1.0 Introduction & Literature Review

1.1 Biology and Taxonomy of Odonata

The order Odonata includes both the dragonflies and damselflies, separated into three suborders, namely Anisoptera (dragonflies with 8 living families), Zygoptera (damselflies with 17 living families) and Anisozygoptera (Williams & Feltmate, 1992) which only can be found in Japan and Himalayas (Hennig, 1969, 1981; Bridges, 1993; Needham *et al*., 2000). This suborder of Anisopzygoptera comprised of two species worldwide (Tsuda, 2000; Silsby, 2001). About 5,500 species of Odonata have been described and they are distributed from the temperate to the tropics, where the greatest numbers and diversity occurred (Williams & Feltmate, 1992).

Dragonflies are among the most recognizable of insects, even have been the subject of extensive folklore (Sarot, 1958). Moreover, this group of odonates have been used in a extensive array of studies dealing with the functional morphology, behavior, ecology, and evolution (Corbet, 1999). Odonates are considered to be among the earliest flying insects and their recognizable progenitors date to the Carboniferous which is 360 to 290 million years ago (Ware *et al.*, 2007) with great mobility (Kiyoshi & Sota, 2006).

Fossils of dragonflies from 250 million years ago have been found. The forbearers of extant odonates which belong to order Protodonata, appeared as early as 325 million years ago. Including in Protodonata is the largest insect known to have existed, *Meganeuropsis americana*, and this species had a wingspans as large as 71 cm (von Ellenrieder, 2003; Corbet, 1999).

The dragonflies and damselflies are denizens of many aquatic ecosystems and their distribution are covered a great deal of range from temporary to permanent water bodies (Corbet, 1999; Johansson & Suhling, 2004). The information on diversity and distribution of odonates of various taxa at habitat, local and regional scale is the key to biodiversity conservation (Subramanian *et al.*, 2008).

1.1.1 Suborder of Anisoptera

For suborder Anisoptera, they are among the species-poor suborders with roughly 2500 species described within all insects. Nevertheless, morphological and ecological variability are truly impressive and inspiring, and the Anisoptera is classified into about 15 families of variable species richness (Misof, 2000). Their earliest known fossils are from the Triassic which is 250 to 200 million years ago and showed they arose later than Zygoptera (Grimaldi & Engel, 2005).

Among suborder Anisoptera, in many studies the family Libellulidae is widely represented in surveys (Lim & Furtado, 1975; Norma-Rashid, 1995a, 1995b, 1998, 1999; Norma-Rashid *et al.*, 2001) which wre also supported by the work done in Bachok Coast, Kelantan, Libellulidae representing 75% while family Coenagrionidae representing 25% (Norma-Rashid, 2010). Libellulidae is divided into 11 to 13 subfamilies depending on researchers (Tillyard, 1917; Fraser, 1957; Davies & Tobin, 1985; Bridges, 1994; Steinmann, 1997) and is the most species-rich family within the suborder Anisoptera with more than 900 described species (Fleck *et al.*, 2008).

For their habitats, the Anisoptera (dragonflies) are always found near freshwater. They lay their eggs in or close to water, and the immature dragonflies which are called nymphs, are fully aquatic. Dragonflies are most abundant and diverse in and around slow-moving freshwater with submerged and emergent vegetation such as small streams and ponds. However, they can also be found in many other freshwater habitats, including small water-filled holes, vernal pools, ditches, dikes, marshes, swamps, rivers, waterfalls and lakes.

Adult dragonflies spend the majority of their time near water, but may travel miles away while hunting. The Anisopterans are agile fliers, and tend to hunt in open areas rather than amongst thick trees or other vegetation (Borror, *et al*., 1989; McGavin, 2001; Silsby, 2001). They can be found worldwide with exception in polar regions and the greatest diversity of dragonflies is found in tropical areas (von Ellenrieder, 2003; Silsby, 2001).

1.1.2 Suborder of Zygoptera

Suborder Zygoptera also known as damselfly accounts for about one-third of the species in Odonata and comprises approximately 95 genera worldwide (Corbet, 1999; McGavin, 2001;"Odonata (Dragonflies and Damselflies)", 2009). Among Zygoptera, Coenagrionidae is the largest family with almost 1100 species, forming a major part of the odonate fauna in all continents (Dijkstra & Kalkman, 2012).

Damselflies depend on the freshwater for oviposition (egg-laying). Other factors that might be important for their habitat include the rate or water flow, water quality such as oxygen content, pH and nutrient load, retreat from predators and variability in water level. Each species of damselfly has distinct habitat preferences for each of the variables. For example, damselflies can be found in freshwater habitats from temporary pools to waterfalls, but individual species occupy only habitats within a certain range of water speeds. (Silsby, 2001; Westfall & May, 1996).

Damselflies are native to all regions of the world, with the exception of Antarctica. Tropical regions host the highest diversity of damselfly species. (Westfall & May, 1996; Silsby, 2001;"Odonata (Dragonflies and Damselflies)", 2009).

1.2 Features of Odonata

The adults of Odonata are medium to large in size. Their head is large with flexible neck, three ocelli and a pair of short, bristle-like antennae. Odonates have very large eyes and more than 80% of their brain is devoted to processing visual information (“Odonata (Dragonflies & Damselflies),” 2009). They have sharp visual acuity and the ability to see under low light levels. Their excellent eyesight is valuable for hunting prey and avoiding predators.

In addition, the mouthparts of odonates are modified for biting. Their prothorax is small, the mesothorax and metathorax are large and fused into a single, strong pterothorax. Besides, the two pairs of wings are long, narrow, and net-veined, and the legs are short which suited for perching and holding their prey.

