3.0 Diversity & Sequence Results of Odonata

3.1 Diversity and Distribution of Odonata in Selangor

Fifty-four odonate species were found in twenty-two localities within the state of Selangor. A total of 1298 individuals were collected from nine families of suborder Anisoptera and Zygoptera. Of 1298 individuals, a total of twenty-three individuals were unidentified and designated as unknown species (Figure 3.1).

This diversity of odonates were represented by Libellulidae (49.11%), Chlorocyphidae (14.96%), Euphaeidae (11.38%), Calopterygidae (10.71%), Coenagrionidae (2.90%), Platycnemididae (2.68%), Aeshnidae (2.23%) Protoneuridae (0.67%), Amphiterygidae (0.22%), and unknown species (5.13%). Among these nine families, Suborder Anisoptera was represented by two families namely Libellulidae and Aeshnidae, while suborder Zygoptera represented by seven other families (Appendix 6).

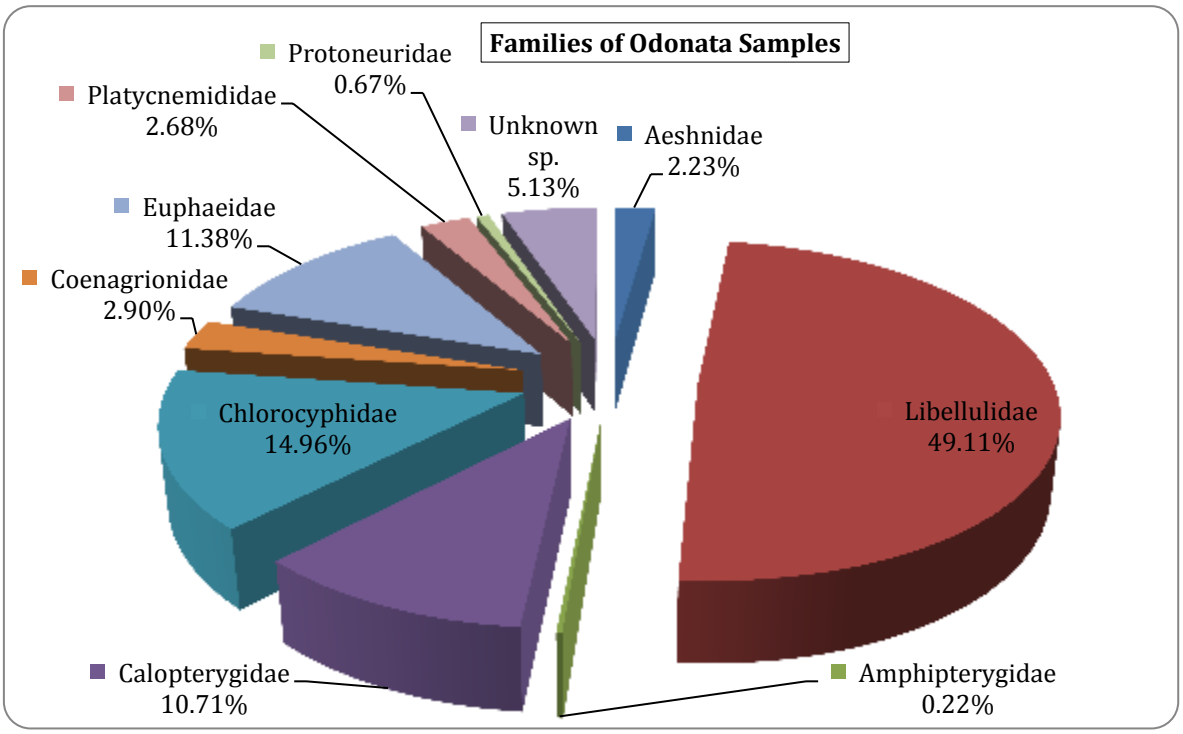


Figure 6: Percentage number of families for all species sampled.

Based on one way Analysis of Variance (ANOVA), there was no significant different in the total abundance $p > 0.05$ of the odonates community in all study sites [$F(1,76) = 0.12$, $p = 0.73$]. Hence, there was no significant variability among the individuals represented at each site.

From the total number of 1298 individuals captured, Libellulidae was the largest family in Selangor compared to other families. It was represented by 750 individuals collected with 29 species. Whereas, family of Protoneuridae seemed to be the smallest family group in Selangor represented by 9 individuals with 2 species.

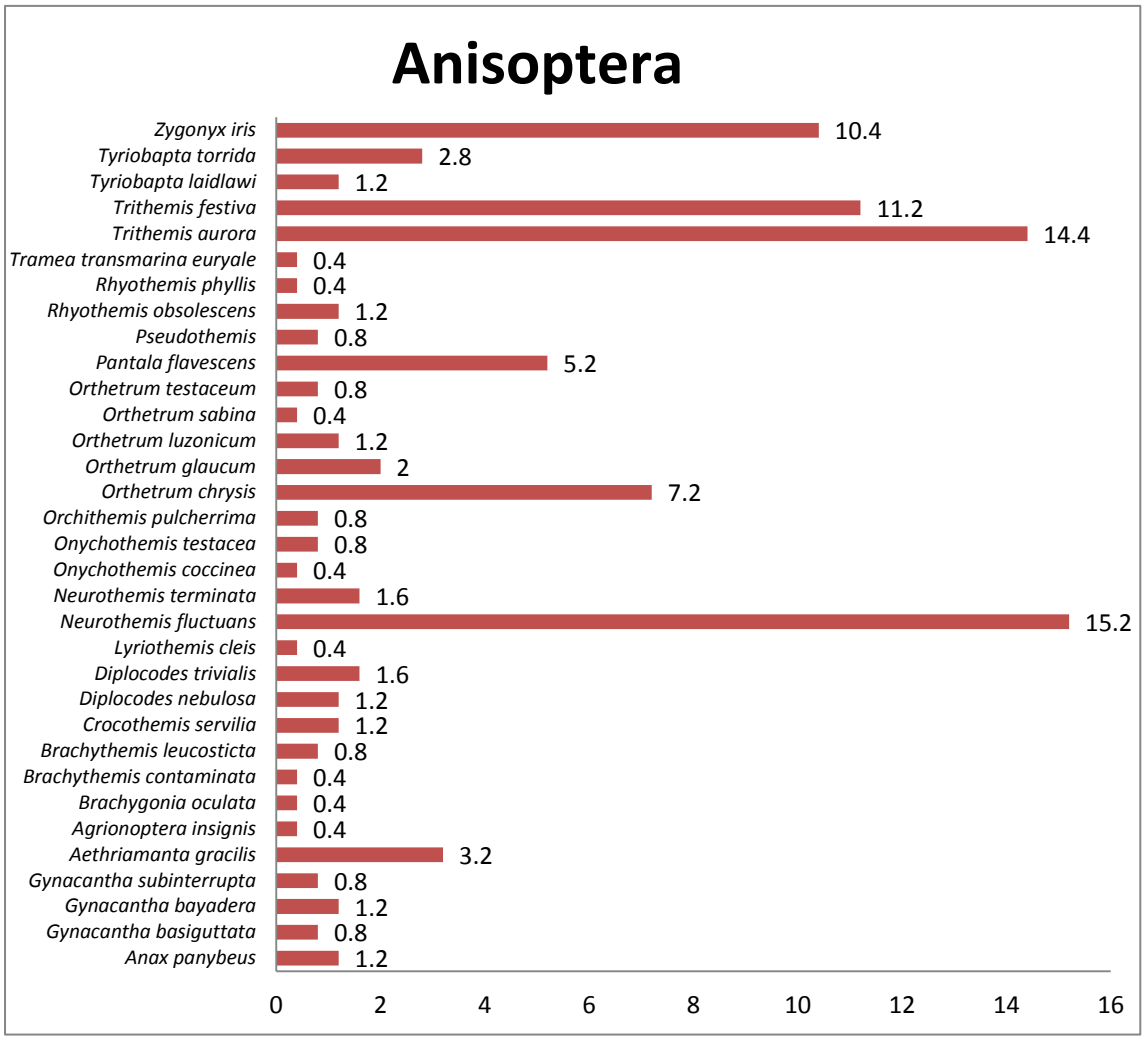


Figure 6.1: Percentage (%) of individuals for all species of Suborder Anisoptera.

Figure 6.1 above shows the percentage of individuals captured for each species of Suborder Anisoptera in all the 22 localities sampled in Selangor. The total number of individuals collected from this suborder was 690 (Appendix 6) which comprises of 2 families, Libellulidae and Aeshnidae. Not surprisingly, Libellulidae was the largest family comprised of 29 species. From the figure, four out of 33 species from the suborder of Anisoptera seemed to be predominant namely *Neurothemis fluctuans* with 15.2% (114 individuals), followed by *Trithemis auroura* with 14.4% (108 individuals), *Trithemis festiva* 11.2% (84 individuals), and *Zygonyx iris* 10.4% (78 individuals).

Additionally, Figure 6.2 below shows the percentage of individuals captured for each species of Suborder Zygoptera. The total individual collected from this suborder was 585 (Appendix 6) which comprises of 7 families, Amphipterygidae, Calopterygidae, Chlorocyphidae, Coenagrionidae, Euphaeidae, Platycnemididae, and Protoneuridae. Chlorocyphidae represented the dominant odonates in Selangor for suborder Zygoptera with six species, *Aristocypha fenestrella,* 15.38% (90 individuals), *Libellago lineata*, 11.79% (69 individuals), *Rhinocypha biforata biforata*, 4.1% (24 individuals), and 1.03% (6 individuals) for species *Heliocypha biforata, Libellago stigmatizans* and *Rhinocypha sp.* each.

Besides, from the figure, four out of 21 species from the suborder of Zygoptera seems to be predominant in all localities, namely *Euphaea ochracea* with 23.59% (138 individuals), followed by *Neurobasis chinensis* with 21.03% (123 individuals), *Aristocypha fenestrella* 15.38% (90 individuals), and *Libellago lineata* 11.79% (69 individuals). The details of percentage for each species sampled from all localities in Selangor for suborder of Zygoptera are shown in Figure 6.2.

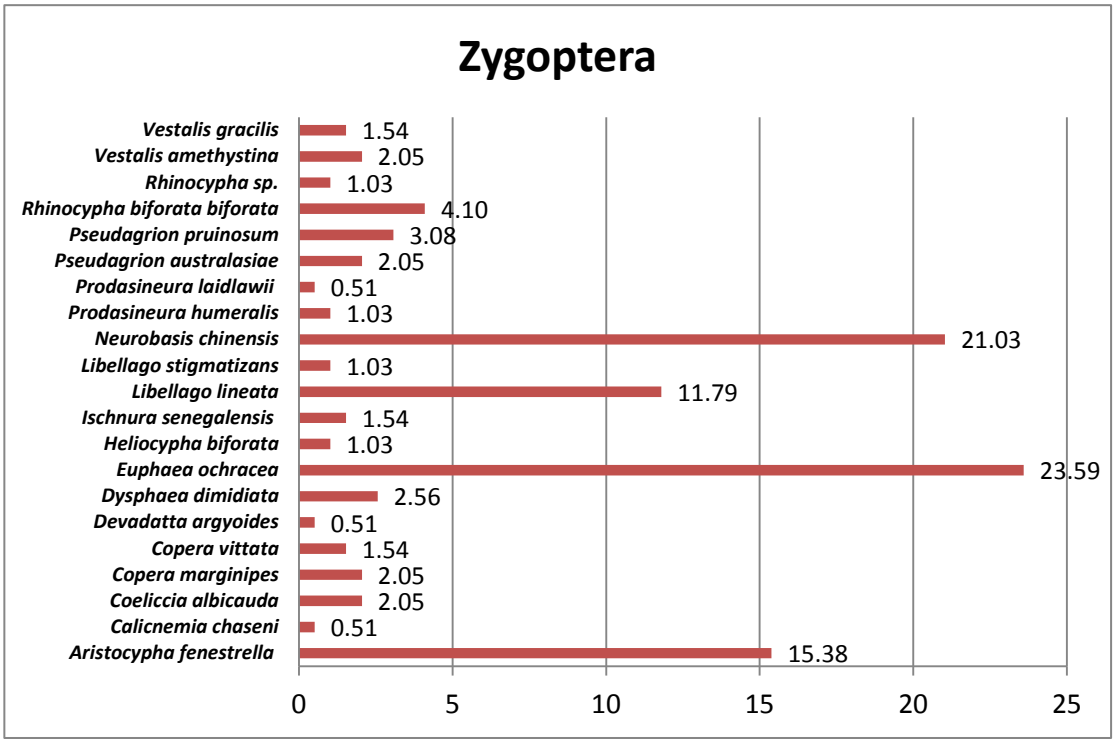


Figure 6.2: Percentage (%) of individuals for all species of Suborder Zygoptera.

