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Investigating leaf beetles (Coleoptera, Chrysomelidae) on the west coast islands of Sabah via checklist-taking and DNA barcoding

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Sabah, northern Borneo is one of the world’s most well-recognized biodiversity hotspots famous for the incredible diversity of its flora and fauna. Plenty of studies of leaf beetle fauna from this region have been conducted over the past 30 years. Yet, our knowledge of the leaf beetle fauna from island habitats remains scarce despite Sabah having the highest number of islands in Malaysia (ca. 500 islands). In this study, we collected leaf beetle fauna from 13 islands off the west coast of Sabah between January 2016 and March 2017. All specimens were identified to species level. Species names were assigned when the specimens fitted the description of species in the literature and morpho-species were assigned when the species names could not be determined. In addition, DNA barcodes – mitochondrial COI gene – of the species were sequenced. A total of 68 species from 31 genera and 5 subfamilies were collected with 12 species name being determined. From the data it was established that Pulau Gaya has the highest species richness (42 species), followed by Pulau Tiga (22 species) and Pulau Sapangar (18 species). Furthermore, a total of 64 Barcode Index Numbers consisting of 101 DNA barcodes were obtained from 60 leaf beetle species. The mean intraspecific and interspecific distances were determined as 0.77 % and 16.11 %, respectively. In addition, DNA barcoding also reveals phenotypic variation in leaf beetle species, particularly in the case of the subfamily Galerucinae. This study provides baseline knowledge and information about the DNA barcodes of leaf beetle species on Sabah’s island habitats for use in future studies.
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Abstract

Sabah, northern Borneo is one of the world’s most well-recognized biodiversity hotspots famous for the incredible diversity of its flora and fauna. Plenty of studies of leaf beetle fauna from this region have been conducted over the past 30 years. Yet, our knowledge of the leaf beetle fauna from island habitats remains scarce despite Sabah having the highest number of islands in Malaysia (ca. 500 islands). In this study, we collected leaf beetle fauna from 13 islands off the west coast of Sabah between January 2016 and March 2017. All specimens were identified to species level. Species names were assigned when the specimens fitted the description of species in the literature and morpho-species were assigned when the species names could not be determined. In addition, DNA barcodes – mitochondrial COI gene – of the species were sequenced. A total of 68 species from 31 genera and 5 subfamilies were collected with 12 species name being determined. From the data it was established that Pulau Gaya has the highest species richness (42 species), followed by Pulau Tiga (22 species) and Pulau Sapangar (18 species). Furthermore, a total of 64 Barcode Index Numbers consisting of 101 DNA barcodes were obtained from 60 leaf beetle species. The mean intraspecific and interspecific distances were determined as 0.77 % and 16.11 %, respectively. In addition, DNA barcoding also reveals phenotypic variation in leaf beetle species, particularly in the case of the subfamily Galerucinae. This study provides baseline knowledge and information about the DNA barcodes of leaf beetle species on Sabah’s island habitats for use in future studies.

Keywords: Barcoding of Life Data System (BOLD)  chrysomelid beetles  colour polymorphism  cryptic diversity  island biodiversity  sexual dimorphism  species distribution
Chrysomelidae Latreille, 1802 is one of the most diverse beetle families, with 35,000 - 60,000 species around the world (Splipnski, Leschen & Lawrence, 2011; Jolivet, 2015). The study of leaf beetle fauna in Borneo started in the 19th century, with the first valid species described by Suffrian (1854). A brief history of leaf beetle studies in Borneo is discussed in *The Leaf Beetle of Borneo* by Mohamedsaid, Salleh and Hassan (1990). Although Borneo is recognized as one of the world’s biodiversity hotspots, taxonomic research on Borneo leaf beetles has been limited to a few publications, possibly due to logistical difficulties and the inaccessibility of forest habitats (Mohamedsaid, Salleh & Hassan, 1990). As of 2004, 635 species of leaf beetle had been recorded in Borneo (Mohamedsaid, 2004).

Over the past a decade and a half, the number of leaf beetle species in Borneo has increased significantly, with more than 100 new species originating from Sabah (Takizawa, 2005, 2011, 2012, 2013, 2014, 2017, Mohamedsaid, 2006, 2010; Beenen, 2007; Medvedev, 2016a,b; Doberl, 2007; Medvedev, 2007, 2009, 2010, 2013; Borowiec, 2009; Moseyko, 2012; Borowiec, Takizawa & Swietojanńska, 2013; Mahadimenakbar & Takizawa, 2013; Bezděk, Romantsov & Medvedev, 2014; Medvedev & Romantsov, 2014, 2015, 2017a; Takizawa & Mohamedsaid, 2015). Most of these new species were discovered in mainland habitats on the west coast district of Sabah. Although Sabah has the highest number of islands in Malaysia, leaf beetle species diversity on islands has been little explored. In view of the fact that island habitats are generally known to have high species endemism (Kier et al., 2009), and that many of the islands on the west coast of Sabah are experiencing a rapid growth in tourism and economic development (Phung, Yu & Liew, 2017), it is important to document leaf beetle species diversity now to obtain baseline biodiversity knowledge.

The above mentioned recent taxonomic works are based on morphological characteristics (Mohamedsaid, 2006; Beenen, 2007; Doberl, 2007; Moseyko, 2012; Medvedev, 2016a; Medvedev & Romantsov, 2017b). Using this conventional taxonomic approach alone is challenging because sexual dimorphism and colour pattern variants or phenotypic polymorphism are common, especially in relation to variables within the subfamily Galerucinae (Crownson, 1981; Helen & Geoff, 2005; Chaboo, 2007; Prado, 2013; Petitpeirre, 2014; Gomez-Zurita et al., 2016). Consequently, DNA barcoding has been added to the taxonomist’s toolkit in order to complement the species identifications that are based on morphological characters (Hebert et al., 2003; Pentinsaari, Hebert & Mutanen, 2014; Gomez-Zurita et al., 2016). To date, there are 73 records of leaf beetles with 15 Barcode Index Numbers (BINs) registered in the Barcoding of Life Data system (BOLD), but none of these records are from Sabah or Borneo.

For all the reasons stated above, this study (1) documented the species richness of leaf beetles from 13 selected islands on the west coast of Sabah, and (2) sequenced DNA barcodes of the leaf beetles to provide phenotypic polymorphism information and baseline DNA barcoding knowledge for future taxonomy study.
Materials and methods

Leaf beetle sampling and processing

Standard plot sampling was carried out between January 2016 and March 2017 on the thirteen islands along the west coast of Sabah (Fig. 1; Table 1) under research permit from Sabah Park (TTS/IP/100-6/2 Jld.4 (49)) and permission from Sapangar Naval Base (MWL2.100-2/2/3-(9)). In each 20 m x 20 m plot, 200 sweeps of shrubs and herbaceous vegetation were conducted using an entomological sweep net (Sánchez-Reyes, Niño-Maldonado & Jones, 2014). This was followed by a manual hand-picking search for leaf beetles over two person-hours. Leaf beetles from each plot were kept in separate Falcon Tubes and brought to the laboratory for further processing. It should be noted that specimens from outside the plots were also collected.