Adult odonates own two pairs of equal (damselflies) or subequal (dragonflies) wings. The wing veins of Odonata are fused at their bases and cannot be folded over the body at rest. The wing design of the Odonata is more primitive than that of most other insects which they have many veins and cells. However, it is very efficient because they use very little energy per unit distance travelled. The wings can differentiate between dragonflies and damselflies.

Furthermore, the odonate abdomen is long and flexible with 10 visible segments, and terminates in clasping organs in both sexes. The female of all damselfly and several dragonflies’ families carry a prominent ovipositor under abdominal segments 9-10. A very peculiar feature of adult odonates is the curious separation in the male of the intromittent organs from the opening of the ejaculatory duct. The former is placed on the underside of the second abdominal segment, while the latter is on the next to the last ventral plate. Therefore, before copulation, male odonates curves their abdomen around beneath, so that the ninth segment of the abdomen is brought into contact with the second, thus transferring the fertilizing fluid to the intromittent organ.

Adult morphologies of Odonata can be contrasted between dragonfly and damselfly as indicated in table below:

Dragonfly (Suborder: Anisoptera)	**Damselfly** **(Suborder: Zygoptera)**
	
• The eyes are broadly rounded and lie mostly flat against the head • The thorax (the green part the wings are attached to in the picture above) ismore broad than the abdomen (the blue part in this dragonfly) • The forewings and hindwings are different shapes • Body is quite large (The dragonfly in the picture, a green darner, is about 3 inches long), though there is a lot of variation in size	• The eyes are largely spherical and protrude off the sides of the head • The thorax (the segment where the wings are attached) is narrow, about the same width as the abdomen • The forewings and hindwings are very similar in size and shape • Usually fairly small (at least compared to the dragonflies)

Table 1: Differences between the adult of Anisoptera and Zygoptera

1.2.1 Physical Descriptions of Anisoptera

Adult dragonflies are simply known by their long thin bodies, two pairs of long wings and very large eyes that seem to cover most of their head. The dragonflies can be distinguished from the closely-related damselflies by the round shape of their heads, their disproportionately large eyes, and the difference in shape between their two pairs of wings which are held horizontally at rest.

Contrasting with damselflies, the rear wings of dragonflies have a broad base. Other characteristics that distinguish dragonflies from damselflies include three anal appendages which are one less than damselflies, and also their females with non-functional or vestigial ovipositors (McGavin, 2001; Silsby, 2001).

Furthermore, the suborder of Anisoptera groups has a short thorax, with three pairs of long, spiny legs that are slanted forward. Their bristles on the legs interlock to form a basket that used for scooping prey out of the air. The abdomens of adult dragonflies are very long with 10 segments, and their often marked with colorful spots, bands and lines.

The long wings of dragonflies can also have distinctive color forms, as well as venation patterns which can be used to classify them into families and species. The abdomens of some tropical species can change color with temperature, from bright red or blue in warm weather to a dull dark gray at the lower temperatures. This may be an adaptation to avoid detection from predators (McGavin, 2001; Silsby, 2001).

In most species of dragonflies, two sexes are similarly colored, though the male may be slightly brighter. Females may also have a slightly more stout abdomen and in a few species in the family of Libellulidae, the two sexes have different patterns on their wings (Borror *et al.*, 1989).

1.2.2 Physical Descriptions of Zygoptera

Zygopterans are long and slender-bodied insects, with two pairs of membranous wings. Though at the first glance, the damselflies appear to be very similar to dragonflies, there are still several characters that can distinguish between two groups.

Damselflies have two pairs of wings that are similar in shape and are held closed when at rest. The rear wings of dragonflies have a much broader base than the front wings, and both pairs are held horizontally at rest. Damselflies have a broad head with eyes that are separated by more than the width of an eye, as opposed to a round head with closely placed eyes (Silsby, 2001; Westfall & May, 1996).

Besides, their thorax is three-segmented, and skewed in a manner that gives the three spiny pairs of legs the exterior of being thrust forward. The legs of damselflies form a basket that is used for scooping prey out of the air. Their abdomens are very slender, ten-segmented and often brightly colored or with distinctly patterned.

Similar to Anisoptera, the color of some species of Zygoptera can change with environmental variables which fade from bright blue to dull purple in response to cool temperatures or darkness. Male of most damselfly species are more brightly colored than female and in some species, female occur in more than one color phase. Frequently, in these species, one of the female color phases is similar to the coloration of the male (Borror, et al., 1989; Silsby, 2001; Westfall & May, 1996).

The smallest known damselfly is the Southeast Asian species named *Agriocnemis femina*. This diminutive damselfly has a wingspan of only 20mm (Silsby, 2001;"Odonata (Dragonflies and Damselflies)", 2009). However, within the suborder of Zygoptera there is one family group which is strikingly large known as Pseudostigmatidae commonly known as forest giant. According to Fincke (1992), this family comprises only of 20 species and includes the largest known extant species, *Megaloprepus caerulatus* (helicopter damselfly) with a wingspan of up to 190mm.

1.3 Development & Life Cyle of Odonata

1.3.1 Suborder of Anisoptera

The lifespan or the longevity of dragonflies varies by species. Once in the adult stage, some species survive for a few weeks and some for several months. The average lifespan of dragonflies probably 6 to 8 weeks once they reach the adult stage and the dragonfly nymphs that spend in the aquatic stage can also range from weeks to years. Generally, tropical species spend less time in the aquatic stage and more time in the adult stage than their temperate counterparts (Borror, *et al*., 1989; von Ellenrieder, 2003).