The table below (Table 5) represents the assessment of the species status categorised as: very abundant to very rare as described in ascending order of total numbers for such categorisation.

Majority of the collections comprised of the species of *Neurobasis chinensis* and *Euphaea ochracea*, while the other five species of odonates that were considered as the abundant species of Odonata in Selangor which were *Zygonyx iris*, *Trithemis festiva*, *Aristocypha fenestrella*, *Trithemis aurora* and *Neurothemis fluctuans*. Other caught species were considered as very rare, rare, scarce and common species in Selangor (Table 5).

Table 5: Categorisation of species status according to the classification of very rare, rare, scarce, common, abundant and very abundant.

Status indicated	Range of individual numbers	Total species	Species names
Very rare	1 – 3	11	*A. insignis, B. oculata, B. contaminate, L. cleis, O. coccinea, O. Sabina, R. Phyllis, T. transmarina Euryale, C. chaseni, D. argyoides, P. laidlawii*
Rare	4 – 10	21	*G. basiguttata, G. subinterrupta, B. leucosticte, O. testacea, O. pulcherrima, O. testaceum, Pseudothemis, H. biforata, L. stigmatizans, P. humeralis, Rhinocypha sp., A. panybeus, G. bayadera, C. servilia, D. nebulosa, O. luzonicum, R. obsolescens, T. laidlawi, C. vittata, I. senegalensis, V. gracilis*
Scarce	11 – 30	12	*D. trivialis, N. terminate, C. albicauda, C. marginipes, P. australasiae, V. amethystina, O. glaucum, D. dimidiate, P. pruinosum, T. torrida, A. gracilis, R. biforata biforata*
Common	31 – 70	3	*P. flavescens, O. chrysis,L. lineata*
Abundant	71 – 120	5	*Z. iris, T. festiva, A. fenestrella, T. aurora N. fluctuans*
Very abundant	> 120	2	*N. chinensis, E. ochracea*

3.2 Habitat Distribution of Odonata in Selangor

Figure 7 shows the total number of species together with the total number of individuals found in every sampling locality in Selangor. The highest distribution recorded of odonates across all the site localities is for site locality SS7 which contained 12 species with 159 total numbers of individuals. The second highest goes to site locality SS2 with 143 individuals from 14 species, while SS1 had the third highest distribution of odonates with 10 species and comprised of 116 individuals as shown in figure below.

In addition, the total abundance and composition of odonates demonstrated variations especially in species number at different study area. Anisopterans were more abundant than the zygopterans at all study sites. The highest number of Anisopterans was recorded at TLR with 413 individuals followed by OA with 277 individuals. On the other hand, the total number of Zygopterans at TLR was 437 individuals while 148 individuals at OA area (Figure 8).

Besides, from the figure it shows that the odonates more prefer the tropical lowland rainforest as their habitat compared to the open area. A total of 865 individuals were found at the tropical lowland rainforest out of 1298 individuals captured in this study. Oppositely, only 433 individuals were found at the open areas (Appendix 7) which shows the species composition of Odonata at the open area (OA) and tropical lowland rainforest (TLR).

A paired-samples t-test was conducted to compare the distribution of odonates in open areas (OP) and tropical lowland rainforest (TLR) study sites. There was a significant difference in the scores for OP (M = 8.49, SD = 14.62) and TLR (M = 16.94,

SD = 24.47); t (50) = -2.00, p = 0.051. These suggest that there is variability of number of species and total of individuals in OP and TLR.

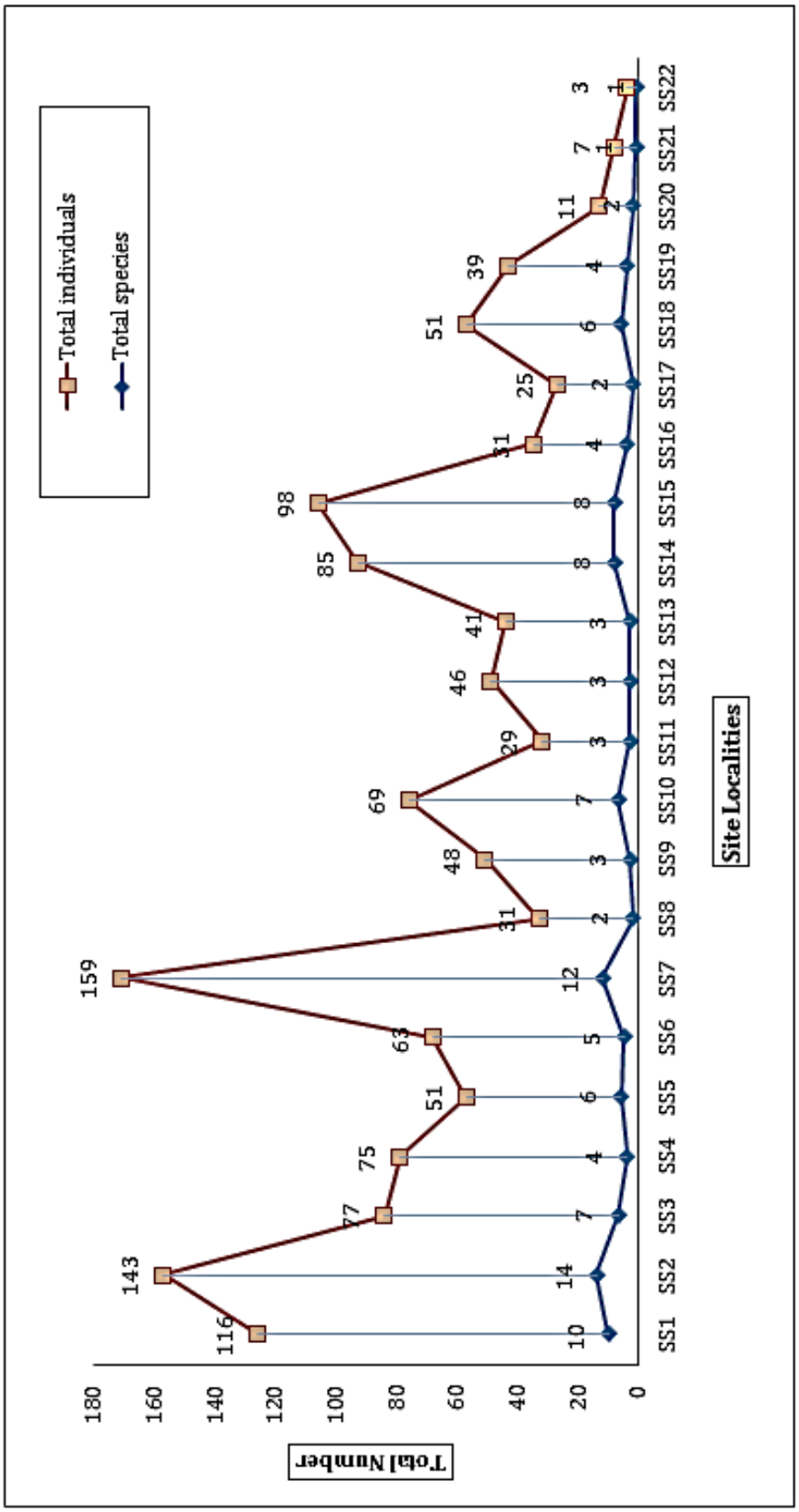


Figure 7: Total number of species and individuals found in every sampling locality.

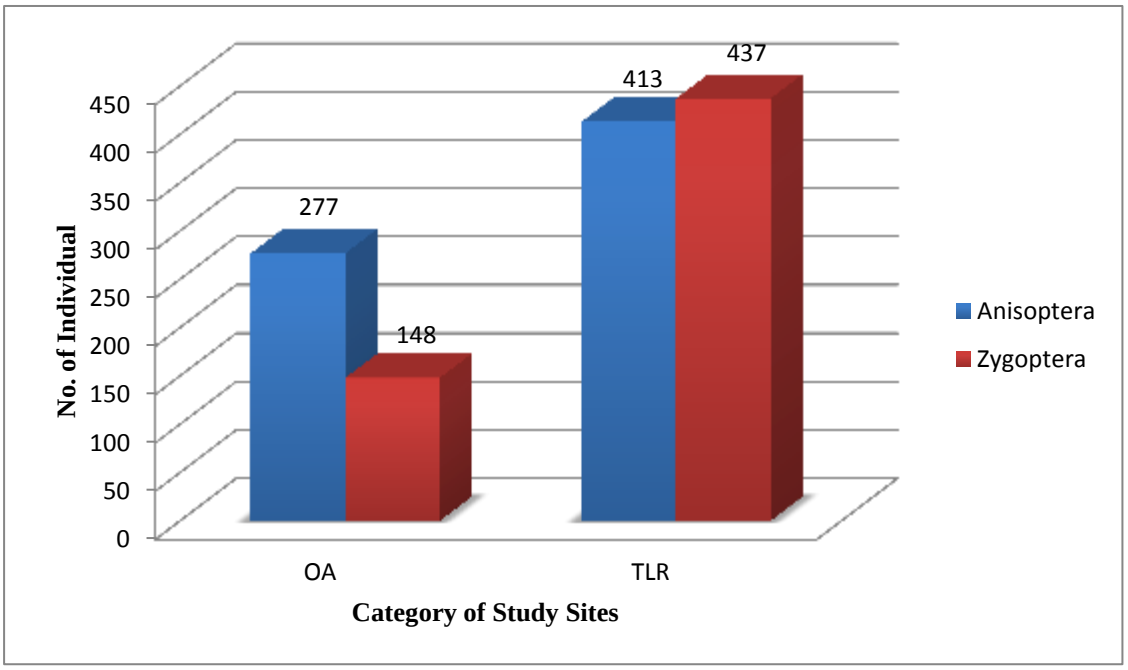


Figure 8: Total number of individuals of odonates in each category of study sites.

Figure 9 shows the analysis of the diversity, species richness and evenness of Odonata for each category of study sites in Selangor. Calculated biological indices indicated that the richness of the species between the two categories of the study sites was significantly varied. TLR demonstrated the highest value of richness index (R) with 7.26 compared to OA with only 4.46.

This R index refers to the different species of Odonata in that certain area representing the observed species richness in an ecosystem, it is usually referred to as species density. However, this R index was a measure on its own, and it takes no account of the number of individuals of each species present. It gives as much weight to those species which were represented with few individuals as to those which had many individuals.