Leaf beetle specimens collected were first killed using 70 % ethanol before being sorted into morphological species under the microscope. All morpho-species were identified to genus and species level by the second author based on morphological characteristics. After that, a few representative specimens of each morpho-species were selected and kept in absolute non-denatured ethanol under – 20 °C for further DNA analysis. Photographs for dorsal and ventral habitus were taken for each morpho-species using a Leica Stereoscope M165C acquired with Leica DFC495 camera and Leica Application Suite ver.4.4.0. All the specimens were deposited in the BORNEENSIS collection at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. Specimen information was catalogued in the BORNEENSIS collection database under the accession number BOR/COL ####.

DNA extraction, PCR amplification, and Sequencing

DNA was extracted from one to three whole legs of the leaf beetles using Qiagen DNeasy Blood and Tissue Kits, following the manufacturer’s protocols. After that, all the DNA extracts were stored under – 20 °C prior to PCR amplification.

The mitochondrial gene region, cytochrome c oxidase subunit I (COI) was PCR-amplified using universal primer LCO 1490 and HCO 2198 (Folmer et al., 1994). The 25 µl PCR reaction mixtures contained 2.5 µl of 10 X GoTaq® PCR buffer with 15 mM MgCl₂, 1.5 µl of 25 mM MgCl₂, 0.5 µl of 2.5 mM dNTP mix, 0.5 µl of 10 pmol each primer, 0.25 µl of 5 u/µl Taq DNA polymerase, 1 µl DNA template and 18.25 µl ddH₂O. PCR amplification was performed in Bio-Rad T100 Thermal Cycler following thermal cycling, an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 47 °C for 45 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 5 min. PCR products were checked for successful amplicon using the 1 % agarose gel with TBE buffer. Successful PCR amplicons were sent to Genomics BioScience and Technology Co., Ltd. (Taipei, Taiwan) for sequencing.

Data analysis
Sequences were checked visually with Bioedit v7.1.9 (Hall, 1999). All the complete sequences were uploaded, registered and managed in the Barcoding of Life Data System, BOLD (Ratnasingham & Hebert, 2007) together with the information about taxonomy and collection, voucher deposition details, original sequence trace files and photographs of the specimens. Each sequence was assigned the Barcode Index Number (BIN) in BOLD (Ratnasingham & Hebert, 2013). Barcode Gap Analysis and distance summary for intraspecific and interspecific distance base on Kimura 2-parameter (K2P) distance model (Kimura, 1980) were performed in BOLD. The nucleotide contents of the sequences are shown in Table 2.

In addition, all sequences were compared to the records in the National Center for Biotechnology Information GenBank using the Basic Local Alignment Search Tool, BLAST (Altschul et al., 1990) in Geneious free trial v11.0.3 (Kearse et al., 2012) to search for similar DNA sequences in the database and to obtain tentative taxa identities. Resulting BLAST top-hits for all the sequences are shown in Table 3.

DNA barcodes
The barcode index numbers (BIN) for each specimen were listed. The intraspecific and interspecific distances of the species were generated using the sequence analysis in BOLD. For intraspecific distance, only species with more than one individual sequence were shown and for interspecific distance, only two or more species under the same genus were shown in the checklist. The “Mean” represents the mean distance, “Max” represents the maximum distance, and abbreviation “N/A” represents data that are not available.

Species checklist
This checklist is comprised of information about the generic diagnosis of the genus: examined materials in BORNEENSIS, species distribution in west coast islands of Sabah, DNA barcode and general remarks on the species (or morphospecies, e.g. Galerucinae sp.). Taxonomy classification of the species in this checklist followed the modified Seeno & Wilcox (1982) system’s as proposed in Bouchard et al. (2011). Morphospecies that could not be identified to genus level were named after the subfamily (e.g. Galerucinae sp.). Photos of dorsal and ventral habitus for each of the species were included.

The generic diagnosis were done using literatures of the original description of the genus, other studies mentioning generic diagnosis characters, or the identification key to the genus level (Baly, 1860, 1865; Clark, 1865; Fairmaire, 1881; Jacoby, 1884, 1908; Lefevre, 1884; Sharp, 1904; Maulik, 1919, 1926, 1936; Laboissiere, 1933; Gressitt & Kimoto, 1963; Samuelson, 1969; Gressitt, 1969; Kimoto & Gressitt, 1979, 1981, 1982; Kimoto, 1989; Mohamedsaid, 1994; Medvedev, 2009; Hazmi & Wagner, 2010; Blanco & Konstantinov, 2013; Borowiec, Takizawa & Swietojanska, 2013; Reid & Beatson, 2015). Then, the distribution of each species on the sampled islands was summarised. Additional information on each of the species such as morphological characteristics, comparison with other species, sampled habitat, phenotypic variation, and possible host plants were provided as remarks.
Results

Species Diversity of Leaf Beetle

A total of 1104 leaf beetle specimens were collected in this study, including 68 species in 33 genera and five subfamilies, with subfamily Galerucinae having the highest number of genera and species recorded (17 genera, 42 species), followed by subfamily Eumolpinae (nine genera, 16 species), subfamily Cassidinae (four genera, seven species), subfamily Chrysomelinae (two genera, two species), and subfamily Criocerinae (one genus, one species). Of all the genera collected, genus Monolepta was the most speciose with 18 species collected, followed by genus Hoplosaenidea with seven species and genus Basilepta with six species. Besides that, in terms of the islands, Pulau Gaya has the highest number of species recorded (42 species), followed by Pulau Tiga (22 species), Pulau Sapangar (18 species), Pulau Dinawan and Pulau Sapi (nine species), Pulau Mantukod and Pulau Manukan (eight species), Pulau Mengalum (seven species), Pulau Mamutik and Pulau Udar Besar (six species), Pulau Udar Kecil (five species), Pulau Peduk (four species), and Pulau Sulug (two species).

DNA barcoding

Whereas DNA from a total of 68 leaf beetle species was extracted, only 60 of these were successfully sequenced resulting in 100 barcode compliant sequences and one non-barcode compliant sequence. These 101 sequences were uploaded and assigned to 64 BINs in BOLD (available at: dx.doi.org/10.5883/DS-BCHRY18). Details of the sequenced leaf beetle species, number of specimens, and respective BINs are listed in the Supplementary file, Table S1. A neighbour-joining tree was constructed based on these 101 sequences via BOLD (Fig. 2), to shows the relationship between these sequences.