Dragonflies lay their eggs in the water, on the surface of the ground near the water or inserted into submerged vegetation. The dragonfly larvae spend at least a few months and sometimes can reach until several years as aquatic predators. Besides, they have gills that allow them to remove oxygen from the water (Gullan & Cranston, 2000; McGavin, 2001).

The dragonfly eggs usually hatch in one to three weeks after they have laid. The amount of time required for eggs to hatch varies within a species as well. For examples, such as in some tropical species, the eggs survive long dry periods and hatch only when the rainy season begins.

After hatching, the aquatic larvae live for six months to five years before they develop into adults and this is depending on the water temperature and food availability. Generally, higher water temperature and food availability help more rapid growth and transformation into the adult stage (Silsby, 2001).

Moreover, the dragonfly nymphs molt 8 to 15 times as they grow. After their final immature stage (instar), the nymphs crawl out of the water, usually early in the morning, and attach themselves to a plant stem or rock.

Here the adult stage frees itself from the nymphal exoskeleton they spend the first half hour or so after emerging drying out and expanding to its full size. This includes expanding and hardening its wings. After this initial expansion as an adult, the dragonflies do not grow, though their exoskeleton may harden some and they may change color over the first few weeks as an adult (McGavin, 2001; Silsby, 2001).

1.3.2 Suborder of Zygoptera

On average, damselflies probably live for 3 to 4 weeks as an adult. However, there is quite a bit of variability between and within species. Once in the adult stage, some species of damselflies survive for a few weeks and some for several months ("Odonata (Dragonflies & Damselflies)," 2009).

Besides, the amount of time that damselfly nymphs spend in the aquatic stage can range from weeks to years depending on the species, temperature, food availability and also photoperiodic regime. In general, tropical species spend less time in the aquatic stage and more time in the adult stage than temperate species (Borror, *et al.*, 1989; Westfall & May, 1996; Silsby, 2001).

Eggs are laid in water and usually hatch after one to three weeks and the length of this period can be varies between and within species. After hatching from the eggs, the larval damselflies called nymphs live in water as aquatic predators. The damselflies live for two months to three years as nymphs and as they grow, they undergo 5 to 15 molts information (Westfall & May, 1996; "Odonata (Dragonflies & Damselflies)," 2009).

After emergence as adults, they are sexually mature in a few days to weeks. Once sexually mature, they seek out freshwater habitat with appropriate oviposition sites and this may be the same habitat from which they emerged as nymphs, or can be a new location. In territorial species, males usually establish a territory that contains good oviposition sites and defend it from other males, and in non-territorial species, males simply find habitat with good oviposition sites and wait for females to arrive (Westfall & May, 1996).

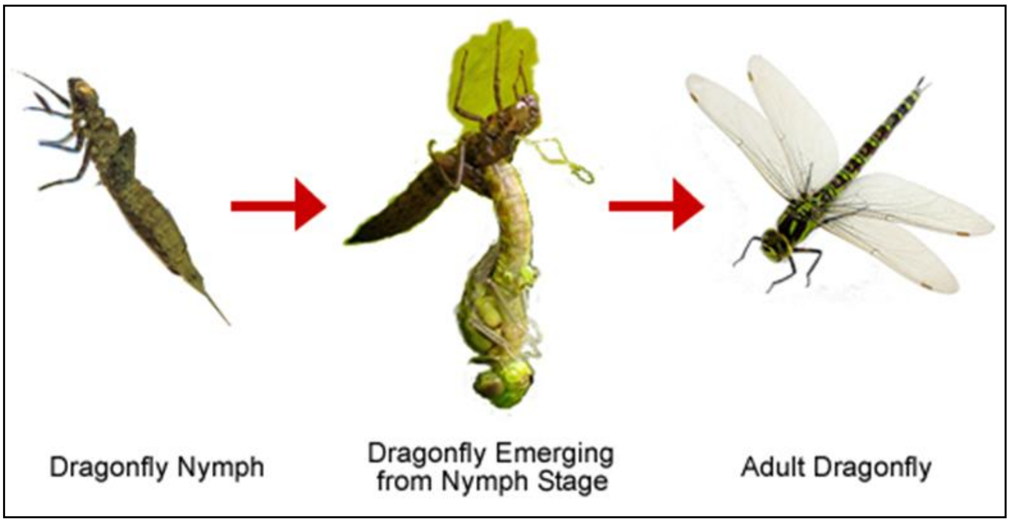

Figure 1: Development of Odonata (Dragonfly & Damselfly)

1.4 The important of Odonata

Most dragonflies and damselflies are regarded as beneficial insects. In terms of ecosystem roles, they are very important in functioning as the biodiversity's indicators which can generally propose to indicate a healthy ecosystem (Corbet, 1999). Besides, they are also important on playing a role in controlling populations of other insects including mosquitoes, and in some tropical areas, dragonfly nymphs are purposely kept in the drinking water storage tanks to control the larval mosquito populations (von Ellenrieder, 2003; McGavin, 2001).

On top of that, the odonates have attracted more attention in recent decades and also have been used as a source of indicator species by several authors such as Carle (1979), Moore (1984), Schmidt (1985), Castella (1987), Clark & Samways (1996). Oertli *et al.* (2008) stated that Odonata is certainly one of the most suitable groups to conduct monitoring studies among the other invertebrates, either alone or, better, in conjunction with another indicator group.

A study done on the identification of dragonflies (Odonata) as indicators of general species richness in boreal forest lakes by Sahlén & Katarina (2001) also used dragonflies as flagship species for conservation biology. Besides, the presence or absence of certain species of odonates will accordingly mirror both of the human activities surrounding the water (Rith-Najarian, 1998; Sahlén, 1999), as well as the structural components of the water (Chovanec & Raab, 1997).