Besides, for the diversity (H') of odonates at each category of the sampling sites shows that the diversity was higher at the TLR areas compared to OA areas with 3.22 at TLR and 2.83 at OA. The diversity indexes offer more information about community composition and structure than simply species richness, and also take the relative abundances of different species into account.

Additionally, from the analysis, the evenness index (E) value of Odonata at TLR was represented with 0.81 while at OA was 0.71. This measured on how equal the community is numerical. The species evenness is also the relative abundance or proportion of individuals among the species.

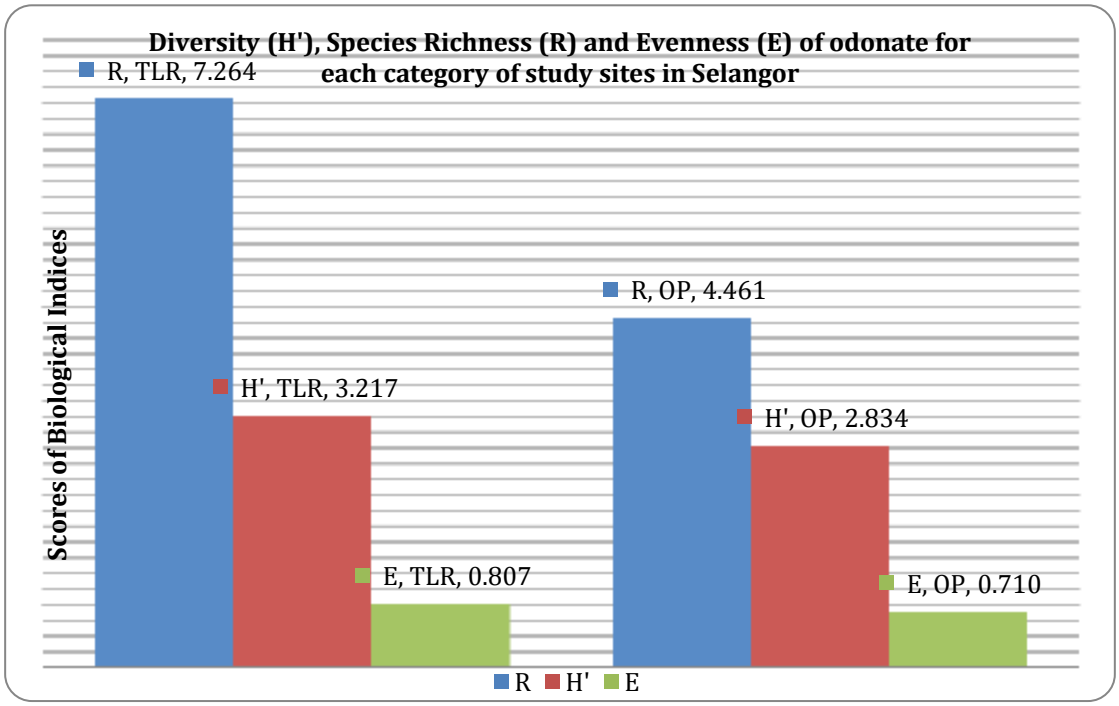


Figure 9: Species richness (R), diversity (H'), and evenness (E) of odonates for each category of study sites in Selangor.

However, changes on environmental variables can affect the diversity and distribution of odonates. Table below (Table 6) shows the descriptive analysis for environmental factors recorded during the study. The mean amount of dissolved oxygen was lower in OA (4.578) compared to TLR (8.583) which indicated that the TLR have a high dissolved oxygen with the minimum value 7.13 than only 1.9 at OA.

Besides dissolved oxygen, the surface water temperature also influenced the distribution of Odonata larvae. The temperature recorded for each of the category of habitat is about the same with the mean of 25.5^{o}C in TLR and 24.1^{o}C in OA. But in OA, the maximum value of temperature reached until 39.8^{o}C which it considered high.

For the pH of water in this study, it seemed that TLR have good pH value with ranged from 6.04 to 7.47, well within the tolerance range indicated for Odonata. For pH of water in OA, the value was towards acidic which is the minimum value reached until 4.8.

Table 6: Environmental factors measured on Odonata for each category of habitat (DO – dissolved oxygen; T – temperature; pH).

Category of Habitat/ Variables		Tropical Lowland Forest (TLR)			Open Areas (OA)		
		DO (ppm)	**T (^{o}C)**	**pH**	**DO (ppm)**	**T (^{o}C)**	**pH**
N	**Valid**	8	8	8	8	8	8
	Missing	0	0	0	0	0	0
Mean		8.583	31.29	6.974	4.578	31.16	5.933
Minimum		7.13	25.5	6.04	1.9	24.1	4.8
Maximum		10.5	35.2	7.47	10.53	39.8	6.56

3.3 Analysis and Characterization the Potential of *ND1* Gene

3.3.1 PCR Amplification

PCR amplifications were performed using primers P850 (fw) and P851 (rev) (Rach *et al*., 2007) for all samples of Odonata. This PCR fragment was encompassing partial 16S rRNA, intervening $tRNA^{Leu}$ and partial of *ND1* gene. After PCR amplification, the quality of PCR products was checked by agarose gel electrophoresis. The example of gel electrophoresis to check the successfulness of PCR amplification is shown in the Figure 10.

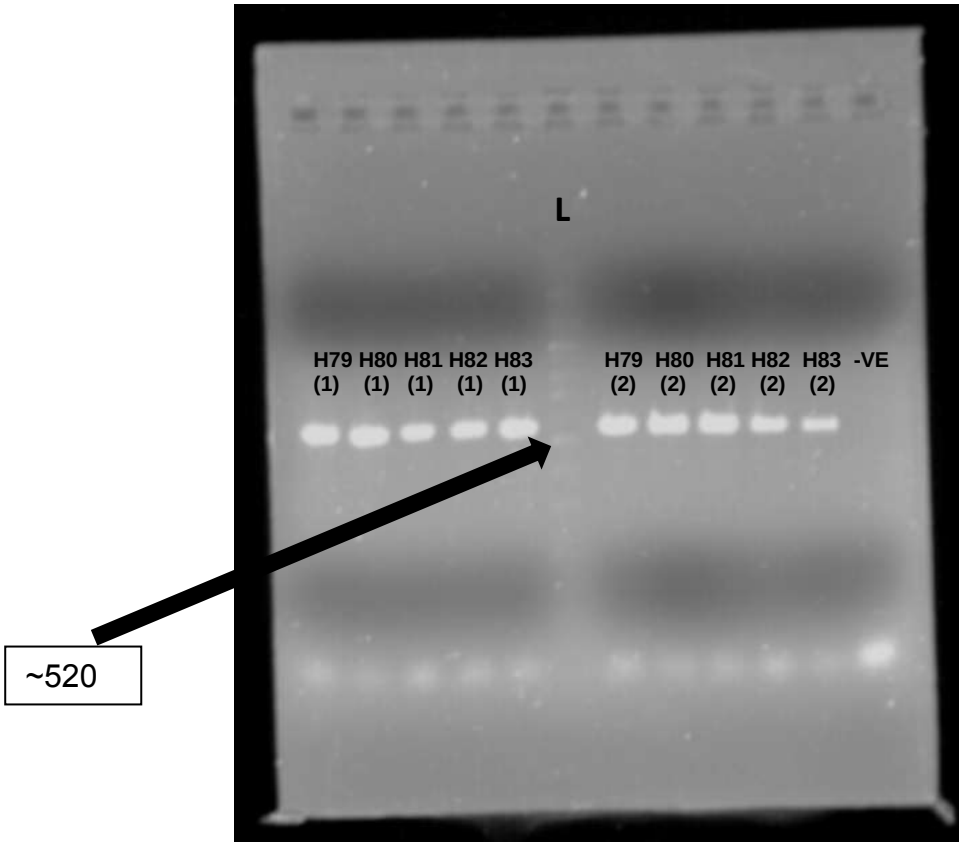


Figure 10: Example of amplification products.

The figure shows the PCR amplifications of partial 16S rRNA, intervening $tRNA^{Leu}$ and partial of *ND1* gene by specific primers of P850 (fw) and P851 (rev) primers at $49^{o}C$.

All the samples above were from the same locality Rimba Ilmu, University of Malaya. H79 and H80 indicated species of *Pantala flavescens,* while H81 and H82 were species of *Pyriobacta torida* and H83 was the sample of species *Pseudothemis.* On the other hand, L was indicates the 100bp ladder while –ve was indicated negative control. Primer-dimers are visible at the bottom of each well. The DNA from a single sample was loaded twice represented by (1) and (2).

3.3.2 PCR Purification

In order to get good results for DNA sequencing, the PCR products were purified to remove the undesired primer-dimers and excessive PCR reagents. The figure below (Figure 11) showed the example of purified products. Sample of H65 and H70 were retrieved from different samples of species *Rhynocypha biforata*, while H71, H72, H73 and H74 represented four different samples of species *Orthetrum chrysis.*

For H80 was the product amplified from *Neurothemis fluctuans*. Besides, the samples of H81 and H82 were purified PCR products of species *Pyriobacta torida* from different individuals, and it was similar to the sample of H83 and H84 where they were the purified PCR products of *Pseudothemis sp.* also from different individuals.

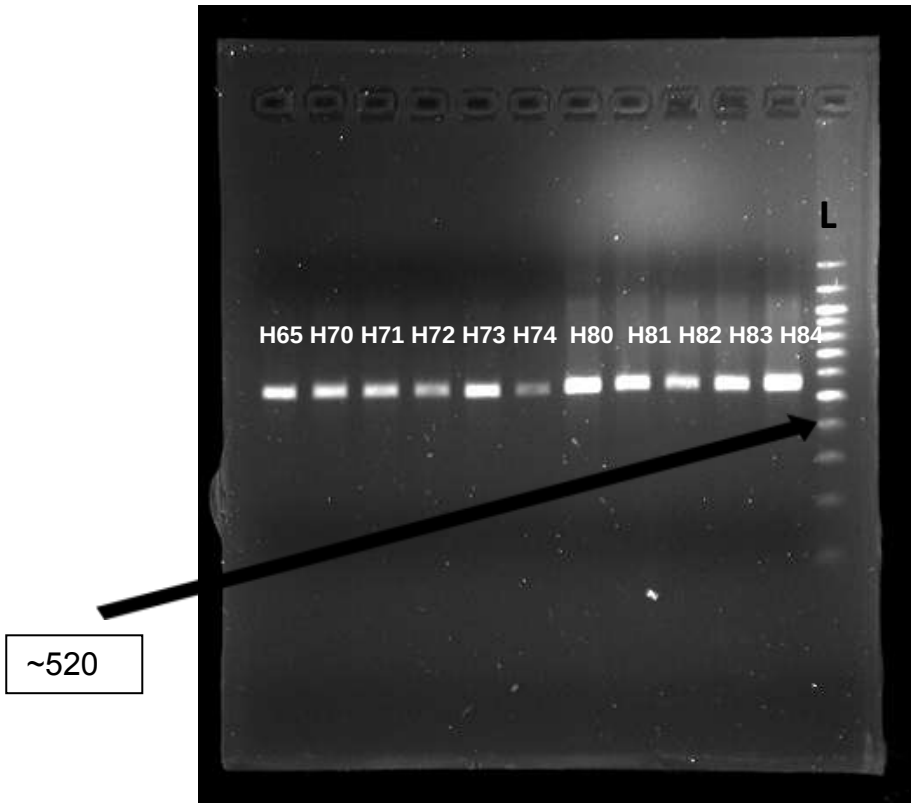


Figure 11: Example of purified PCR products. Primer-dimers and unspecific bands were removed. L indicates 100bp ladder (SeeGene, Korea).

