 Structure of Microhabitat and Vegetation

Generally, many stonefly species are established only in certain type of streams, often in specific microhabitats (Knight and Gaufin, 1966; Stoneburner 1997, Sheldon, 1985). Helesic and Sedlack (1995) also documented the importance of substrate types in relation to the stonefly community. In broader habitat, many species are found at certain elevations or in large or in small or other stream types (Knight and Gaufin, 1967). A few stoneflies in temperate region such as *Brachyptera* and the gripopterygid *Trinotoperla* like to live on large rocks (Hynes, 1976), *Protonemura* in moss (Hynes, 1961; Egglishaw, 1969) and *Nemoura cinerea* and *Leuctra nigra* on silt substratum in slow water (Brinck, 1949).

A few examples of substratum-stoneflies relationships in tropical region showed that *Neoperla* spp. and *Kamimuria* spp. (Perlidae) are adapted to fast and medium flowing waters and rocky substrates (Dudgeon, 2000; Subramaniam and Sivaramakrishnan, 2005; Otsuki and Iwakuma, 2008). Species of Perlidae and Perlodidae represent a highly sensitive group of which species explicitly prefer gravel or cobbles-boulder bottom (Helesic and Sedlack, 1995). According to Buffagni and Comin (2000), *Amphinemura* (Nemouridae) shows preference to live in pool.

2.4.2 Temperature and Oxygen

Generally, the distribution of Plecoptera is closely linked to the temperature (Illies, 1964; Bispo et al., 2002). Hynes (1941) and Macan (1962), states that altitudinal influences the temperature is lower where at higher altitudes. The direct effect of temperature on the aquatic organisms will be the increased of oxygen solubility. Temperature and oxygen solubility are negatively correlated and their combination can restrict the occurrence of some Plecoptera taxa to definite altitudes or latitudes (Pennak, 1978). For example, in Brazil, Gripoterygidae genera are characteristics of the mountainous areas, where the altitudes are above 950 metre a. s. l., Perlidae are found at lower altitudes between 800 metre and 700 metre a. s. l. with exception of *Tupiperla* spp. (Bispo et al., 2002).

2.4.3 pH and Conductivity

The pH value or the concentration of hydrogen ions is used to measure the strength of acid and alkali in water samples while conductivity of natural water is a measurement of the water ability to conduct electricity (Boyd, 1990). Distribution of aquatic insects is strongly affected by the variations in the pH values (Potts and

Fryer, 1979) and decreased in pH (less than 5.5) is related to a general decrease in diversity of aquatic insects (Winner et al., 1980; Coimbra et al., 1996). However, high pH was reported to affect the structure of the benthic communities (Coimbra et al., 1996). Studies by previous researchers, Bispo et al., 2006 found that pH and electrical conductivity affect the distribution of Ephemeroptera, Plecoptera and Trichoptera (EPT) only at extreme values or when they are associated with organic pollution. Thomsen and Friberg (2002) documented that slow development occurred in *Leuctra nigra* (Plecoptera; Leuctridae) due to increased pH in coniferous forest, Denmark.

2.4.4 Total Suspended Solid

Suspended solids are defined as residue acquired after the filtering process. These residues usually consist of organic and inorganic substances such as plankton, silt and clay (Twort et al., 1994). The amount of suspended solids in surface water can be influenced by water current and seasonal changes. For example, during rainy season or flood season the amount of suspended solids can increase a few thousand mg/L (Twort et al., 1994). The increase in suspended solids can also be caused by human activities. This includes changes in land use, such as farming (Allan et al., 1997; Hellawell, 1986; Murakami et al., 2001), road construction (Barton, 1977; Cline et al., 1982; Extence, 1978), forestry activities (Platts et al., 1989) and mining (Hellawell, 1986).