In Yangon (Rangoon), Myanmar (Burma), dragonflies are have been used in pest control programs. They use the larvae of Libellulid *Crocothemis servilia* to control the Yellow Fever Mosquito, *Aedes aegypti*, which was responsible for the transmission of Dengue Fever in that locality. They can rapidly depress the mosquito populations to a level lower than any other method including the treatment of chemical insecticides. This effectiveness of this approach has been described by Sebastian *et al.* (1990).

On the other hand, in several eastern countries, the dragonflies are considered as a delicacy and have also been used in traditional medicine in China and Japan (Corbet, 1999; McGavin, 2001). Furthermore, they served as food for many aquatic and terrestrial organisms (Dunn, 1996; McGavin, 2001).

1.5 Diversity & Distribution of Odonata

According updated records reported by Kalkman *et al*. (2008), a total of 5680 species of Odonata are known worldwide, of which 2739 species belong to the suborder Zygoptera and 2941 species to the suborder Anisoptera. A speculative estimated by Tennessen (1997) indicated that there are less than 10000 extant species of Odonata. However in Malaysia, according to Orr *et al.* (2004), the Malaysian odonate fauna comprises of 342 named species, which include 161 species of Zygoptera from 88 families and 181 species of Anisoptera from 5 families.

It is known that 239 species are recorded from Sabah, Sarawak and Brunei, and 226 species from Peninsular Malaysia which includes Singapore. The current checklist of Singapore odonates comprised of 117 species (Norma-Rashid *et al*., 2008). A total of 123 species or 36% are common to Peninsular Malaysia, Sabah and also Sarawak (Table 2).

Table 2: Species richness and endemicity of odonate families in Peninsular Malaysia and Sabah-Sarawak (Orr *et al.*, 2004)

Taxon	Peninsular Malaysia		Sabah & Sarawak		Total Species	% common to Peninsula-Borneo
	No. spp	**% endemic**	**No. spp**	**% endemic**		
ZYGOPTERA	**88**	**6**	**105**	**66**	**161**	**20**
Amphipterygidae	1	0	1	100	2	0
Caleopterygidae	6	0	9	78	13	15
Chlorocyphidae	10	0	18	67	23	22
Euphaeidae	3	0	8	75	9	22
Lestidae	5	0	3	33	6	33
Megapodagrionidae	4	25	5	80	8	13

Table 2: Continued

Coenagrionidae	33	9	28	43	47	30
Platycnemididae	10	10	11	82	19	11
Platystictidae	9	100	11	100	20	0
Protoneuridae	7	14	11	5	14	31
ANISOPTERA	**138**	**8**	**134**	**19**	**181**	**50**
Gomphidae	32	19	24	46	46	21
Chlorogomphidae	3	33	1	0	3	33
Aeshnidae	15	13	27	1	30	43
Corduliidae	14	14	13	46	18	47
Libellulidae	74	0	69	7	84	67
TOTAL	**226**	**11**	**239**	**40**	**342**	**3**

Progressive research is very much recommended as when more research conducted will contribute to additional records found in the areas, for example, locally in Malaysia, a study conducted in Tasek Bera (Norma-Rashid *et al*., 2001) on the distribution and diversity of Odonata (dragonflies and damselflies) reported 59 species of odonates in contrast to 33 species found to exist previously.

Examining habitats and subhabitats at different times of the day and in different months of the year provide useful information about specific factors that affect distribution of larvae within subhabitats. According to Miller (1987), such factors include the pH of water, amount and type of aquatic vegetation, and the mobility of the water. For example, the ponds that have no Odonata tend to be temporary, shaded heavily by trees, or have little to no aquatic vegetation (Corbet, 1980). In Britain, a study had showed a trend of specific species found in different areas. For instance, acid bogs and peaty areas with *Sphagnum* moss are likely to contain larvae of *Libellula* *quadrimaculata*, *Pyrrhosoma nymphula*, *Aeshna juncea*, and *Sympetrum danae* while for species *Cordulegaster boltonii*, *Orthetrum coerulescens*, and *Calopteryx virgo* they prefer small streams whereas small ponds contain *Libellula depressa*, *Aeshna cyanea*, and *Sympetrum striolatum* (Miller, 1987).

On the other hand, both water dissolved oxygen concentration and temperature directly affect the abundance of Odonata larvae. The amount of dissolved oxygen in water affects the behavior, metabolism, and survival of Odonata larvae (Corbet, 1999; Hofmann & Mason, 2005). Variations in oxygen availability in lacustrine (low oxygenation) and lotic (high oxygenation) environments determine the diversity of Odonata species (Voshell & Simmons, 1978; Corbet, 1999; Fulan *et al*., 2008) while the water temperature also has an effect on the abundance and development of Odonata larvae (Lutz, 1974; Ward, 1992; Corbet, 1999).

Sites with high temperatures, such as thermal springs, tend to present low abundance and diversity of Odonata species (Corbet, 1999). However, when considering aquatic insects, Odonata are characterized by a high tolerance to changes of surface water temperature (Kondratieff & Pyott, 1987; Cobert, 1999). For example, the importance of abiotic factors like water dissolved oxygen and temperature for the presence of Odonata larvae near macrophytes was also shown for a lake lateral to Paranapanema River, Brazil (Fulan & Henry, 2006). According to the study, *Enallagma*, *Homeura* and *Telebasis* were highly sensitive to variations in dissolved water oxygen concentration and temperature during the year. Nevertheless, besides the environmental variables, biotic factors such as predation risk can also affect the macroinvertebrate distribution during the 24-hours period.