From the sequence nucleotide composition analysis in BOLD, the average percentage of all the sequences G, C, A, and T were 16.38 % (14.58 – 18.13 %), 17.19 % (13.23 – 24.02 %), 29.86 % (27.11 – 33.13 %) and 36.57 % (30.66 – 40.99 %), respectively (See Table 2). The overall mean AT content of the 101 sequences was 66.43% (57.85 –71.66 %) and strongly AT biased at the third codon position with mean AT content of 85.09 % (63.64 – 96.51%). Intraspecific and interspecific K2P distances were easily distinguishable from each other, with overall means 0.77 % (range 0 - 1.99 %) and 16.11 % (range 4.71 – 24.6 %), respectively. Further details of the intraspecific and interspecific distances are available in the Supplement File Table S2 and Table S3.

All the 101 sequences submitted to GenBank through BLAST to search for identical results and the top-hit results was shown in Table 3. The pairwise identity percentage of the 101 sequences with records in GenBank ranges from 82.6 % - 100 %. These BLAST top-hits results were summarized and grouped into two categories (≥ 90 % and < 90%) based on their pairwise identity percentages, as shown in Table 4. Of the 101 sequences, 21 sequences (15 species) match existing sequences in Genbank with pairwise identity percentage higher than 90 % whereas 80 sequences (47 species) with pairwise identity percentages below 90 %. However,
those 21 with high pairwise identity percentages can only be identified into subfamily (three
records) or genus (nine records) based on the taxonomic information available for the records in
the Genbank. For the remaining 80 sequences, two records can be identified to family level and
29 records identified to subfamily level.

Discussion

Species Diversity of Leaf Beetle

The dominant leaf beetle on these islands are species of subfamily Galerucinae (excluding Tribe
Alticini) and subfamily Eumolpinae which is accordant to the general trend throughout the
Oriental region (Kimoto, 1988). It seems that leaf beetle species richness is greater on islands
situated closer to the mainland and with a larger land area.

Although this study sampled only 13 islands out of the 500 islands (~3 %) from Sabah,
the checklist documented 68 species, comprising 9 % of the current known leaf beetle species in
Borneo. This suggests that species richness on the islands is comparable to that on mainland
habitats and that more species remain to be discovered. In addition, this checklist also reveals the
distribution of agricultural pest species on the islands, which is vital for the control of their
dispersal. For example, Brontispa longissima, one of the coconuts pests in the Pacific region, is
commonly found on the sampled islands noted for human habitation, tourist activities and
resorts.

DNA barcoding

Out of the 64 generated BINs, 60 unique BINs are new to BOLD and 4 non-unique BINs
are existing records in BOLD. As compared to the existing 73 records and 15 BINs of
Chrysomelidae from Malaysia in BOLD, all the 101 sequences and 64 BINs from this study are
new to Malaysia. Through the Barcode Gap Analysis and distance summary analysis in BOLD,
six sequenced Nodina sp. specimens were revealed to be five different species. However, these
cfive species are morphologically hard to distinguish from one another and thus, all these five
species are collectively treated as a single species (Kishimoto-Yamada, Takizawa &
Mahadimenakbar, 2016) and excluded from the overall mean intraspecific and interspecific
distance analysis.

Barcode Gap Analysis in BOLD also revealed those leaf beetle species with phenotypic
variation through the distribution of distances within each species and the distance to the nearest
neighbour of each species (Puillandre et al., 2012). All the leaf beetle species that exhibited
phenotypic variation in the study are from the subfamily Galerucinae, which corroborates
previous reports (Maulik, 1936; Beenen, 2007; Prado, 2013). Referring to the neighbour joining
tree (see Fig. 2), leaf beetles with phenotypic variation (highlighted in red colour) are genus
indet. nr. Monolepta (Figs. 8G, 8H, 9A), Hoplosaenidea sp. 5 (Figs. 5A – 5B), Monolepta sp. 5
(Figs. 6A – 6D), and Monolepta sp. 14 (Figs. 7E – 7F) with mean intraspecific distances of 0.2
%, 0 %, 0.96 %, and 0.41 %, respectively. The Hoplosaenidea sp. 5 and Monolepta sp. 14 are
possibly sexual dimorphics, and genus indet. nr. Monolepta and Monolepta sp. 5 are possibly
colour polymorphics, yet, further investigations are needed before conclusions can be made. The phenotypic variation for both sexual dimorphism and colour polymorphism in leaf beetles is possibly caused by genetics, environment or a combination of both factors (Jolivet, Petitpierre & Hsiao, 1988).

Nonetheless, 97 % of the sequences obtained from this study are new to GenBank. On top of that, out of the 21 analysed sequences with pairwise identity percentage higher than 90 %, only five sequences were identified to species level in BLAST top-hits results. These are Brontispa longissima, Altica birmanensis, and Altica engstroemi with pairwise identity percentages of 100 %, 99.2 %, and 99.0 %, respectively (see Table 3). However, both Altica birmanensis and Altica engstroemi were previously not recorded in Borneo and the latter species’ known distribution was only from northern Europe (Mohamedsaid, 2004; Reid & Beatson, 2015; GBIF, 2017). This has become complicated by the fact that the pairwise identity percentage of these two species sequences in GenBank is 99.5 %, suggesting that they should be the same species, and that they were possibly misidentified as the locality of both record specimens were from Karala, India. This conforms with previous reports on the poor quality of taxonomic identifications in GenBank (Bridge et al., 2003; Vilgalys, 2003; James Harris, 2003; Kristiansen et al., 2005).

SPECIES CHECKLIST

SUBFAMILY GALERUCINAE

Tribe ALTICINI Newman 1835

Genus Altica Geoffroy, 1762.

Refer to Appendix A, page 1 for the generic diagnosis of this genus.

Altica aenea (Olivier, 1808)


Distribution in Sabah. Pulau Tiga, Pulau Gaya.

Barcode Index Number (BIN). BOLD:AAP8616

Intra-specific distance (%). Mean: 0 Max: 0

Remarks. BLAST top-hit result shows 99 % similarity with Altica bermanensis and Altica engstroemi. However, both species not recorded in Sabah (Mohamedsaid, 2004; Reid & Beatson, 2015). So, the records in GenBank probably misidentified.

Genus Aphthona Chevrolat, 1837.

Refer to Appendix A, page 1 for the generic diagnosis of this genus.

Aphthona sp.
Examined materials (1). *Pulau Mamutik*: BOR/COL 9602.

Distribution in Sabah. Pulau Mamutik.

Barcode Index Number (BIN). BOLD:ADH3773

Intraspecific distance (%). N/A

Remarks. Only found in Pulau Mamutik.

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Genus *Argopistes* Motschulsky, 1860.

Refer to Appendix A, page 1 for the generic diagnosis of this genus.

*Argopistes* sp. 1


Barcode Index Number (BIN). BOLD:ADH5650

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.38 Max: 15.38

Remarks. Differentiate from *Argopistes* sp. 2 by black dorsum and yellow venter.

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*Argopistes* sp. 2

Examined materials (2). *Pulau Mamutik*: BOR/COL 9608 – 9609.