Previous research works had demonstrated that fine sediments would have significant adverse effects on aquatic biota and lotic ecosystem (Newcombe and Macdonald, 1991; Ryan, 1991; Wood and Armitage, 1997; Yamada and Nakamura, 2002). Malmqvist (1999) and Miserendino and Pizzolon (2004) reported that

Plecoptera community decreased when fine sediments accumulation increased due to the deposition of fine sediments on the gills of nymphs. Besides, sedimentation also reduced the food availibity for Plecoptera community (Deegan et al., 2007).

2.4.5 Water Movement in Relation to River Width and Depth

The current speed is a factor of major importance in running water. It controls the occurrence and abundance of aquatic insect and hence the whole structure of the animals community (Che Salmah et al., 1999). It plays an important role in maintaining high concentrations of dissolved oxygen (Hollmann and Miserendino, 2006). Froehlich (1969) and Froehlich and Oliveira (1997) observed the preference of some Plecoptera species for different stream sizes. Froehlich and Oliveira (1997) suggested that in some cases the preference may be related to water velocity, as often stream size and water velocity are related (Hynes, 1970). Robinson et al., (1992), reported that fast water current will accelerate the development of Plecoptera (*Hesperoperla pacifica*) from univoltine to bivoltine.

2.4.6 Biological Oxygen Demand

Biological Oxygen Demand (BOD) is defined as the quantity of dissolved oxygen which is able to oxidize the organic components in the water with the assistance of microorganisms under defined experimental conditions. The BOD is an empirical test in which the water conditions such as temperature, oxygen concentration or type of bacteria play a decisive role (Rump and Krist, 1992). Weitz et al. (2002) demonstrated behavioural avoidance to higher BOD in Ephemeroptera, Plecoptera and Trichoptera and attributed higher mortality of Plecoptera to the deficient oxygen.

2.4.7 Chemical Oxygen Demand

Chemical oxygen demand (COD) is the amount of oxygen in the form of oxidizing agent consumed in the oxidation of organic water components. The degree of oxidation depends upon the type of substances, pH value, temperature, reaction time and concentration of oxidizing agent as well as the type of added accelerators, if any. Higher COD indicates higher amount of pollution in the test sample. COD is related to biochemical oxygen demand, another standard test for assaying the oxygen-demanding strength of waste water.

2.4.8 Ammonium

Ammonium ions can be formed in water by the microbiological degradation of nitrogen-containing organic compounds as well as by nitrate reduction under certain condition. Considerable concentration (up to 50mg/L) is found in waste water. Very high concentration (up to 1000mg/L) can be encountered in seepage from refuse dumps. For this reason and within certain limits, ammonium may be regarded as a pollution indicator in ground water and drinking water (Rump and Krist, 1992).

Equilibrium exist in aqueous solutions between free ammonia (toxic or fish) and ammonium ions depending on the pH value (Rump and Krist, 1992). When ammonium-containing water is brought into contact with oxygen over a long period of time, the ammonium can be microbiologically oxidized from nitrite to nitrate (Rump and Krist, 1992). A study done by Hickey and Vickers (1994) found that raise of ammonium concentration will reduce the community of stonefly nymph in Rangitukia Stream, New Zealand.

2.5 Life Histories of Stoneflies

Life history theory is the schedule and duration of key events in an organism's lifetime (life cycle) shaped by natural selection to produce the largest possible number of surviving offspring based on some kind of evolutionary reasoning (Stearns 1982, Grafen 1988, Kozlowski and Janzur 1994). A list of life history traits would typically include those having to do with reproduction and development, such as number of offspring, offspring mass, as well as age and mass at first reproduction (Nylin, 2001). Generally, the life cycle of tropical insects in permanent waters is typically continuous with multiple generations and cohorts per year due to the monsoon and flood (Dudgeon, 2000).

The environmental conditions, such as water temperature, food quality and quantity as well as biotic interactions are important factors influencing the development and the number of instars in Plecoptera (Anderson and Cummins 1979; 1980; Rossillon, 1988; Giberson and Rosenberg, 1992; Williams and Feltmate, 1992; Huryn, 1996). Riparian vegetation has been also shown to be important to the life history of stream invertebrates, as both food quality and quantity reflect riparian forest type (Cummins et al., 1989). Moreover, biotic interactions such as competition and predation pressure, also affect the life histories of macroinvertebrates (Peckarsky and Cowan, 1991).