1.6 History of Odonata Classification

The relationships of the Odonatoidea which comes from Odonata and Protodonata to other pterygote insects are continue to be much debated. Mackerras (1970) placed the Odonata as the sister to the other pterygotes (Figure 2) and it was supported by Boudreaux (1979). On the other hand, a study made by Hennig (1981) and Kukalova-Peck (1991) argued for a sister-group relationship between the Odonata and Ephemeroptera (Figure 2.1).

```
                 |=====  Ephemeroptera
           |=====|
     |=====|     |=====  Neoptera
     |     |
=====|     |===========  Palaeodictyopterodea
     |
     |=================  Odonatoidea
```

Figure 2: The relationship proposed by Mackerras (1970)

```
                 |=====  Odonatoidea
           |=====|
     |=====|     |=====  Ephemeroptera
     |     |
=====|     |===========  Palaeodictyopterodea
     |
     |=================  Neoptera
```

Figure 2.1: The relationship proposed by Hennig (1981) and Kukalova-Peck (1991)

The other possible relationship regarding the Odonata has been made by Kristensen (1981, 1991), Wheeler (1989) and Bechly (1996). They stated three possibilities of the relationship which favored a sister-group relationship between Odonata and Neoptera, but disagreed on the placement of the other branches (Figure 2.2)

```
                 |=====  Odonatoidea
           |=====|
     |=====|     |=====  Neoptera
     |     |
=====|     |===========  Palaeodictyopterodea
     |
     |=================  Ephemeroptera
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or