Distribution in Sabah. Pulau Mamutik.

Barcode Index Number (BIN). BOLD:ADH5651

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.38 Max: 15.38


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Genus *Erystus* Jacoby, 1885.

Refer to Appendix A, page 1 & 2 for the generic diagnosis of this genus.

*Erystus villicus* (Weise, 1892)

Examined materials (37). *Pulau Gaya*: BOR/COL 8134 – 8141, BOR/COL 9332 – 9334, BOR/COL 9394 – 9395, BOR/COL 9400 – 9416.

Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). BOLD:ADH6322

Intraspecific distance (%). N/A

Remarks. Usually found on *Hibiscus tiliaceus* near the beach with a great number of individuals. Heavily defoliate the host plant.

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Genus *Hemipyxis* Chevrolat, 1836.

Refer to Appendix A, page 2 for the generic diagnosis of this genus.

*Hemipyxis* sp.

(Fig. 3F)
Examined materials (10). *Pulau Gaya*: BOR/COL 8187, BOR/COL 8213, BOR/COL 8236, BOR/COL 8325 – 8326, BOR/COL 9397, BOR/COL 9814 – 9815, BOR/COL 9924, BOR/COL 9961.

**Distribution in Sabah.** Pulau Gaya.

**Barcode Index Number (BIN).** N/A

**Intraspecific distance (%).** N/A

**Remarks.** Only collected from Pulau Gaya. Body is yellow in colour.

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Genus *Hyphasis* Harold, 1877

Refer to Appendix A, page 2 for the generic diagnosis of this genus.

*Hyphasis* sp.

(Fig. 3G)

Examined materials (1). *Pulau Dinawan*: BOR/COL 8449.

**Distribution in Sabah.** Pulau Dinawan.

**Barcode Index Number (BIN).** BOLD:ADH5610

**Intraspecific distance (%).** N/A

**Remarks.** Only found in Pulau Dinawan, near to deforested area.

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Genus *Lanka* Maulik, 1926.

Refer to Appendix A, page 2 & 3 for the generic diagnosis of this genus.

*Lanka* sp.

(Fig. 3H)

Examined materials (1). *Pulau Gaya*: BOR/COL 8097.

**Distribution in Sabah.** Pulau Gaya.

**Barcode Index Number (BIN).** BOLD:ADH7255

**Intraspecific distance (%).** N/A

**Remarks.** Collected from a plant near to the river in Pulau Gaya.

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Genus *Schenklingia* Csiki & Heikertinger, 1940

Refer to Appendix A, page 3 for the generic diagnosis of this genus.

*Schenklingia* sp.

(Fig. 4A)

Examined materials (1). *Pulau Gaya*: BOR/COL 9429.

**Distribution in Sabah.** Pulau Gaya.

**Barcode Index Number (BIN).** BOLD:ADH3903

**Intraspecific distance (%).** N/A

**Remarks.** Body colour dark red, first three and 11th antennal segment orange-brown colour and remaining antennal segments black in colour.

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Tribe LUPERINI Leng 1920

Subtribe AULACOPHORINA Wilcox 1972

Section Aulacophorites Chapius 1875

Genus *Aulacophora* Dejean, 1835
Refer to Appendix A, page 3 for the generic diagnosis of this genus.

*Subtribe LUPERINA Wilcox 1965

**Section Doryscites Wilcox 1973**

**Genus Strobiderus Jacoby, 1884**

Refer to Appendix A, page 3 for the generic diagnosis of this genus.

*Strobiderus sp.*

(Fig. 4C)

Examined materials (7). *Pulau Gaya*: BOR/COL 8103, BOR/COL 8184, BOR/COL 8321, BOR/COL 8331, BOR/COL 9462 – 9464.

Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). BOLD:ADH4212

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 14.84 Max: 22.94

Remarks. Found on the plants near river area in Pulau Gaya.

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*Subtribe LUPERINA Wilcox 1965

**Section Luperites Chapius 1875**

**Genus Hoplosaenidea Laboissiere, 1933.**

Refer to Appendix A, page 3 & 4 for the generic diagnosis of this genus.

*Hoplosaenidea malayensis* (Jacoby, 1884)

(Fig. 4D)


Barcode Index Number (BIN). BOLD:ADH4031

Intraspecific distance (%). Mean: 0.1 Max: 0.15

Interspecific distance (%). Mean: 17.70 Max: 22.35

Remarks. Whole body yellow in colour, usually found in few of individuals on a single plant.

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*Hoplosaenidea sp. 1*

(Fig. 4E)

Examined materials (1). *Pulau Tiga*: BOR/COL 7000.

Distribution in Sabah. Pulau Tiga.

Barcode Index Number (BIN). BOLD:ADH3897

Intraspecific distance (%). N/A
Interspecific distance (%). Mean: 17.70  Max: 22.35

Remarks. Body completely creamy white in colour.

Hoplosaenidea sp. 2
(Fig. 4F)
Distribution in Sabah. Pulau Tiga, Pulau Gaya.
Barcode Index Number (BIN). BOLD:ADH4030
Intraspecific distance (%). N/A
Interspecific distance (%). Mean: 17.70  Max: 22.35
Remarks. Whole body banana yellow in colour, and elytra with two longitudinally black stripes.

Hoplosaenidea sp. 3
(Fig. 4G)
Examined materials (1). Pulau Gaya: BOR/COL 8268.
Distribution in Sabah. Pulau Gaya.
Barcode Index Number (BIN). N/A
Intraspecific distance (%). N/A
Remarks. Whole body red-orange in colour.

Hoplosaenidea sp. 4
(Fig. 4H)
Examined materials (1). Pulau Gaya: BOR/COL 8095.
Distribution in Sabah. Pulau Gaya.
Barcode Index Number (BIN). BOLD:ADH4029
Intraspecific distance (%). N/A
Interspecific distance (%). Mean: 17.70  Max: 22.35
Remarks. Similar to Hoplosaenidea sp. 6, different by thorax and elytra colouration, and the 9th antennae segment on basal half white and on apical half black.

Hoplosaenidea sp. 5
(Figs. 5A – 5B)
Examined materials (2). Pulau Mantukod: BOR/COL 9720 – 9721.
Distribution in Sabah. Pulau Mantukod.
Barcode Index Number (BIN). BOLD:ADH4033
Intraspecific distance (%). Mean: 0  Max: 0
Interspecific distance (%). Mean: 17.70  Max: 22.35
Remarks. Possible sexual dimorphism, with difference in size and body colour.

Hoplosaenidea variabilis (Jacoby, 1894)
(Fig. 5C)
Examined materials (1). Pulau Udar Besar: BOR/COL 9638.
Distribution in Sabah. Pulau Udar Besar.
Barcode Index Number (BIN). BOLD:ADH4032
Intraspecific distance (%). N/A
Interspecific distance (%). Mean: 17.70  Max: 22.35
Remarks. Head and thorax maroon red colour, and elytra with metallic bluish-green colour.