3.3.3 Sequence Analysis

3.3.3.1 Sequence Reading

40 samples were successfully sequenced and the sequence readings obtained were in the range of 533 bp to 574 bp. Owing to the unclear results at the fragments ends close to the primers, the sequence were trimmed at the beginning (forward primer) and at the end (reverse primer) of the sequences (Appendix 8).

The sequences of all samples were compared with each other. It shows the sequence data for *ND1* region of the same species were matched very closely to each other. The sequence result is shown in the table below (Figure 12).

```
#A.gracilis_C14_ND1              TGT-TAGTTC TTAGTACGAA AGCACCAGG- ACTAT-AAAA TATTTTTTT-
#A.gracilis_C14_ND1(2)           ...-...... .......... .........- .....-.... .........-
#A.gracilis_C16_ND1              ...-...... ......A... ..G......- .....-.... .........-
#A.gracilis_H87_ND1              ...-...... .......... ..G......- .....-.... .........-
#Euphaea_ochracea_C20_ND1        .TGG..A..T .......... ..G....TT- .T..C-.... .........A
#Euphaea_ochracea_C21_ND1        .TGG..A..T .......... ..G....TT- .T..C-.... .........A
#Libellago_lineata_A14_ND1       ATAT..A.AT .......... ..GG..CTT- TT..A-.TT. .....A..A-
#Libellago_lineata_A15_ND1       ATAT..A.AT .......... ..GG..CTT- TT..A-.T.. .........-
#Libellago_lineata_A16_ND1       ATAT..A.AT .......... ..GG..CTT- .T..A-.T.. .....A..A-
#Libellago_lineata_A25_ND1       ATAT..A.AT ......A... ..GC..CTT- .T..A-.T.. .....A..A-
#Libellago_lineata_A27_ND1       ATAT..A.AT .......... ..GG..CTT- .T..A-.T.. .....A..A-
#Neurobasis_chinensis_A18_ND1    .T.G..A..T .......... ..G....TT- TT..C-..G. ....C....A
#Neurobasis_chinensis_A21_ND1    .T.G..A..T .......... ..G....TT- TT..C-..G. ....C....A
#Neurobasis_chinensis_A24_ND1    .T.G..A..T .......... ..G....TT- TT..C-..G. ....C....A
#Neurobasis_chinensis_A26_ND1    .T.G..A..T .......... ..G....TT- TT..C-..G. ....C....A
#Neurobasis_chinensis_A28_ND1    .T.G..A..T .......... ..G....TT- TT..C-..G. ....C....A
#Neurobasis_chinensis_C22_ND1    .T.G..A..T .......... ..G....TT- TT..C-..G. ....C....A
#Neurobasis_chinensis_C23_ND1    GT.-..AC.T .......... ..G....AA- GT...-G... ..G......-
#Neurothemis_fluctuans_A13_ND1   GT.-..AC.T .......... ..GC...AA- GT...-G... ..G......-
#Neurothemis_fluctuans_C18_ND1   GT.-..AC.T .......... ..GC...AA- GT...-G... ..G......-
#Neurothemis_fluctuans_C19_ND1   GT.-..AC.T .......... ..G....AA- GT...-G... ..G......-
#Orthetrum_glaucum_A34_ND1       AT.-C....T ...C...... ..G....AA- .T.G.-.... ..A.....A-
#Pantala_flavescens_E8_ND1       AT.-..A..T .......... ..G....AA- .T..A-G... ..A......-
#Pantala_flavescens_E9_ND1       AT.-..A..T .......... ..G....AA- .T.G.-G... ..A......-
#Pantala_flavescens_GU323081.1   ---------- ------.... ..G....AA- .T..A-G... ..A......-
#Rhyothemis_obsolescens_C12_ND1 AAC-..AC.T .......... ..G....AA- GT.GG-G... ..A......-
#Trithemis_aurora_A10_ND1        AC.-..AC.A .......... ..G....TA- GT...-.T.. ..A..A...A
#Trithemis_aurora_A5_ND1         AC.-..AC.A .......... ..G....TA- GT...-.T.. ..A..A...A
#Trithemis_aurora_C17_ND1        AC.-..AC.A .......... ..G....TA- GT...-.T.. ..A..A...A
#Trithemis_aurora_E7_ND1         AT.-..A..T .......... ..G....AA- .T..A-G... ..G...CC.-
#Trithemis_aurora_GU323085.1     ---------- ------.... ..G....TA- GT...-.T.. ..A..A...A
#Trithemis_aurora_H89_ND1        AT.-..A..T .......... ..G....AA- .T..A-G... ..G...C..-
#Trithemis_festiva_A1_ND1        .T.-...C.A .......... ..G....TA- G...A-.T.. ..A..A...-
#Trithemis_festiva_GU323099.1    ---------- ------.... ..G....TA- G...A-.T.. ..A..A...-
#Trithemis_festiva_H7_ND1        .T.-...C.A .......... ..G....TA- G...A-.T.. ..A..A...-
#Trithrmis_aurora_C9_ND1         AT.-..A..T .......... ..G....AA- .T..A-G... ..G...C..-
#Zygonyx_iris_A32_ND1            AT.-C.T... .......... ..G......- .A.GG-G... ..A......-
#Zygonyx_iris_A7_ND1             AT.-C.T... .......... ..G......- .A.GG-G... ..A......-
#Zygonyx_iris_G15_ND1            CT.-C.T... .......... ..GC.....- .A.GG-G... ..A......-
#Zygonyx_iris_H91_ND1            AA.-.....T .......... ..G....AA- ....A-G... ..A...C..-
```

```
#A.gracilis_C14_ND1              -ATATTTGAA TATTATTAA- CTATTTTGGC AGAAAAGTGC CATGGATTTA
#A.gracilis_C14_ND1(2)           -......... .........- .......... .......... ..........
#A.gracilis_C16_ND1              -......... .........- .......... .......... ..........
#A.gracilis_H87_ND1              -......... .........- .......... .......... ..........
#Euphaea_ochracea_C20_ND1        C.GT....TT ..A......- .......... .......... T...A.....
#Euphaea_ochracea_C21_ND1        C.GT....TT ..A......- .......... .......... T...A.....
#Libellago_lineata_A14_ND1       -T.T...... ..C.....-- .......... ...T...G.T G...A.....
#Libellago_lineata_A15_ND1       -T.T.....T .TC.....-- .......... ...T.....T G...A.....
#Libellago_lineata_A16_ND1       -T.T.....T ..A.....-- .......... ...T.....T G...A.....
#Libellago_lineata_A25_ND1       -T.T.....T ..C.....-- .......... ...T.....T G...A.....
#Libellago_lineata_A27_ND1       -T.T.....T ..C.....-- .......... C..T.....T G.A.A.....
#Neurobasis_chinensis_A18_ND1    ---T.....T .........- .......... .......... ....A.....
#Neurobasis_chinensis_A21_ND1    ---T.....T .........- .......... .......... ....A.....
#Neurobasis_chinensis_A24_ND1    ---T.....T .........- .......... .......... ....A.....
#Neurobasis_chinensis_A26_ND1    ---T.....T .........- .......... .......... ....A.....
#Neurobasis_chinensis_A28_ND1    ---T.....T .........- .......... .......... ....A.....
#Neurobasis_chinensis_C22_ND1    ---T.....T .........- .......... .......... ....A.....
#Neurobasis_chinensis_C23_ND1    -TCTG..... ..CC.....- ...C...... .......... A...A.....
#Neurothemis_fluctuans_A13_ND1   -TCTG..... ..CC.....- ...C...... .......... A...A.....
#Neurothemis_fluctuans_C18_ND1   -TCTG..... ..CC.....- ...C...... .......... A...A.....
#Neurothemis_fluctuans_C19_ND1   -TCTG...G. ..CC.....- ...C...... .......... A...A.....
#Orthetrum_glaucum_A34_ND1       -T.TG....T ..A......- .......... .......... ....A.....
#Pantala_flavescens_E8_ND1       -T-.A..... ..CC.....- .......... .......... ....A.....
#Pantala_flavescens_E9_ND1       -T-.A..... .-CC.....- .......... .......... ....A.....
#Pantala_flavescens_GU323081.1   -T..A..... ..CC.....- .......... .......... ....A.....
#Rhyothemis_obsolescens_C12_ND1 -G.TG....T ..A......- ......?... .......... ....A.....
#Trithemis_aurora_A10_ND1        -T.TA....T ..A......- .......... .......... ....A.....
#Trithemis_aurora_A5_ND1         -T.TA....T ..A......- .......... .......... ....A.....
#Trithemis_aurora_C17_ND1        -T.TA....T ..A......- .......... .......... ....A.....
#Trithemis_aurora_E7_ND1         -G.TG..... .....A...- .......... .......... ....A.....
#Trithemis_aurora_GU323085.1     -T.TA....T ..A......- .......... .......... ....A.....
#Trithemis_aurora_H89_ND1        -G.TG..... .....A...- .......... .......... ....A.....
#Trithemis_festiva_A1_ND1        -T.TG....T ..A......- .......... .......... ....A.....
#Trithemis_festiva_GU323099.1    -T.TG....T ..A......- .......... .......... ....A.....
#Trithemis_festiva_H7_ND1        -T.TG....T ..A......- .......... .......... ....A.....
#Trithrmis_aurora_C9_ND1         -G.TG..... .....A...- .......... .......... ....A.....
#Zygonyx_iris_A32_ND1            -TATA....T ..A......A .......... ......A... ....A.....
#Zygonyx_iris_A7_ND1             -TATA....T ..A......A .......... ......A... ....A.....
#Zygonyx_iris_G15_ND1            -TATA....T ..A......A .......... ......A... ....A.....
#Zygonyx_iris_H91_ND1            -TA.C....T ..A......- .......... ....G..... ....A.....
```

Figure 12: Comparison of *ND1* sequences data of various species of Odonata.

. = similar **?** = missing site **-** = indel site

In addition, results of the standard sequence obtained from three species were compared with the sequences that published previously in the GenBank. The species were *Pantala flavescens*, *Trithemis aurora* and *Trithemis festiva.* It is shown as in Figure 12.1, Figure 12.2 and Figure 12.3 below. The conserved and variable sites were counted for each species.