A previous study done by Robinson et al. (1992), in Idaho Stream, United State, showed *Hasperola pacifica* (Plecoptera) had slow asynchronous development and was influenced by low water temperature. Huryn and Wallace (2000) stated that habitats with warm temperature usually have small size instars because their growth and life cycles complete quickly. Thomsen and Friberg (2002) reported positive growth of *Leuctra nigra* (Plecoptera; Leuctricidae) in water temperature and acidity at four streams of coniferous forest in central Jutland, Denmark.

Jackson and Sweeney (1995), documented growth rates and developmental time for 35 species of Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae for tropical region in Costa Rican streams. They showed that most taxa had multivoltine life cycles (32 of 35 taxa), overlapping cohorts and complex size structure. Rapid growth rates and short developmental times for *Kamimuria tibialis* was also reported by Otsuki and Iwakuma (2008) in Northern Japan. This species had six generations per year and its nymph showed asynchronous development throughout the year.

Although there is a large literature on the ecology of Plecoptera (Hynes, 1976; Zwick, 1980, 2000), there is a scarcity of information on the number of larval instars. Plecoptera instars determination is particularly difficult due to large number of instar (13-33 instars) and prolonged immature life cycle. Two old studies for carnivorous species report 22 larval instars for *Perla abdominalis* Burm. (Samal, 1923) (now *P. burmeisteriana* Claasen), and 33 for *Dinocras cephalotes* (Curtis) (Schoenemund, 1925). However, a more recent study on the latter species estimated the number of larval instars from a growth model as 17 for males and 18 for females, fewer than in the earlier study (Frutiger, 1987). According to Fink (1980), the role of development and number of instars for insects are probably controlled by genetic and environmental factors and generally poorer nutrition seems to result in greater number of instars.

CHAPTER 3

TAXONOMIC STUDY OF THE STONEFLIES (INSECTA; PLECOPTERA) IN THE RIVERS OF ROYAL BELUM STATE PARK (RBSP).[1]

3.1 Introduction

Plecoptera is a small order of insects with more than 3400 described species in the world. It constitutes a significant ecological component of running water ecosystems (Fochetti and Tierno de Figuera, 2008). In general the plecopteran fauna in the Southeast Asian region is not thoroughly studied. However previous studies on Plecopteran fauna (Perlidae) in Southeast Asia including Vietnam, Laos, Cambodia, Thailand, western Malaysia and Myanmar by several researchers had identified 41 species. (Kawai, 1968, Stark & Szczytko 1979, Zwick 1982a, 1982b, 1983c, 1986b, 1988, Stark 1983, 1987, Sivec & Zwick 1989, Stark & Sivec 1991, Stark & Sivec 2005, Cao & Bae 2006).The known stonefly fauna of Malaysia includes members of Leuctridae, Nemouridae, Peltoperlidae and Perlidae (Sivec & Yule, 2004). Of these, Perlidae is thought to be the most widespread and diverse group in Malaysia with five genera (*Kamimuria, Etrocorema, Neoperla, Phanoperla and Tetropina*) and potentially as many as 17 species already reported by Fochetti & Tierno de Figueroa in 2008. The number of recorded species is low in Malaysia (Wan Nur Asiah et al., 2009; Sivec & Stark, 2008; Sivec & Yule, 2004; Zwick, 1988; Jewett, 1975) when compared those from Indonesia (27 species) and Thailand (29 species)(Fochetti & Tierno de Figueroa, 2008) .

[1] Wan Nur Asiah, W. M. A., Che Salmah, M. R., and Sivec, 1. (2009). Description of *Etrocorema belumensis* sp. n. from Royal Belum State Park, Perak, Malaysia. *Illiesia* **5(17)**:182-187.