Section MONOLEPTITES Chapius 1875

Genus *Metrioidea* Fairmaire, 1881.

Refer to Appendix A, page 4 for the generic diagnosis of this genus.

*Metrioidea grandis* (Allard, 1889)

(Fig. 5D)


Distribution in Sabah. Pulau Gaya, Pulau Sapangar.

Barcode Index Number (BIN). BOLD: ADH7177

Intraspecific distance (%). Mean: 1.99 Max: 1.99


Genus *Monolepta* Erichson, 1843

Refer to Appendix A, page 4 for the generic diagnosis of this genus.

*Monolepta* sp. 1

(Fig. 5D)


Barcode Index Number (BIN). BOLD: ADH4138

Intraspecific distance (%). Mean: 0.92 Max: 0.92

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Whole body yellow in colour with the brown or orange tibia.

*Monolepta* sp. 2

(Fig. 5F)


Distribution in Sabah. Pulau Gaya, Pulau Tiga.

Barcode Index Number (BIN). BOLD: ADH7139

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60
Remarks. Body length around 2 – 3 mm. Black colour elytra with two distinct white bands. Last ventrite segment black.

**Monolepta sp. 3**

(Fig. 5G)


Distribution in Sabah. Pulau Gaya, Pulau Tiga.

Barcode Index Number (BIN). BOLD:ADH4196

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. This species especially abundant during the flowering season, with deep red colour head, thorax and abdomen, and black colour elytra, last antennae segment black in colour.

**Monolepta sp. 4**

(Fig. 5H)


Barcode Index Number (BIN). BOLD:ADH6840

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Heavily defoliate *Citrus* sp., *Mangifera* sp. and *Sauropus androgynous* plants young shoots.

**Monolepta sp. 5**

(Figs. 6A-6D)


Barcode Index Number (BIN). BOLD:ADH4050

Intraspecific distance (%). Mean: 0.96 Max: 1.69

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. This species exhibit phenotypic polymorphism, with four different phenotypic characters, one fully milky white in colour, one with suture and elytra edge black in colour, one elytra with two dark brown bands separated by light brown bands, and one elytra with two dark brown bands interconnected by dark brown suture but separated by two light brown bands.
**Monolepta sp. 6**
(Fig. 6E)

Examined materials (1). *Pulau Tiga*: BOR/COL 8531.

Distribution in Sabah. *Pulau Tiga*.

Barcode Index Number (BIN). BOLD: ADH6249

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Found resting on the beach *Ipomoea* species. Head and elytra deep red in colour with thorax creamy white in colour.

**Monolepta sp. 7**
(Fig. 6F)

Examined materials (6). *Pulau Sapangar*: BOR/COL 8426, BOR/COL 8437 – 8439, BOR/COL 9677, BOR/COL 9717.

Distribution in Sabah. *Pulau Sapangar*.

Barcode Index Number (BIN). BOLD: ADH4051

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Usually found on the highest point in *Pulau Sapangar*.

**Monolepta sp. 8**
(Fig. 6G)

Examined materials (22). *Pulau Gaya*: BOR/COL 8314, BOR/COL 9299 – 9301, BOR/COL 9824, BOR/COL 9826 – 9835, BOR/COL 9841, BOR/COL 9939 – 9944.

Distribution in Sabah. *Pulau Gaya*.

Barcode Index Number (BIN). BOLD: ADH7150

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Only collected from *Pulau Gaya*, light yellow in colour.

**Monolepta sp. 9**
(Fig. 6H)


Distribution in Sabah. *Pulau Gaya*, *Pulau Tiga*, *Pulau Sapangar*.

Barcode Index Number (BIN). BOLD: ADH7149

Intraspecific distance (%). Mean: 1.32 Max: 1.83

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Black colour head with milky white colour thorax and black colour elytra with a white band in the middle of the elytra.

**Monolepta sp. 10**
(Fig. 7A)

Distribution in Sabah. Pulau Gaya, Pulau Mantukod.

Barcode Index Number (BIN). BOLD:ADH7148

Intraspecific distance (%). Mean: 0 Max: 0

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Orange colour head and thorax, semi-transparent elytra with light green abdomen.

**Monolepta** sp. 11

(Fig. 7B)

Examined materials (1). *Pulau Gaya*: BOR/COL 8119.

Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). BOLD:ADH7140

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Similar to *Monolepta* sp. 18, with the difference on the elytra patterns.

**Monolepta** sp. 12

(Fig. 7C)

Examined materials (1). *Pulau Tiga*: BOR/COL 9201.

Distribution in Sabah. Pulau Tiga.

Barcode Index Number (BIN). BOLD:ADH4195

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Collected from random sweeping along the Pagong-Pagong trail in Pulau Tiga.

**Monolepta** sp. 13

(Fig. 7D)

Examined materials (1). *Pulau Sapangar*: BOR/COL 9678.

Distribution in Sabah. Pulau Sapangar.

Barcode Index Number (BIN). N/A

Intraspecific distance (%). N/A

Remarks. Body length 2-3mm. Only collected from Pulau Sapangar.

**Monolepta** sp. 14

(Figs. 7E-7F)


Distribution in Sabah. Pulau Sapi, Pulau Gaya, Pulau Manukan, Pulau Tiga.

Barcode Index Number (BIN). BOLD:ADH4966

Intraspecific distance (%). Mean: 0.41 Max: 0.62

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Possible exhibit sexual dimorphism.

**Monolepta** sp. 15

(Fig. 7G)


Barcode Index Number (BIN). BOLD:ADH4198

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Black colour head with the yellow thorax, black elytra with one yellow band in the middle.

Monolepta sp. 16

(Fig. 7H)

Examined materials (3). Pulau Gaya: BOR/COL 9424, BOR/COL 9445, BOR/COL 9958.

Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). N/A

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Whole body brown in colour, only found in Pulau Gaya.

Monolepta sp. 17

(Fig. 8A)

Examined materials (1). Pulau Gaya: BOR/COL 9449.

Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). BOLD:ADH7141

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Whole body white in colour, meso- and metasternum light brown in colour.

Monolepta sp. 18

(Fig. 8B)

Examined materials (1). Pulau Sapangar: BOR/COL 9679.

Distribution in Sabah. Pulau Sapangar.

Barcode Index Number (BIN). BOLD:ADH4197

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 15.88 Max: 24.60

Remarks. Differentiate from Monolepta sp. 11 by the dark colour patterns on the elytra.

Genus Ochralea Clark, 1865

Refer to Appendix A, page 4 for the generic diagnosis of this genus.

Ochralea nigripes (Olivier, 1808)

(Fig. 8C)

Tribe GALERUCINI Laboissiere 1921

Genus Clitena Baly, 1864.

Refer to Appendix A, page 4 & 5 for the generic diagnosis of this genus.