```
                 |=====  Odonatoidea
           |=====|
     |=====|     |=====  Neoptera
     |     |
=====|     |===========  Ephemeroptera
     |
     |=================  Palaeodictyopterodea
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or

```
           |=====  Odonatoidea
     |=====|
     |     |=====  Neoptera
=====|
     |     |=====  Ephemeroptera
     |=====|
           |=====  Palaeodictyopterodea
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Figure 2.2: The relationship proposed by Kristensen (1981, 1991), Wheeler (1989), and Bechly (1996).

The difficulties in placing the Odonata in relation to the other orders are because the authors disagree about the pattern of the wing venation of Odonata relating to the others. However, previous study done by Linnaeus (1958) suggested that the dragonflies were originally classified in the genus of *Libellula* within the order of Neuroptera, while Fabricius (1775) divided this genus of *Libellula* into three which were *Libellula, Aeshna* and *Agrion.*

The most notable pre-cladistic phylogenetic studies of the Odonata were performed by Needham (1903) on the entire group, and Zygoptera by Munz (1919). These were mostly based on the patterns in the wing venation and the theory that "ontogeny recapitulates phylogeny" (Haeckel, 1866). Needham in his study stated that there is a dichotomy between the suborder of Anisoptera and Zygoptera. Anisoptera are further divided into Libellulidae (modern Libelluloidea) and Aeshnidae (the remainder of the Anisoptera), with Aeshnidae considered representing a primitive branch. On the other hand, the Zygoptera are in turn divided into Calopterygidae (Calopterygoidea) and the other Zygoptera lumped into Agrionidae.

Munz (1919) also argued for a dichotomy between Zygoptera and Anisoptera, where the Agrionidae (Calopterygoidea in modern taxonomic terms) are a grade including monophyletic Coenagrionidae (the remainder of the Zygoptera). Zygoptera are seen as being derived from Anisozygoptera. In other study made by Fraser (1957), he interpreted that Zygoptera are a paraphyletic group. Below is the Fraser's phylogeny from a cladistics perspective.

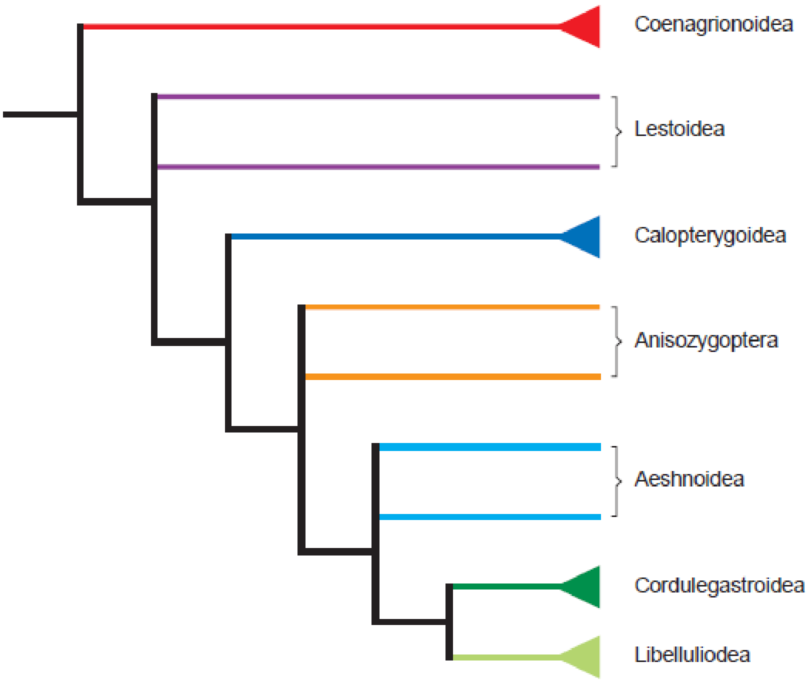

Figure 2.3: Interpretation of Fraser's tree (1957)

As late in 1996, the first formal cladistic study on Odonata has been published in Jon Trueman's modestly titled "A preliminary cladistic analysis of odonate wing venation". In this study, the author placed superfamily Hemiphlebioidea in its own clade which appeared as a sister taxon of all extant Odonata. All other superfamilies except Libelluloidea are found to be paraphyletic (Figure 2.4).

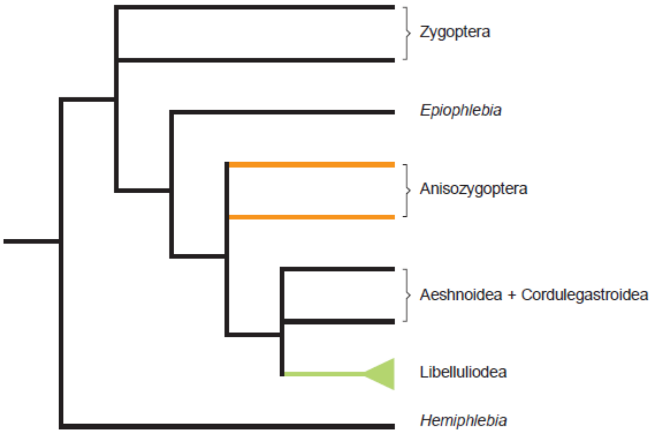

Figure 2.4: Trueman's tree (1996)

To date, the most ambitious study on the phylogeny of Odonata was performed by Rehn (2003). In this morphological cladistic study, the author was focused on the resolving of higher-level relationships in Zygoptera. He found strong support for monophyly of extant Zygoptera as the sister group of Epiprocta (Figure 2.5).

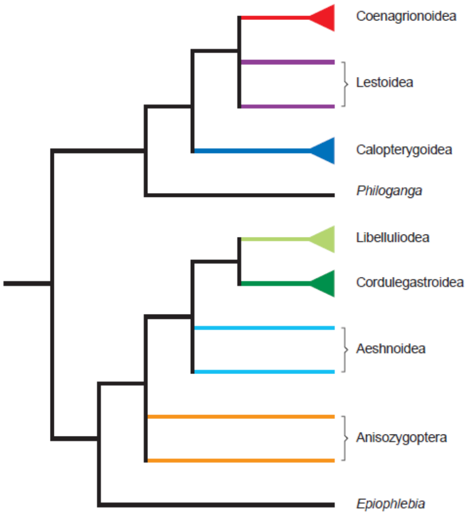

Figure 2.5: Phylogeny of Odonata according to Rehn (2003)

1.7 Identification of Odonata

The adult odonates (Anisoptera & Zygoptera) can be identified based on their wing venation. As for Anisoptera, the hindwing is broader than the forewing. In both wings of dragonflies, the crossvein devides the discoidal cell into a triangle and supertriangle shape (Figure 1.6). Oppositely, in Zygoptera, the two pairs of wings are almost exactly equal in shape, size and venation. The venations are with numerous crossveins (Figure 3.1).

Important wing characters used in identification of Odonata adapted from Watson & O”Farrell (1991).

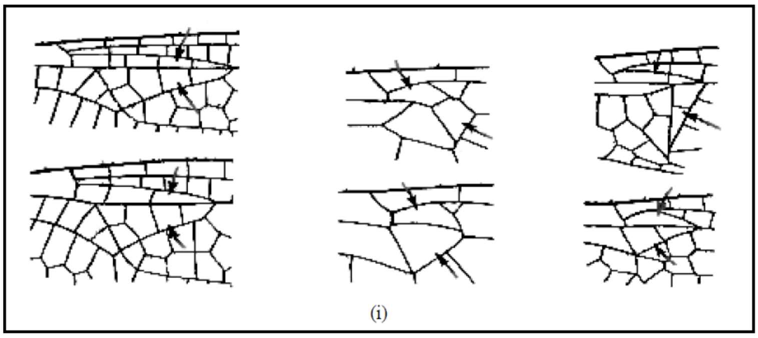

Figure 3: Wing venation of dragonflies (Anisoptera). The arrows show the discoidal cell.

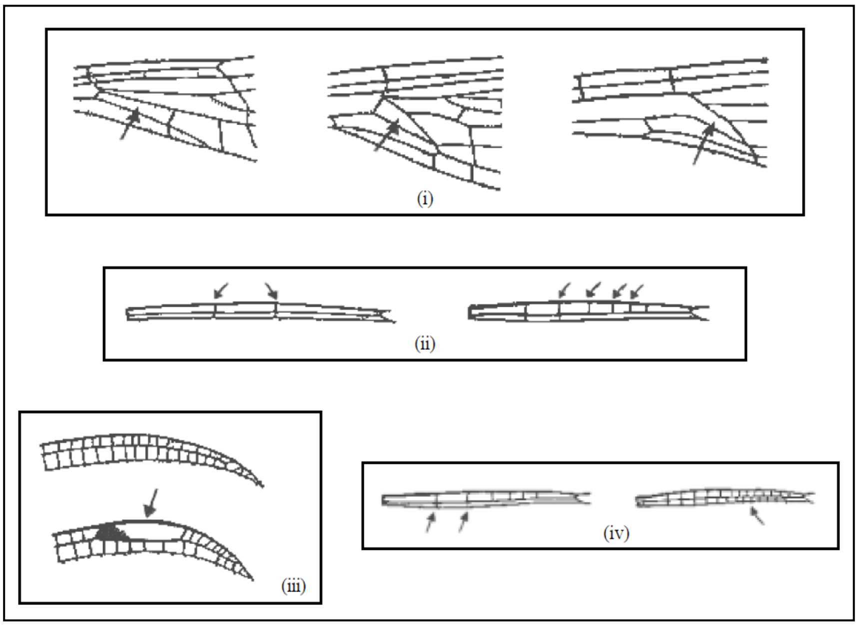

Figure 3.1: Wing venation of damselflies (Zygoptera). The arrows showing (i) discoidal cell, (ii) antenodal crossveins, (iii) presence and absence of pterostigma, and (iv) crossings of basal antenodal crossveins, (Watson & O”Farrell, 1991).

Therefore, the phylogeny of Odonata cannot be estimated separately from the evolution of the odonate wing. A variety of wing-vein naming systems have been proposed during the past 100 or so years, and each implies its separate view of odonate higher relationships. Correspondingly, a variety of phylogenetic hypotheses have been proposed and these imply a view of historical developments in the venation (e.g, Comstock & Needham, 1898; Tillyard, 1917; Forbes, 1943; Hamilton, 1972; Trueman, 1993; etc).