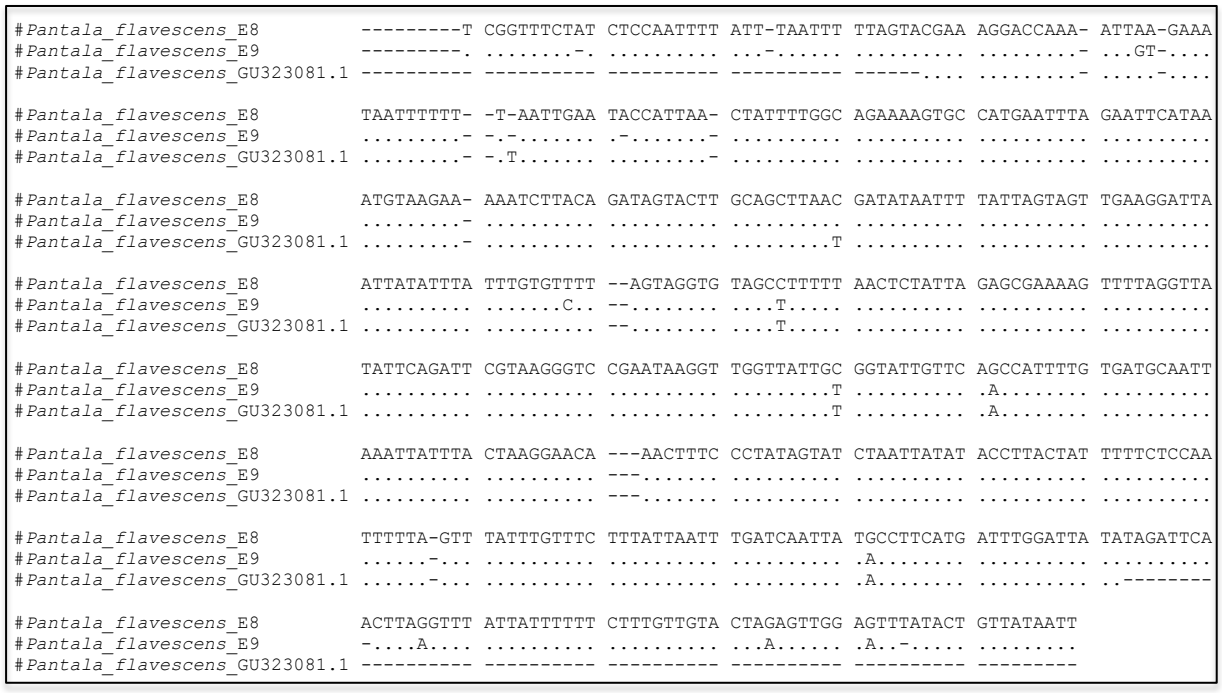


Figure 12.1: Comparison of sequence obtained (*Pantala flavescens*) with previously published sequence.

(Accession Number: GU323081)

Conserved Site = 512 / 544

Variable Site = 11 / 544

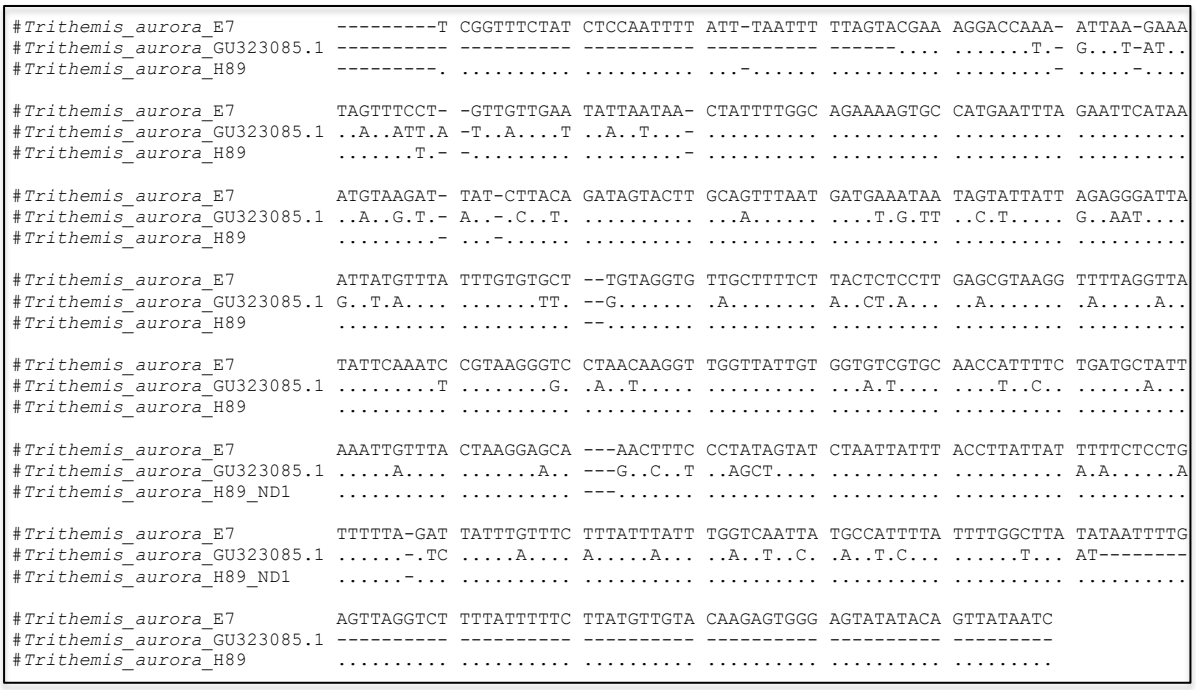
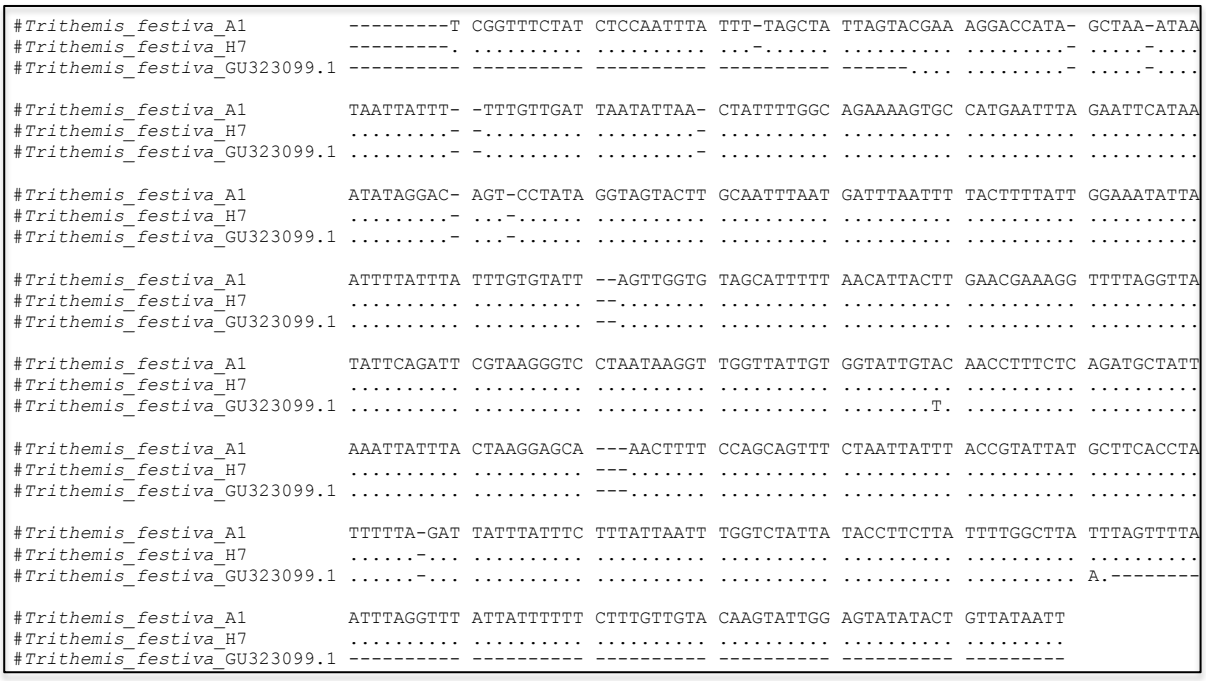


Figure 12.2: Comparison of sequence obtained (*Trithemis aurora*) with previously published sequence.

(Accession Number: GU323085)

Conserved Site = 446 / 544

Variable Site = 80 / 544



```
#Trithemis_festiva_A1         ----------T CGGTTTCTAT CTCCAATTTA TTT-TAGCTA TTAGTACGAA AGGACCATA- GCTAA-ATAA
#Trithemis_festiva_H7         ----------. .......... .......... ...-...... .......... ..........- .....-....
#Trithemis_festiva_GU323099.1 ---------- ---------- ---------- ---------- ------.... ..........- .....-....

#Trithemis_festiva_A1         TAATTATTT- -TTTGTTGAT TAATATTAA- CTATTTTGGC AGAAAAGTGC CATGAATTTA GAATTCATAA
#Trithemis_festiva_H7         ..........- -.......... ..........- .......... .......... .......... ..........
#Trithemis_festiva_GU323099.1 ..........- -.......... ..........- .......... .......... .......... ..........

#Trithemis_festiva_A1         ATATAGGAC- AGT-CCTATA GGTAGTACTT GCAATTTAAT GATTTAATTT TACTTTTATT GGAAATATTA
#Trithemis_festiva_H7         ..........- ...-...... .......... .......... .......... .......... ..........
#Trithemis_festiva_GU323099.1 ..........- ...-...... .......... .......... .......... .......... ..........

#Trithemis_festiva_A1         ATTTTATTTA TTTGTGTATT --AGTTGGTG TAGCATTTTT AACATTACTT GAACGAAAGG TTTTAGGTTA
#Trithemis_festiva_H7         .......... .......... --........ .......... .......... .......... ..........
#Trithemis_festiva_GU323099.1 .......... .......... --........ .......... .......... .......... ..........

#Trithemis_festiva_A1         TATTCAGATT CGTAAGGGTC CTAATAAGGT TGGTTATTGT GGTATTGTAC AACCTTTCTC AGATGCTATT
#Trithemis_festiva_H7         .......... .......... .......... .......... .......... .......... ..........
#Trithemis_festiva_GU323099.1 .......... .......... .......... .......... ........T. .......... ..........

#Trithemis_festiva_A1         AAATTATTTA CTAAGGAGCA ---AACTTTT CCAGCAGTTT CTAATTATTT ACCGTATTAT GCTTCACCTA
#Trithemis_festiva_H7         .......... .......... ---........ .......... .......... .......... ..........
#Trithemis_festiva_GU323099.1 .......... .......... ---........ .......... .......... .......... ..........

#Trithemis_festiva_A1         TTTTTA-GAT TATTTATTTC TTTATTAATT TGGTCTATTA TACCTTCTTA TTTTGGCTTA TTTAGTTTTA
#Trithemis_festiva_H7         ......-... .......... .......... .......... .......... .......... ..........
#Trithemis_festiva_GU323099.1 ......-... .......... .......... .......... .......... .......... A.--------

#Trithemis_festiva_A1         ATTTAGGTTT ATTATTTTTT CTTTGTTGTA CAAGTATTGG AGTATATACT GTTATAATT
#Trithemis_festiva_H7         .......... .......... .......... .......... .......... .........
#Trithemis_festiva_GU323099.1 ---------- ---------- ---------- ---------- ---------- ---------
```

Figure 12.3: Comparison of sequence obtained (*Trithemis festiva*) with previously published sequence.

(Accession Number: GU323099)

Conserved Site = 524 / 544

Variable Site = 2 / 544

3.3.3.2 Sequence Editing

All the DNA sequences obtained from the sequencing were processed by CHROMAS software version 2.31. Both of the forward and reverse primers sequences were excluded and the forward and reverse sequences were then merged in order to get the full length of sequence. The table below (Table 7) shows the length of DNA sequences obtained for each species.

Table 7: DNA sequence length obtained from different Odonata species.

SUBORDER	FAMILY	SPECIES	SEQUENCE LENGTH (bp)
Anisoptera	Libellulidae	*Aethriamanta gracilis C14*	505
Anisoptera	Libellulidae	*Aethriamanta gracilis C15*	526
Anisoptera	Libellulidae	*Aethriamanta gracilis C16*	526
Anisoptera	Libellulidae	*Aethriamanta gracilis H87*	526
Anisoptera	Libellulidae	*Neurothemis fluctuans A13*	526
Anisoptera	Libellulidae	*Neurothemis fluctuans C18*	526
Anisoptera	Libellulidae	*Neurothemis fluctuans C19*	526
Anisoptera	Libellulidae	*Orthetrum glaucum A34*	526
Anisoptera	Libellulidae	*Pantala flavescens E8*	526
Anisoptera	Libellulidae	*Pantala flavescens E9*	522
Anisoptera	Libellulidae	*Rhyothemis obsolescens C12*	527
Anisoptera	Libellulidae	*Trithemis aurora A10*	490
Anisoptera	Libellulidae	*Trithemis aurora A5*	527
Anisoptera	Libellulidae	*Trithemis aurora C17*	527

Table 7: Continued.