Clitena sp.

Examined materials (2). Pulau Manukan: BOR/COL 8399, BOR/COL 9580.

Distribution in Sabah. Pulau Manukan.

Barcode Index Number (BIN). BOLD:ADH4213

Intraspecific distance (%). Mean: 0.7 Max: 1.06

Remarks. 8 -10 mm body length, with colour variations of yellow and yellow-orange body colour. Very abundant especially in Pulau Gaya and Pulau Tiga. Few individuals collected in between leaf litters and twigs from the ground.

Tribe METACYCLINI Leng 1920

Genus Sumatrasia Jacoby, 1884

Refer to Appendix A, page 5 for the generic diagnosis of this genus.

Sumatrasia sp.

Examined materials (1). Pulau Sapi: BOR/COL 6938.

Distribution in Sabah. Pulau Sapi.

Barcode Index Number (BIN). BOLD:ADH4430

Intraspecific distance (%). N/A

Remarks. Whole body yellow in colour. Collected along the trail in Pulau Sapi.

Tribe SERMYLINI Wilcox 1965


Refer to Appendix A, page 5 for the generic diagnosis of this genus.

Dercetina sp.

(Fig. 8F)
Examined materials (5). *Pulau Gaya*: BOR/COL 8150. *Pulau Sapangar*: BOR/COL 8428, BOR/COL 8434, BOR/COL 9671, BOR/COL 9713. 

**Distribution in Sabah.** Pulau Gaya, Pulau Sapangar.

**Barcode Index Number (BIN).** BOLD:ADH3896

**Intraspecific distance (%).** Mean: 0.3 Max: 0.3

**Remarks.** Body divided into two colour: head, thorax and the basal half of elytra red colour, and apical half black. Last ventrite visible from dorsal.

Genus indet. nr. *Monolepta*  
(Figs. 8G–8H, 9A)


**Distribution in Sabah.** Pulau Gaya, Pulau Peduk.

**Barcode Index Number (BIN).** BOLD:ADH3996

**Intraspecific distance (%).** Mean: 0.2 Max: 0.3

**Remarks.** Possibly exhibit phenotypic polymorphism with three different patterns and colourations on the elytra. These three patterns also observed at UMS hill based on second author collection.

**SUBFAMILY EUMOLPINAE**

**Tribe ADOXINI Jacoby 1908**

**Section Scelodontites Chapius 1874**

Genus *Scelodonta* Westwood, 1837.  
Refer to Appendix A, page 5 for the generic diagnosis of this genus.

*Scelodonta granulosa* Baly, 1867  
(Fig. 9B)


**Distribution in Sabah.** Pulau Mengalum, Pulau Sapangar.

**Barcode Index Number (BIN).** BOLD:ADE7488

**Intraspecific distance (%).** N/A

**Remarks.** Iridescent body colour with the red colour tibia.

**Tribe COLASPOSOMINI Springlova 1960**

**Section Colasposomites Wilcox 1982**

Genus *Colasposoma* Laporte, 1833.  
Refer to Appendix A, page 6 for the generic diagnosis of this genus.

*Colasposoma auripenne* Motschulsky, 1860  
(Fig. 9C)

Examined materials (2). *Pulau Dinawan*: BOR/COL 9753 – 9754. 

**Distribution in Sabah.** Pulau Dinawan.

**Barcode Index Number (BIN).** BOLD:ADH6210
Intraspecific distance (%). N/A

Remarks. This species was found on the cultivated sweet potatoes plant, *Ipomoea batatas*.

**Tribe EUMOLPINI Jacoby 1908**

**Section Endocephalites Chapius 1874**

**Genus Aulacia Baly, 1867.**
Refer to Appendix A, page 6 for the generic diagnosis of this genus.
*Aulacia* sp.
(Fig. 9D)

Examined materials (2). *Pulau Tiga*: BOR/COL 9154, BOR/COL 9200.
Distribution in Sabah. Pulau Tiga.
Barcode Index Number (BIN). N/A
Intraspecific distance (%). N/A

**Genus Colaspoides Laporte, 1833.**
Refer to Appendix A, page 6 for the generic diagnosis of this genus.
*Colaspoides* sp. 1
(Fig. 9E)

Distribution in Sabah. Pulau Tiga, Pulau Gaya, Pulau Sapangar.
Barcode Index Number (BIN). BOLD:ADH4442
Intraspecific distance (%). N/A
Interspecific distance (%). Mean: 23.03 Max: 23.03
Remarks. 1st to 8th antennae segments yellow-brown, 9th to 11th antennae segments black, dorsum and leg yellow-brown.

*Colaspoides tuberculata* Baly, 1867
(Fig. 9F)

Examined materials (1). *Pulau Gaya*: BOR/COL 9858.
Distribution in Sabah. Pulau Gaya.
Barcode Index Number (BIN). BOLD:ADH4443
Intraspecific distance (%). N/A
Interspecific distance (%). Mean: 23.03 Max: 23.03
Remarks. Antennae black, body colour iridescent colour.

**Tribe NODININI Chen 1940**

**Section Nodostomini Jacoby 1908**

**Genus Basilepta Baly, 1860**
Refer to Appendix A, page 6 for the generic diagnosis of this genus.
*Basilepta* sp. 1

Distribution in Sabah. Pulau Gaya, Pulau Mantukod.

Barcode Index Number (BIN). BOLD:ADH5567

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 18.02 Max: 21.63

Remarks. Pronotum strongly punctate, body dark brown in colour.

*Basilepta* sp. 2

(Fig. 9H)


Distribution in Sabah. Pulau Gaya, Pulau Sapangar.

Barcode Index Number (BIN). BOLD:ADH5568

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 18.02 Max: 21.63

Remarks. Thorax strongly punctate, elytra weakly punctate than pronotum.

*Basilepta* sp. 3

(Fig. 10A)

Examined materials (6). *Pulau Tiga*: BOR/COL 9158, BOR/COL 9170, BOR/COL 9187, BOR/COL 9757, BOR/COL 9769. *Pulau Sapangar*: BOR/COL 9714.

Distribution in Sabah. Pulau Tiga, Pulau Sapangar.

Barcode Index Number (BIN). BOLD:ADI3390

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 18.02 Max: 21.63

Remarks. Pronotum impunctate, elytra not strongly punctate.

*Basilepta* sp. 4

(Fig. 10B)

Examined materials (10). *Pulau Tiga*: BOR/COL 8064 – 8065, BOR/COL 9758 – 9765.

Distribution in Sabah. Pulau Tiga.

Barcode Index Number (BIN). BOLD:ADH4287

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 18.02 Max: 21.63


*Basilepta* sp. 5

(Fig. 10C)


Distribution in Sabah. Pulau Gaya, Pulau Sapangar.

Barcode Index Number (BIN). N/A
Genus *Nodina* Motschulsky, 1858.

Refer to Appendix A, page 7 for the generic diagnosis of this genus.