Furthermore, over 54 years several groups of researchers have attempted to resolve the relationships within Odonata groups (Anisoptera and Zygoptera), especially for the larval stages using morphological characters (Fraser, 1957; Hennig, 1969, 1981; Carle, 1982; Pfau, 1991; Trueman, 1996; Bechley, 2002). Within the Suborder Anisoptera only, many studies have worked on the phylogenies based on morphological characters (Pfau, 1991; Carle, 1995; Lohmann, 1996; Trueman, 1996; Bechly, 1996; Bechly *et al.*, 1998) and molecular characters (Artiss *et al.*, 2001; Misof *et al.*, 2001).

Many morphological studies have attempted to use different characters to resolve the relationships of the odonates based on the flight apparatus and copulatory structures (Pfau, 1991) as well as morphology based on wing venation (Fraser, 1957; Carle, 1982; Trueman, 1996). However, none of them have been able to come to a robust conclusion. It is difficult to see how further progress in odonate higher phylogeny can be made, at least on the basis of morphological characters, without greater agreement on the identification of wing veins across the odonatoid orders and suborders.

On top of that, larval identification remains problematic, thus it is perceived that taxonomic research should not be confined solely to morphological identification. Additional techniques such as the molecular, phylogenetic analysis combined with ecological and behavioral data can reveal the evolutionary history, origin, climatology, dispersal or migratory ability of these species.

1.8 Molecular Systematics

1.8.1 Phylogenetic Species Concept

Hennig (1996) summarized a phylogenetic species concept as ‘a species is the smallest recognizable monophyletic group or lineage’. The phylogenetic studies as a basis for systematic reconstructions and analyses of direction and rate of evolution take an important place in biological sciences (Mayr, 1963).

In recent years, molecular genetics has provided techniques that allow researchers to understand and study the relationships among the organisms at a molecular level. Molecular markers have become instrumental to efforts aimed at understanding the patterns and processes resulting in extant global biological diversity (Rubinoff & Holland, 2005). A large number of these studies have obtained the phylogenetic information from the mitochondrial DNA. Researchers place trust in this molecule and expect it generally to be a reliable marker for addressing questions ranging from population genetics to phylogenies among distantly related lineages (Zardaya & Meyer, 1996).

In several animal groups, species have been assigned cryptic species discovered and clusters within species detected by short DNA sequences of a standardized gene region (Hebert *et al.*, 2003; Ward *et al.*, 2005; Smith *et al.*, 2006; Gomez *et al.*, 2007). In the context of species identification, DNA barcoding does not rely on a species concept. In fact, species identification using DNA barcoding is consistent with any species concept that a taxonomist uses to establish a named species and can serve as a complement to the approaches of traditional morphological identification systems (Rach *et al.*, 2007).

In a classic work of Spirin 55 years ago, it was shown that “DNA has pronounced species specificity, close species having fewer differences in DNA composition compared to systematically distant ones” (Spirin *et al.*, 1957). The development of this inference has resulted in wide use of comparative analysis of nucleotide sequences to solve taxonomic and systematic problems in all groups of the organic world (Hills, 1996).

Traditionally, the evolutionary application of mitochondrial DNA (mtDNA) has been limited to population- level questions (Avise, 1994) and the use of it in the phylogenetics has been argumentative since it became clear that individual gene and species phylogenetic trees are not always congruent (Avise, 2004; Avise *et al*., 1983; Avise & Saunders, 1984), and discrepancies between nuclear and mtDNA inheritance patterns have been well documented (Funk & Omland, 2003).

Nevertheless, since the introduction of the polymerase chain reaction, PCR (Saiki *et al*., 1988) and direct sequencing techniques (Kocher *et al*., 1989) the use of mtDNA as a phylogenetic marker has been extended too much greater levels of phylogenetic inclusiveness (Meyer & Wilson 1990). On top of that, the mtDNA has been one of the most widely used molecular markers for phylogenetic studies in animals because of its simple genomic structure (Avise, 2004).

1.8.2 Mitochondrial DNA Genes