Anisoptera	Libellulidae	*Trithemis aurora C9*	526
Anisoptera	Libellulidae	*Trithemis aurora E7*	526
Anisoptera	Libellulidae	*Trithemis aurora H89*	526
Anisoptera	Libellulidae	*Trithemis festiva A1*	526
Anisoptera	Libellulidae	*Trithemis festiva H7*	526
Anisoptera	Libellulidae	*Zygonyx iris A32*	527
Anisoptera	Libellulidae	*Zygonyx iris A6*	525
Anisoptera	Libellulidae	*Zygonyx iris H91*	526
Anisoptera	Libellulidae	*Zygonyx iris A7*	527
Anisoptera	Libellulidae	*Zygonyx iris G15*	527
Zygopetra	Calopterygidae	*Neurobasis chinensis A18*	525
Zygopetra	Calopterygidae	*Neurobasis chinensis A21*	525
Zygopetra	Calopterygidae	*Neurobasis chinensis A24*	525
Zygopetra	Calopterygidae	*Neurobasis chinensis A26*	525
Zygopetra	Calopterygidae	*Neurobasis chinensis A28*	525
Zygoptera	Calopterygidae	*Neurobasis chinensis C22*	525
Zygoptera	Calopterygidae	*Vestalis amethystina C23*	526
Zygopetra	Chlorocyphidae	*Libellago lineata A15*	526
Zygopetra	Chlorocyphidae	*Libellago lineata A16*	526
Zygopetra	Chlorocyphidae	*Libellago lineata A14*	526
Zygopetra	Chlorocyphidae	*Libellago lineata A27*	526
Zygoptera	Euphaeidae	*Euphaea ochracea C20*	531
Zygoptera	Euphaeidae	*Euphaea ochracea C21*	531

From the table above, the DNA sequence length varied among the species with a range of 490bp (*Trithemis auroura*) to 531bp (*Euphaea ochracea*).

Furthermore, the DNA sequence lengths were shown to vary within the species even though they were identified as the same species morphologically. The PCR amplification and gel electrophoresis revealed a size difference of approximately 40 base pairs (bp) between *Trithemis aurora,* while *Aethriamanta gracilis* with 21 base pairs and 4 base pairs between *Pantala flavescens*. Additionally, the species of *Zygonyx iris* had matched very closely to each other with only difference 2 base pairs over 527 base pairs of *ND1* sequence.

On the other hand, there were also individuals with successful amplifications that did not conform to the size difference. These individuals were originally classified as *Neurothemis fluctuans*, *Trithemis festiva* and *Libellago lineata* with 526 base pairs, while, for species of *Neurobasis chinensis* with 525 base pairs, and species of *Euphaea ochracea* with 531 base pairs.

3.3.3.3 Sequence Alignment

To understand the relationship between the sequences, all the sequence data were then analysed using MEGA software version 4.0.1 (Tamura *et al.,* 2007). From the alignment result, the final sequence length was 532bp. Three foreign DNA sequences from GenBank database were included to ensure that the DNA sequences obtained from this study were correct regions of partial 16S rRNA, intervening $tRNA^{Leu}$ and partial of *ND1* gene. These DNA sequences included were under accession number of GU323085.1 (*Trithemis auroura*), GU323099.1 (*Trithemis festiva*) and GU323081.1 (*Pantala flavescens*).

Table 7.1: *ND1* gene alignment showing total alignment length, number of conserved sites, variable sites, singleton sites, parsimony-informative sites and average nucleotide frequencies

***ND1* region**								
Alignment length = 544 bp	Conserved (monomorphic) Sites		Variable (polymorphic) Sites		Singleton Sites		Parsimony Informative Sites	
Samples = 40	75	13.8%	458	84.2%	153	28.1%	305	56.1%
Average nucleotide frequencies (%) **T** : 45.7 **C** : 11.0 **A** : 27.0 **G** : 16.3								

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From the table above, the total alignment length was 544 bp or 544 sites. There were 458 variable sites (84.2%) with remaining of 75 conserved sites (13.8%). The singleton sites totalled was 153 (28.1%) with 305 parsimony-informative sites (56.1%). As expected, this region of mtDNA was observed to have a strong AT bias (72.7%), which is characteristic of insect mitochondrial DNA (Crozier & Crozier, 1993), while

the GC content was 27.3%. In addition, the nucleotide compositions were T (45.7%), C (11.0%), A (27.0%) and G (16.3%).

3.3.4 Base Nucleotide Composition

The detailed descriptions of base nucleotide composition for each species are presented in the table below (Table 8). The average nucleotides for all the samples recorded were 518.1 bp.

Table 8: Nucleotide composition of partial 16S rRNA, $tRNA^{Leu}$ and *ND1* region of sampled.

Species / Nucleotide	**T(U)**	**C**	**A**	**G**	**Total**
Aethriamanta gracilis C14	45.2	11.8	28.9	14.1	5**26**
Aethriamanta gracilis C15	45.2	11.8	28.9	14.1	526
Aethriamanta gracilis C16	45.2	11.4	29.1	14.3	526
Aethriamanta gracilis H87	45.1	11.6	28.9	14.4	526
Bactrocera carambolae AY037453	50.7	8.3	**23.5**	**17.6**	**533**
Bactrocera dorsalis FN400901	47.9	8.6	24.2	19.3	524
Euphaea ochracea C20	48.0	10.5	23.5	17.9	531
Euphaea ochracea C21	48.0	10.5	23.5	17.9	531
Libellago lineata A14	48.9	10.3	24.0	16.9	526
Libellago lineata A15	50.6	9.9	22.4	17.1	526
Libellago lineata A16	48.9	9.9	24.3	16.9	526
*Libella**go lineata*** A25	49.4	9.7	23.8	17.1	526
Libellago lineata A27	49.4	10.6	22.8	17.1	526
Neurobasis chinensis A18	46.3	10.9	27.2	15.6	525
Neurobasis chinensis A21	46.3	10.9	27.2	15.6	525
*Neurobasi**s chinensis*** A24	46.3	10.9	27.2	15.6	525
Neurobasis chinensis A26	46.3	10.9	27.2	15.6	525
Neurobasis chinensis A28	46.3	10.9	27.2	15.6	**525**
Neurobasis chinensis C22	46.3	10.9	27.2	15.6	525

Table 8: Continued.

Neurobasis chinensis C23	45.1	13.1	24.9	16.9	526
Neurothemis fluctuans A13	44.9	13.5	24.7	16.9	526
Neurothemis fluctuans C18	45.1	13.3	24.7	16.9	526
Neurothemis fluctuans C19	45.1	13.1	24.7	17.1	526
Orthetrum glaucum A34	45.6	10.8	29.5	14.1	526
Pantala flavescens E8	43.3	11.0	30.6	15.0	526
Pantala flavescens E9	43.9	10.9	30.8	14.4	522
Pantala flavescens GU323081.1	41.7	10.6	32.8	14.9	424
Rhyothemis obsolescens C12	45.1	9.9	26.8	18.3	526
Trithemis aurora A10	43.3	12.4	30.2	14.1	490
Trithemis aurora A5	45.0	11.4	28.7	15.0	527
Trithemis aurora C17	45.0	11.6	28.7	14.8	527
Trithemis aurora E7	43.9	11.6	27.6	16.9	526
Trithemis aurora GU323085.1	43.6	11.6	30.2	14.6	424
Trithemis aurora H89	44.1	11.4	27.6	16.9	526
Trithemis festiva A1	45.8	10.8	28.7	14.6	526
Trithemis festiva GU323099.1	44.0	11.3	29.8	14.9	423
Trithemis festiva H7	45.8	10.8	28.7	14.6	526
Trithrmis aurora C9	44.1	11.4	27.6	16.9	52**6**
Zygonyx iris A32	45.2	10.4	27.5	16.9	527
Zygonyx iris A7	45.2	10.4	27.5	16.9	527
Zygonyx iris G15	45.2	10.8	26.9	17.1	527
Zygonyx iris H91	43.2	10.8	30.0	16.0	526
Average.	**45.8**	**11.0**	**27.1**	**16.0**	**518.1**

Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism were subjected to the Tajima's Neutrality Test (Table 8.1). All positions containing gaps and missing data were eliminated from the data set (Complete deletion option). The number of segregating sites was 323 while the nucleotide diversity π = 0.215506.

Table 8.1: Tajima's Neutrality Test for 42 sequences. (m = number of sites, S = number of segregating sites, p_s = S/m, $\boldsymbol{\Theta} = p_s/a_1$, π = nucleotide diversity, and D = Tajima test statistic.

m	S	p_s	Θ	π	D
42	323	0.859043	0.199641	0.215506	0.294877

3.3.5 Nucleotide Substitution.

The nucleotide substitution was analysed using all the combined dataset. Rates of different transitional substitutions are shown in bold: [A-G] = 17.99, [T-C] = 20.24, [C-G] = 5.32, [G-A] = 10.34, and those of transversional substitutions are shown in italics (Table 9). The nucleotide frequencies are A = 0.283, T/U = 0.439, C = 0.115, and G = 0.163. The transition/transversion rate ratios are k_1 = 2.756 (purines) and k_2 = 2.001 (pyrimidines). The overall transition/transversion bias is R = 0.805, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

All positions containing gaps and missing data were eliminated from the dataset (Complete-deletion option). There were a total of 376 positions in the final dataset. All the calculations were done in Mega 4 (Tamura *et al.*, 2007) and the nucleotide pair frequencies were then analyzed which resulted the average of identical pairs from all the dataset = 392, transitional pairs = 49, transversional pairs = 64, and transitional pairs/transversional pairs = 0.8.

Table 9: Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution (Tamura *et al.*, 2007).