*Nodina* sp.

(Figs. 10E – 10H, 11A)


**Distribution in Sabah.** Pulau Tiga, Pulau Gaya, Pulau Sapi, Pulau Manukan, Pulau Sapangar, Pulau Dinawan, Pulau Mantukod.

**Barcode Index Number (BIN).** BOLD:ADI2797, BOLD:ADI2798, BOLD:ADI3779, BOLD:ADI3780, BOLD:ADI3781

**Intraspecific distance (%).** Excluded

**Remarks.** These species are so closely similar on outer morphological traits that we refrain from sorting them into morphological species at present. Six random individuals selected for sequencing results in five different species.

Section Pagriites Lefevre 1885

**Genus Pagria Lefevre, 1884.**

Refer to Appendix A, page 7 for the generic diagnosis of this genus.

*Pagria* sp.

(Fig. 11B)

**Examined materials (1).** Pulau Gaya: BOR/COL 9479.
Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). BOLD:ACW8270

Intraspecific distance (%). N/A

Remarks. Head and thorax black in colour and elytra brown in colour.

Section Metachromites Chapius 1874

Genus Rhyparida Baly, 1861.

Refer to Appendix A, page 7 for the generic diagnosis of this genus.

Rhyparida sp. 1


Barcode Index Number (BIN). BOLD:ADH5562

Intraspecific distance (%). Mean: 0.91 Max: 1.53

Interspecific distance (%). Mean: 16.91 Max: 17.79

Remarks. Anterior femora with or without weak spine.

Rhyparida sp. 2


Barcode Index Number (BIN). BOLD:ADH5563

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 16.91 Max: 17.79

Remarks. Anterior femora with well-developed spine on inner margin.

Section Typophorites Chapius 1874

Genus Cleorina Lefevre, 1885.
Refer to Appendix A, page 7 for the generic diagnosis of this genus.

**Cleorina malayana** (Jacoby, 1896)


Distribution in Sabah. Pulau Manukan, Pulau Sulug.

Barcode Index Number (BIN). BOLD: ADH5352

Intraspecific distance (%). N/A

Remarks. Found feeding on the family *Zingiberaceae* plants.

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**SUBFAMILY CASSIDINAE**

**Tribe CRYPTONYCHINI** Weise 1911

**Genus Brontispa** Sharp, 1904.

Refer to Appendix A, page 7 for the generic diagnosis of this genus.

**Brontispa longissima** (Gestro, 1885)


Distribution in Sabah. Pulau Tiga, Pulau Manukan, Pulau Dinawan, Pulau Mengalum, Pulau Mamutik.

Barcode Index Number (BIN). BOLD: AAL7691

Intraspecific distance (%). Mean: 0.10 Max: 0.15

Remarks. Only found on the islands with tourism activities and/or resorts.

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**Tribe GONOPHORINI** Weise 1911

**Genus Gonophora** Baly, 1858.

Refer to Appendix A, page 8 for the generic diagnosis of this genus.

**Gonophora sp.**

Examined materials (21). Pulau Tiga: BOR/ COL 8016, BOR/ COL 8519, BOR/ COL 8532 – 8533, BOR/ COL 9169, BOR/ COL 9207 – 9209, BOR/ COL 9770, BOR/ COL 9803 – 9804.


Distribution in Sabah. Pulau Tiga, Pulau Gaya, Pulau Sapi, Pulau Sapangar.

Barcode Index Number (BIN). BOLD: ADH6672

Intraspecific distance (%). Mean: 0.69 Max: 1.38

Remarks. Usually found on the leaf surface of *Oncosperma tigillarium*. 

---
Tribe HISPINI Weise 1911

Genus *Dactylispa* Weise, 1897.

Refer to Appendix A, page 8 for the generic diagnosis of this genus.

*Dactylispa* sp. 1

(Fig. 11H)

Examined materials (1). *Pulau Tiga*: BOR/COL 9777.

Distribution in Sabah. *Pulau Tiga*.

Barcode Index Number (BIN). BOLD:ADH5880

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 21.32 Max: 21.32

Remarks. Different from *Dactylispa* sp. 2 by the spine branching on the prothorax and smaller in size.

*Dactylispa* sp. 2

(Fig. 12A)

Examined materials (1). *Pulau Gaya*: BOR/COL 8305.

Distribution in Sabah. *Pulau Gaya*.

Barcode Index Number (BIN). BOLD:ADH6349

Intraspecific distance (%). N/A

Interspecific distance (%). Mean: 21.32 Max: 21.32

Remarks. Generally bigger in size than *Dactylispa* sp. 1.

Tribe NOTOSACANTHINI Hincks 1952

Genus *Notosacantha* Chevolat, 1836.

Refer to Appendix A, page 8 for the generic diagnosis of this genus.

*Notosacantha* sp. 1

(Fig. 12B)

Examined materials (4). *Pulau Gaya*: BOR/COL 8312, BOR/COL 9446 – 9448.

Distribution in Sabah. *Pulau Gaya*.

Barcode Index Number (BIN). BOLD:ADH5640

Intraspecific distance (%). N/A

Remarks. Found on *Ardisia* sp. plant.

*Notosacantha* sp. 2

(Fig. 12C)


Distribution in Sabah. *Pulau Tiga*, *Pulau Mengalum*.

Barcode Index Number (BIN). BOLD:ADH5641

Intraspecific distance (%). N/A

Remarks. Found on *Ardisia* sp. plant.

*Hispinae* sp.
Examined materials (1). *Pulau Gaya*: BOR/COL 9417.

Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). N/A

Intraspecific distance (%). N/A

Remarks. Elytra dilated at side, regularly with four interstices of two regular rows of punctures.

SUBFAMILY CHRY SOMELINAE

Tribe CHRYSOMELINI Reitter 1912

Subtribe CHRYSOMELINA Chen 1936

Genus *Plagiodera* Chevrolat, 1837.

Refer to Appendix A, page 8 & 9 for the generic diagnosis of this genus.

*Plagiodera* sp.

Examined materials (1). *Pulau Tiga*: BOR/COL 8514.

Distribution in Sabah. Pulau Tiga.

Barcode Index Number (BIN). BOLD:ADH0536

Intraspecific distance (%). N/A

Remarks. Found on plants near southeast mud volcano of Pulau Tiga.

Subtribe PHYLLOCHARINA Weise 1915

Genus *Phola* Weise, 1890.

Refer to Appendix A, page 9 for the generic diagnosis of this genus.

*Phola sedecimpustulata* (Stal, 1857)

Examined materials (1). *Pulau Peduk*: BOR/COL 9882.

Distribution in Sabah. Pulau Peduk.

Barcode Index Number (BIN). BOLD:ADH6695

Intraspecific distance (%). N/A

Remarks. Pronotum with three spots forming a triangular shape, elytra with nine yellow spots and one of the spots at the tip of the elytra.