According to Kocher *et al.* (1989), in conducting molecular phylogenetic studies with highlighting more on investigation of evolutionary relationships among species, and less on the evolution of the sequenced genes itself, a trade-off is exercised at the moment when a particular gene is chosen over an alternative one. In many recent phylogenetic studies, DNA fragments of 300-600 base pairs from a single gene or from a small number of genes are routinely amplified and sequenced from different species in order to infer their evolutionary relationships.

One of the most widely used approaches nowadays is comparative analysis of mitochondrial DNA (Semina *et al.*, 2007). The mtDNA is characterized by a number of traits that make it suitable for these purposes (Avise, 2000). They include the small size of the molecule (Attardi, 1985) and the high rate of evolution of mitochondrial gene sequences, which is 5-10-fold faster than in nuclear loci (Brown *et al.*, 1979). On the other hand, mtDNA had haploid maternal inheritance and also lack of recombination (Birky, 2001). The analysis of mutations in mtDNA and their distribution in the species habitat allow not only the differentiation of the taxa but also the retrospective reconstruction of the consecution of the origin of taxa and intra-species groups as well as the estimation of the approximate time of their divergence (Avise, 2000).

Though mtDNA sequence data have proved valuable information in determining phylogenetic relationships, the choice of gene is also of great significance (Simon *et al*. 1994; Lunt *et al*. 1996). While current barcoding studies have primarily focused on a single marker gene namely the mitochondrial *cytochrome c oxidase I* (*CO1*) gene as a source for identifying diagnostic barcodes (Hebert *et al.*, 2003b; Armstrong & Ball

2005; Blaxter *et al.*, 2005; Janzen *et al.*, 2005; etc), other markers have been suggested as equally well suited (Markmann & Tautz 2005; Monaghan et al. 2005; Savolainen et al. 2005; etc).

One study done by Groeneveld *et al*. (2007) found that generally very high distance values of the *ND1* gene fragment across all taxa analyzed, highlight its fast evolutionary rate compared to the 16S rDNA and the *EF1* fragment. The *ND1* has been successfully applied to the phylogenetic and population genetic studies in Odonates before and seems to be well suited as an alternative or complement to *CO1* (Hadrys *et al*., 2006; Dijkstra *et al.*, 2007; Groeneveld *et al*., 2007).

Therefore, in this study, the mitochondrial *ND1* gene region (*NADH dehydrogenase 1*) will be discovered for finding character-based DNA barcodes specific for taxonomic units within the insect order Odonata that includes dragonflies and damselflies (Figure 4). Moreover, the odonates provide an ideal platform for exploring the potential of character-based DNA barcoding because of they represent a species rich, yet tractable with approximately 5600 described species, insect order.

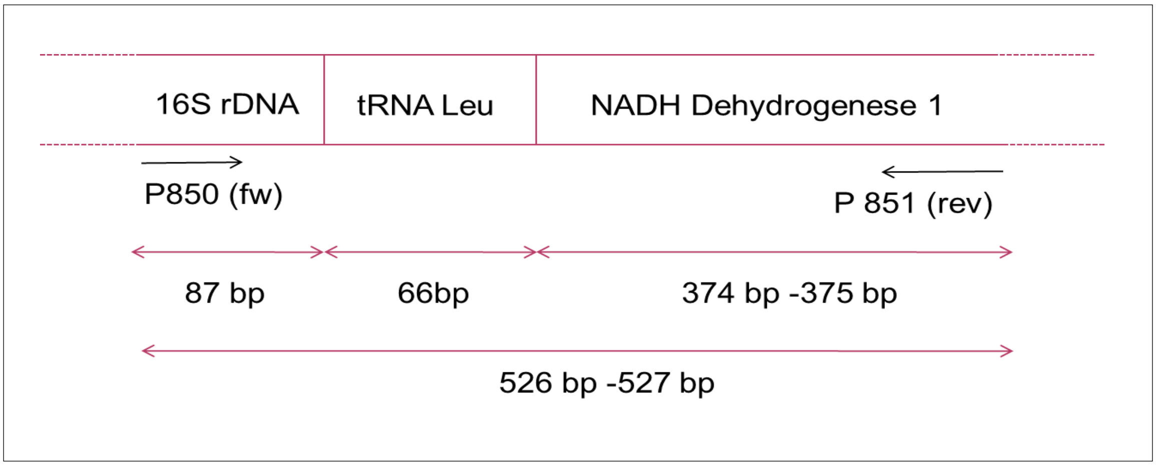

Figure 4: The fragment of *NADH dehydrogenase 1* region, including partial of 16S rDNA and $\mathrm{tRNA}^{\mathrm{Leu}}$ sequence.

Moreover, in the GenBank only a few species of odonates from South America and Africa are reported with partial sequences of *ND1* (Appendix 1) compared to other genes. No sequences of Asian odonates are available on the GenBank using this gene fragment.

Here in this work, presented the results of a molecular systematic and phylogenetic approach of the odonates that were inferred from mitochondrial DNA, *NADH dehydrogenase subunit 1* (*ND1*) gene, as well as the diversity and distribution of odonates in Selangor.

The DNA sequences were used as markers for a priori established species with the species identification using this DNA barcoding consistent with any concept to establish a named species (Rach *et al.*, 2007).

1.9 Significance of Study / Justification

This study will contribute as baseline information for the monitoring of Odonata in the Selangor areas in which aspects of diversity, abundance, distribution as well as effects of physical of parameter were investigated. Besides, it also will present the results of molecular systematic and phylogenetic approaches of the odonates that were inferred from mitochondrial gene region *NADH dehydrogenase subunit 1* (*ND1*) gene included the partial fragment of 16S rRNA and intervening tRNA^{Leu}.

1.9.1 Objectives

The main objectives of this study are as follows:

i. To document the diversity and distribution of Odonata from numerous habitats throughout Selangor.

ii. To evaluate and determine the dragonfly habitat preference in the study areas.

iii. To analyze and characterize the potential use of character-based DNA barcode of Odonata using mitochondrial gene – partial regions of *NADH dehydrogenase subunit 1* (*ND1*) gene and 16S rDNA as well as full sequence of intervening tRNA^{Leu} region for species discrimination and phylogenetic relationship studies.