	A	**T**	**C**	**G**
A	-	*10.11*	*2.66*	**10.34**
T	*6.53*	-	**5.32**	*3.75*
C	*6.53*	**20.24**	-	*3.75*
G	**17.99**	*10.11*	*2.66*	-

3.3.6 Genetic Distance Evaluation

ND1: Sequence Divergence vs. No. of transitions and transversions

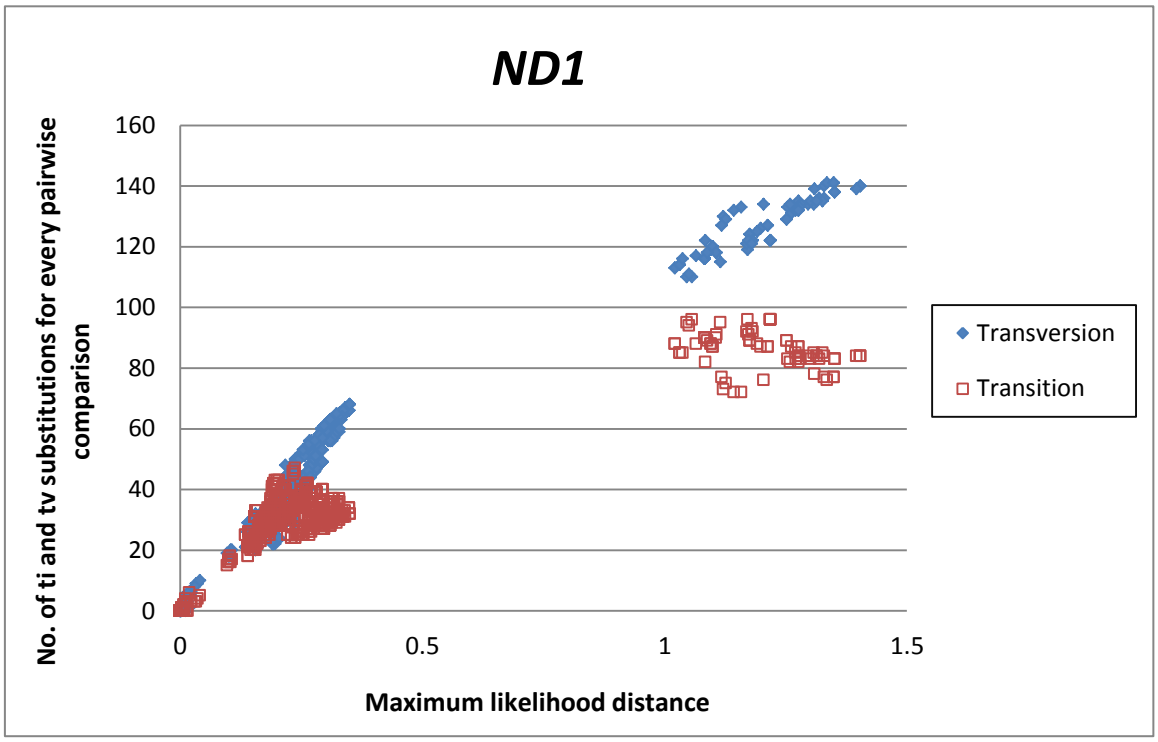

Figure 13: No. of transversional (tv) and transitional (ti) substitutions plotted against sequence divergence (calculated using maximum likelihood distance) for 42 samples in the *ND1* region.

The scatter plot above shows the number of transversional (tv) and transitional (ti) substitutions plotted against the sequence divergence of the *ND1* region (Figure 13). From the graph, the pattern distributions of both substitution types are separated with a distinct gap in the middle. Furthermore, the number of transversional (tv) substitutions gather more rapidly than the transitional (ti) substitutions.

Analysing at the first part of the graph showed a low transition – transversion rate (<0.1), where they represented the intraspecific comparison among the dragonflies

(Anisoptera) and the damselflies (Zygoptera). At this point, the distance range was 0 – 0.35. Meanwhile at the second region of the graph, the distance values ranged from 1.013 – 1.404, which showed quite small number of transversion – transition substitutions fall within this region. These reflected the data of interspecific comparison (pairwise comparisons with outgroup taxa).

Besides, the graph also shows that these two substitutions have only a slightly different of rate. There is a low increment of transition as genetic divergence progresses. Henceforth the transition: transversion ration can be considered as 0.8. (Refer appendix 13). Moreover, both transition and transversion substitutions did not reach saturation as the divergence increases. Therefore, there was no need to analyse the transitional and transversional changes separately.

For the genetic distances between all the samples, the values revealed the genetic differences between the species (Table 10). From the pairwise divergence dataset, the lowest intra-specific genetic distance value is zero (*Trithemis aurora, Euphaea ochracea, Aethriamanta gracilis, Neurobasis chinensis, Zygonyx iris* and *Trithemis festiva*) which were all within the same species.

The highest genetic distance value (1.503) was observed between the *Aethriamanta gracilis* from suborder Anisoptera with *Bactrocera dorsalis* (Order: Diptera) which was the outgroup. While the highest genetic distance within Odonata was 0.338 (*Neurothemis fluctuans – Libellago lineata*), which indicates the distance between two suborders of Odonata.

Table 10: Pairwise distance between Species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
[1]																																										
[2]	0.155																																										
[3]	0.003	0.158																																									
[4]	0.215	0.202	0.219																																								
[5]	0.155	0.018	0.158	0.201																																							
[6]	0.232	0.198	0.235	0.005	0.197																																					
[7]	0.000	0.155	0.003	0.215	0.155	0.232																																				
[8]	0.215	0.202	0.219	0.005	0.201	0.005	0.215																																			
[9]	0.155	0.156	0.162	0.197	0.165	0.194	0.155	0.197																																		
[10]	0.179	0.162	0.182	0.173	0.159	0.175	0.179	0.175	0.151																																	
[11]	0.171	0.161	0.173	0.174	0.153	0.171	0.171	0.174	0.154	0.145																																
[12]	0.210	0.206	0.213	0.202	0.196	0.198	0.210	0.202	0.180	0.175	0.182																															
[13]	0.174	0.180	0.177	0.216	0.180	0.213	0.174	0.216	0.211	0.192	0.155	0.198																														
[14]	0.173	0.180	0.177	0.216	0.180	0.212	0.173	0.216	0.211	0.198	0.155	0.198	0.005																													
[15]	0.247	0.265	0.251	0.259	0.267	0.255	0.247	0.259	0.247	0.240	0.255	0.240	0.240	0.236																												
[16]	0.170	0.177	0.174	0.213	0.176	0.209	0.170	0.213	0.207	0.195	0.152	0.194	0.003	0.003	0.236																											
[17]	0.247	0.265	0.251	0.259	0.267	0.255	0.247	0.259	0.247	0.240	0.255	0.240	0.240	0.236	0.000	0.236																										
[18]	0.268	0.257	0.272	0.283	0.256	0.280	0.268	0.283	0.258	0.276	0.244	0.271	0.253	0.253	0.173	0.249	0.173																									
[19]	0.235	0.202	0.239	0.000	0.201	0.005	0.215	0.005	0.197	0.175	0.174	0.202	0.216	0.216	0.259	0.213	0.259	0.285																								
[20]	0.190	0.183	0.193	0.206	0.182	0.203	0.190	0.206	0.168	0.168	0.177	0.106	0.177	0.177	0.270	0.174	0.270	0.276	0.206																							
[21]	0.213	0.203	0.217	0.198	0.193	0.195	0.213	0.198	0.183	0.172	0.179	0.003	0.195	0.194	0.257	0.191	0.257	0.275	0.198	0.103																						
[22]	0.173	0.158	0.176	0.172	0.153	0.171	0.173	0.172	0.145	0.005	0.142	0.172	0.195	0.195	0.256	0.192	0.256	0.269	0.172	0.166	0.169																					
[23]	0.259	0.239	0.262	0.234	0.232	0.230	0.259	0.234	0.212	0.210	0.213	0.028	0.232	0.232	0.273	0.229	0.273	0.300	0.234	0.140	0.031	0.206																				
[24]	0.177	0.183	0.180	0.216	0.183	0.213	0.177	0.216	0.211	0.182	0.159	0.195	0.003	0.008	0.256	0.005	0.256	0.257	0.216	0.181	0.191	0.195	0.229																			
[25]	0.208	0.535	0.502	0.561	0.543	0.553	0.208	0.561	0.306	0.276	0.313	0.206	0.529	0.532	0.249	0.528	0.249	0.281	0.361	0.503	0.202	0.273	0.526	0.533																		
[26]	0.282	0.518	0.286	0.540	0.525	0.532	0.282	0.540	0.289	0.231	0.288	0.273	0.512	0.515	0.253	0.511	0.253	0.278	0.340	0.287	0.270	0.250	0.502	0.516	0.020																	
[27]	0.286	0.526	0.290	0.543	0.534	0.537	0.286	0.543	0.289	0.231	0.282	0.260	0.524	0.527	0.253	0.524	0.253	0.280	0.345	0.282	0.265	0.250	0.287	0.528	0.020	0.010																
[28]	0.268	0.257	0.272	0.283	0.256	0.280	0.268	0.283	0.258	0.276	0.244	0.271	0.253	0.253	0.173	0.249	0.173	0.000	0.285	0.276	0.275	0.269	0.300	0.257	0.291	0.278	0.290														
[29]	0.268	0.257	0.272	0.283	0.256	0.280	0.268	0.283	0.258	0.276	0.244	0.271	0.253	0.253	0.173	0.249	0.173	0.000	0.285	0.276	0.275	0.269	0.300	0.257	0.291	0.278	0.290	0.000														
[30]	0.268	0.257	0.272	0.283	0.256	0.280	0.268	0.283	0.258	0.276	0.244	0.271	0.253	0.253	0.173	0.249	0.173	0.000	0.285	0.276	0.275	0.269	0.300	0.257	0.291	0.278	0.290	0.000	0.000													
[31]	0.291	0.527	0.293	0.350	0.341	0.291	0.341	0.298	0.256	0.301	0.277	0.320	0.328	0.241	0.295	0.324	0.241	0.295	0.350	0.291	0.274	0.258	0.306	0.524	0.023	0.013	0.008	0.295	0.295	0.295												
[32]	0.268	0.257	0.272	0.283	0.256	0.280	0.268	0.283	0.258	0.276	0.244	0.271	0.253	0.253	0.173	0.249	0.173	0.000	0.285	0.276	0.275	0.269	0.300	0.257	0.291	0.278	0.290	0.000	0.000	0.000	0.295											
[33]	0.303	0.540	0.307	0.363	0.348	0.354	0.303	0.363	0.310	0.270	0.313	0.289	0.338	0.341	0.252	0.337	0.252	0.307	0.363	0.303	0.285	0.270	0.319	0.342	0.028	0.018	0.013	0.307	0.307	0.307	0.015	0.307									
[34]	0.268	0.257	0.272	0.283	0.256	0.280	0.268	0.283	0.258	0.276	0.244	0.271	0.253	0.253	0.173	0.249	0.173	0.000	0.285	0.276	0.275	0.269	0.300	0.257	0.291	0.278	0.290	0.000	0.000	0.000	0.295	0.000	0.307								
[35]	0.173	0.158	0.176	0.172	0.153	0.171	0.173	0.172	0.145	0.005	0.142	0.172	0.195	0.195	0.256	0.192	0.256	0.269	0.172	0.166	0.169	0.000	0.206	0.195	0.273	0.250	0.269	0.269	0.269	0.269	0.258	0.269	0.270	0.269							
[36]	0.190	0.180	0.193	0.166	0.168	0.166	0.190	0.166	0.135	0.133	0.152	0.180	0.171	0.170	0.257	0.167	0.257	0.238	0.166	0.156	0.177	0.150	0.215	0.174	0.285	0.276	0.268	0.238	0.238	0.238	0.277	0.238	0.289	0.238	0.150						
[37]	0.190	0.185	0.193	0.206	0.182	0.203	0.190	0.206	0.168	0.168	0.177	0.106	0.177	0.177	0.270	0.174	0.270	0.276	0.206	0.000	0.103	0.164	0.140	0.181	0.303	0.287	0.282	0.276	0.276	0.276	0.291	0.276	0.303	0.276	0.164	0.156				
[38]	0.207	0.200	0.210	0.198	0.190	0.195	0.207	0.198	0.180	0.175	0.176	0.005	0.198	0.198	0.247	0.194	0.247	0.268	0.198	0.100	0.008	0.172	0.034	0.201	0.289	0.266	0.261	0.268	0.268	0.268	0.270	0.268	0.282	0.268	0.172	0.174	0.100			
[39]	0.190	0.181	0.193	0.203	0.178	0.199	0.190	0.203	0.168	0.164	0.174	0.106	0.174	0.174	0.266	0.171	0.266	0.272	0.203	0.003	0.103	0.161	0.140	0.177	0.307	0.291	0.286	0.272	0.272	0.272	0.295	0.272	0.307	0.272	0.161	0.155	0.003	0.101		
[40]	0.146	0.013	0.149	0.198	0.010	0.194	0.146	0.198	0.153	0.135	0.131	0.190	0.174	0.173	0.261	0.170	0.261	0.233	0.198	0.172	0.187	0.152	0.223	0.177	0.326	0.310	0.295	0.253	0.253	0.253	0.319	0.253	0.331	0.253	0.152	0.171	0.172	0.184	0.169	
[41]	1.371	1.328	1.383	1.503	1.299	1.479	1.371	1.468	1.319	1.282	1.293	1.273	1.274	1.301	1.423	1.282	1.423	1.250	1.503	1.152	1.263	1.310	1.387	1.297	1.250	1.221	1.219	1.250	1.250	1.192	1.250	1.241	1.250	1.310	1.343	1.132	1.263	1.146	1.302	
[42]	1.317	1.277	1.331	1.440	1.250	1.418	1.317	1.407	1.270	1.266	1.244	1.252	1.238	1.252	1.366	1.243	1.366	1.226	1.440	1.110	1.240	1.270	1.362	1.250	1.236	1.207	1.203	1.226	1.226	1.226	1.139	1.226	1.226	1.226	1.279	1.291	1.110	1.240	1.124	1.228	0.015

3.4 Phylogenetic Relationship among the Taxa

Phylogenetic trees were constructed based on the 532bp aligned DNA sequences using MEGA software version 4.0.1 (Tamura *et al.,* 2007). For Figure 14.1, the evolutionary history was inferred using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987), while Figure 14 was inferred using the Maximum Parsimony (MP) method (Eck & Dayhoff`, 1966).