SUBFAMILY CRIOCERINAE

Tribe LEMIINI Heinze 1962

Genus *Lema* Fabricius, 1798.

Refer to Appendix A, page 9 for the generic diagnosis of this genus.

*Lema* sp.

Examined materials (1). *Pulau Gaya*: BOR/COL 9393.

Distribution in Sabah. Pulau Gaya.

Barcode Index Number (BIN). BOLD:ADH6230
Intraspecific distance (%). N/A

Remarks. Found after a shower rain near the Padang Point Restaurant at Pulau Gaya.

Conclusions
A total of 68 leaf beetle species was collected from 13 islands off Sabah west coast, representing leaf beetle species richness on a small portion (~ 3 %) of island habitat in Sabah and indicates that many species yet to be discovered from the island habitats. This study also provides baseline knowledge and information about the DNA barcodes of leaf beetle species on Sabah’s island habitats for use in future studies.

Acknowledgements
We thank various agencies for providing permission to access and conduct this study on the islands: Sabah Park for Tunku Abdul Rahman Park and Pulau Tiga Park (permit TTS/IP/100-6/2 Jld.4 (49)); Sapangar Navy Base (TLD) for Pulau Udar Kecil (MWL2.100-2/2/3- (9)). We are also grateful to the staff of various agencies for providing logistic support throughout the fieldwork: Justinus Guntabid, Sukur B. Sukardi, Muhammad Aliff B. Suhaimin, Simon Limbawang, Victor Siam and others (Sabah Park); Prof. Dr. Charles and ITBC staff, Assoc. Prof. Dr. Rossita Hj. Shapawi and IPMB boathouse staff (Universiti Malaysia Sabah); Marudu Express Travel Service staff (Pulau Dinawan); Mr. Balan and family (Pulau Sapangar). We appreciate assistance from Simon Kuyun, Foo She Fui, Phung Chee Chean, Phung Kin Wah, Jasrul Dulipat, and Choo Ming Huei during fieldwork. Special thanks to Foon Junn Kitt and Phung Chee Chean for manuscript checking.

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Table 1 (on next page)

Coordinate, area (km$^2$), distance from nearest mainland (km) and number of plot(s) on the 13 sampled west coast islands of Sabah.
Table 1: Coordinate, area (km$^2$), distance from nearest mainland (km) and number of plot(s) on the 13 sampled west coast islands of Sabah.

<table>
<thead>
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<th>Island name (pulau)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Area (km$^2$)</th>
<th>Distance from nearest mainland (km)</th>
<th>No. of plot(s)</th>
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<td>1. Pulau Dinawan</td>
<td>5.8472</td>
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Table 2 (on next page)

Nucleotide composition of the 101 sequences.
**Table 2:** Nucleotide composition of the 101 sequences.

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Table 3 (on next page)

BLAST top-hits result from NCBI GenBank for each sequence.
Table 3: BLAST top-hits result from NCBI GenBank for each sequence.

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Summary of BLAST top-hits result based on identical percentage.
**Table 4**: Summary of BLAST top-hits result based on identical percentage.

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<tr>
<td></td>
<td>Genus</td>
<td>11</td>
<td></td>
</tr>
<tr>
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<td>Species</td>
<td>38</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>101</strong></td>
<td><strong>60</strong></td>
</tr>
</tbody>
</table>

* Number in bracket shows the number of species shared in both identical percentage.
Figure 1 (on next page)

The 13 Sabah west coast islands selected and sampled in this study.

A Sabah overview map with the location of each island group (highlighted in red and selected in box); B Sapangar bay island group; C Tunku Abdul Rahman Park; D Pulau Dinawan and Pulau Mantukod; E Pulau Tiga; F Pulau Mengalum. Pulau = Island.
Figure 2 (on next page)

Neighbour-joining tree for all the 101 analysed COI sequences (performed on the BOLD).

Clade highlighted in red colour represents leaf beetle species with phenotypic variation.
Figure 3

Dorsal and ventral habitus of leaf beetle species.

**A** Altica aenea; **B** Aphthona sp. 1; **C** Argopistes sp. 1; **D** Argopistes sp. 2; **E** Erystus villicus; **F** Hemipyxis sp.; **G** Hyphasis sp.; **H** Lanka sp.
Figure 4

Dorsal and ventral habitus of leaf beetle species.

A Schenklingia sp.; B Aulacophora sp.; C Strobiderus sp.; D Hoplosaenidea malayensis; E Hoplosaenidea sp. 1; F Hoplosaenidea sp. 2; G Hoplosaenidea sp. 3; H Hoplosaenidea sp. 4.
Figure 5

Dorsal and ventral habitus of leaf beetle species.

A-B Hoplosaenidea sp. 5; C Hoplosaenidea variabilis; D Metrioidea grandis; E Monolepta sp. 1; F Monolepta sp. 2; G Monolepta sp. 3; H Monolepta sp. 4.
Figure 6

Dorsal and ventral habitus of leaf beetle species.

A-D *Monolepta* sp. 5; E *Monolepta* sp. 6; F *Monolepta* sp. 7; G *Monolepta* sp. 8; H *Monolepta* sp. 9.
Figure 7

Dorsal and ventral habitus of leaf beetle species.

A Monolepta sp. 10; B Monolepta sp. 11; C Monolepta sp. 12; D Monolepta sp. 13; E – F Monolepta sp. 14; G Monolepta sp. 15; H Monolepta sp. 16.
Figure 8

Dorsal and ventral habitus of leaf beetle species.

A Monolepta sp. 17; B Monolepta sp. 18; C Ochralea nigripes; D Clitena sp.; E Sumatrasia sp.; F Dercetina sp.; G-H genus indet. nr. Monolepta.
Figure 9

Dorsal and ventral habitus of leaf beetle species.

A genus indet. nr. *Monolepta*; B *Scelodonta* sp.; C *Colasposoma auripenne*; D *Aulacia* sp.; E *Colaspoides* sp. 1; F *Colaspoides tuberculata*; G *Basilepta* sp. 1; H *Basilepta* sp. 2.
Figure 10

Dorsal and ventral habitus of leaf beetle species.

A *Basilepta* sp. 3; B *Basilepta* sp. 4; C *Basilepta* sp. 5; D *Basilepta* sp. 6; E – H *Nodina* sp.
Figure 11

Dorsal and ventral habitus of leaf beetle species.

A Nodina sp.; B Pagria sp.; C Rhyparida sp. 1; D Rhyparida sp. 2; E Cleorina malayana; F Brontispa longissima; G Gonophora sp.; H Dactylispa sp. 1.
Figure 12

Dorsal and ventral habitus of leaf beetle species.

A *Dactylispa* sp. 2; B *Notosacantha* sp. 1; C *Notosacantha* sp. 2; D *Hispinae* sp.; E *Plagiodera* sp.; F *Phola sedecimpustulata*; G *Lema* sp.