For both trees, the bootstrap consensus trees inferred from 5000 replicates were taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). All the branches which correspond to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (5000 replicates) is shown next to the branches.

The Maximum Parsimony tree was obtained using the Close-Neighbor-Interchange algorithm (Nei & Kumar, 2000) with search level 3, in which the initial trees were obtained with the random addition of sequences (10 replicates). On the other hand, the evolutionary distances for Neighbor-Joining were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data for both trees were eliminated from the dataset (Complete Deletion option). There were a total of 376 positions in the final dataset of MP and 398 positions in the final dataset of NJ.

The analysis of the phylogenetic trees revealed that there are almost similar topologies were recovered from the NJ and MP analyses (Figure 14 & 14.1). The phylogeny of Odonate was separated into 3 clades (A-C) that were supported by high bootstrap values = >50%. Clade A is comprised of *Trithemis aurora, Pantala*

flavescens, Zygonyx iris, Rhyothemis obsolescens, Neurothemis fluctuans, Orthetrum glaucum, Aethriamanta gracilis and *Trithemis festiva.* Clade B is consists of two species which are *Euphaea ochracea* and *Neurobasis chinensis,* while clade C is comprised of species *Libellago lineata*. Two species from Order Diptera (Family Tephritidae) were used as the outgroup in both phylogenetic trees namely *Bactrocera dorsalis* and *Bactrocera carambolae*.

As observed in both trees, each species in suborder Anisoptera are clustered well into their own specific cluster. Opposite to suborder Zygoptera, they were resolved clustered into 2 clusters. There is a distinct separation between cluster Anisoptera and Zygoptera with confidence level 72% in the NJ analyses while 90% in MP analyses. The separation between cluster Zygoptera clade B and clade C in NJ analyses was with the confidence level of 63% and 64% in the MP analyses.

The relationships among Zygoptera are more clearly resolved. One result consistent across all the analyses was the finding of Suborder Anisoptera as a monopyletic, with the exception of *Trithemis aurora*, where *Trithemis aurora* A5, A10, C17 and GU323085 (Gen Bank) are more closely related to *Trithemis festiva* which were resolved as a sister groups. Besides, the other clade of *Trithemis aurora* is successfully clustered well in another cluster with 100% of confidence level.

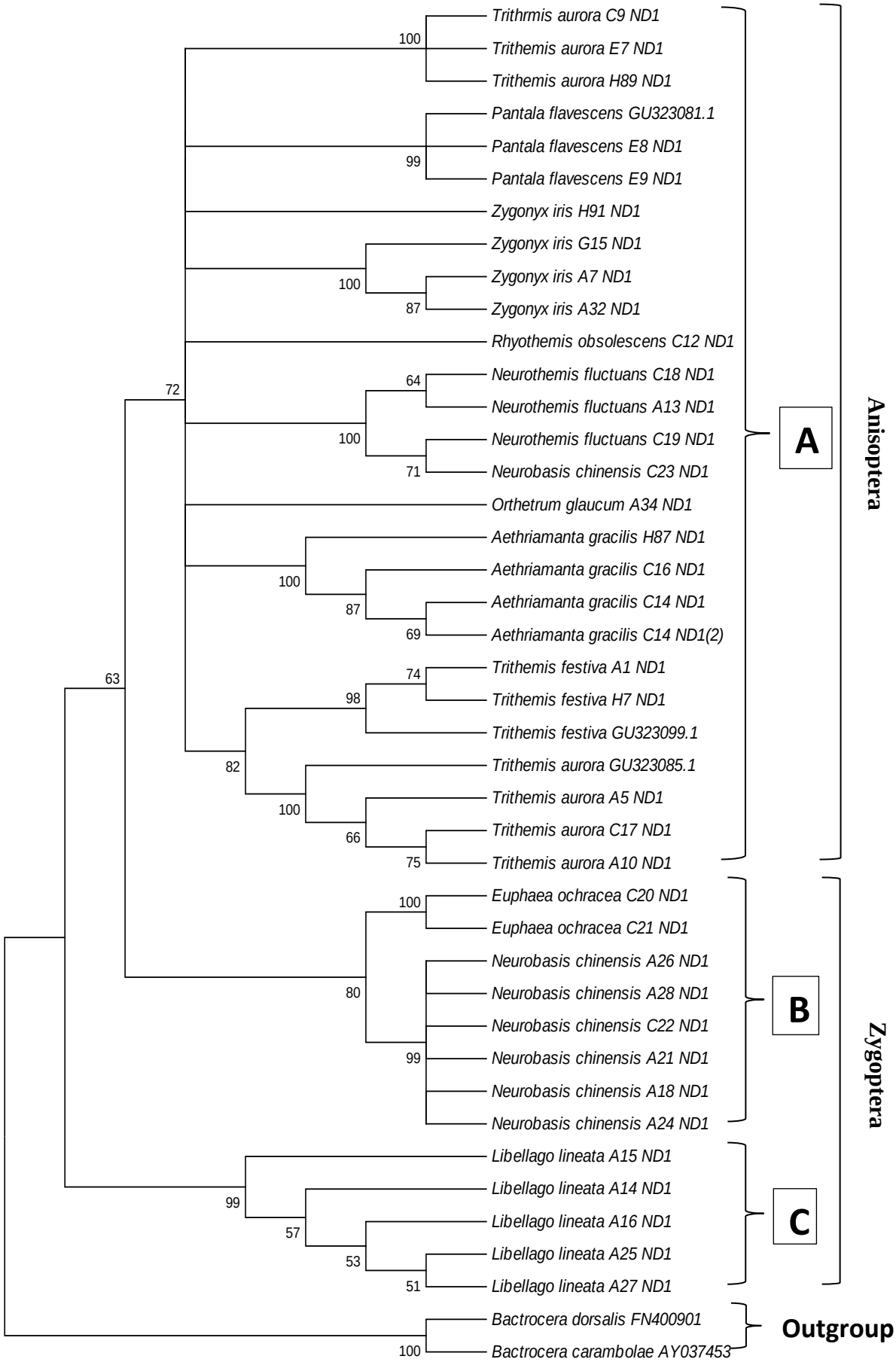

Figure 14: Neighbor-joining phylogram

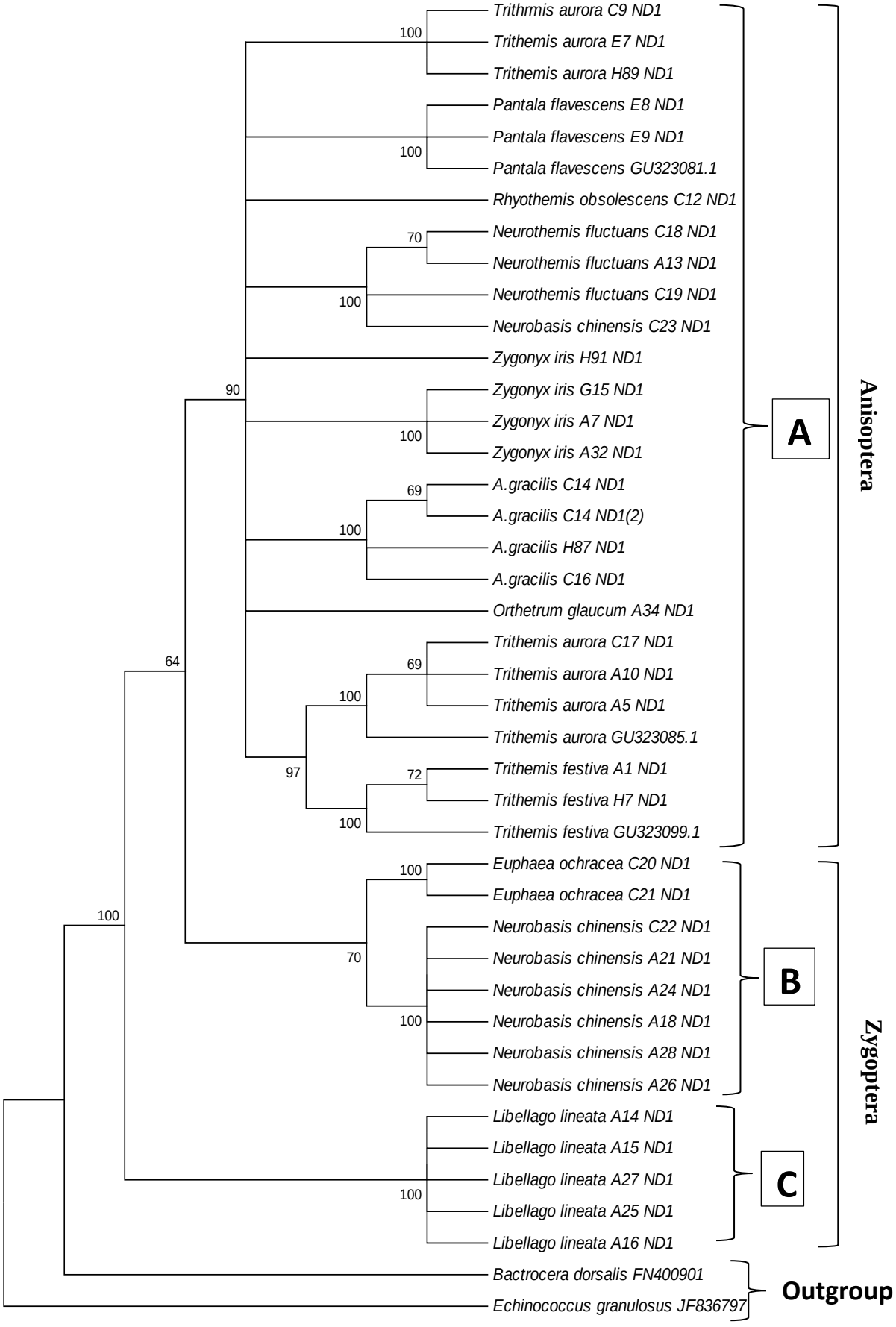

Figure 14.1: Maximum Parsimony phylogram