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Updating Knowledge on Species Richness of the Tortoise Beetles (Coleoptera: Cassidinae) from Peninsular Malaysia through Their DNA Barcoding

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ABSTRACT

The Cassidinae family, comprising unique and beautiful leaf beetles, has been the subject of limited research regarding its diversity and richness in Malaysia. Consequently, this study aimed to perform DNA barcoding on the Cassidinae species collected from Peninsular Malaysia by using the cytochrome *c* oxidase subunit I (*COI*) gene. Prior to molecular work, each species was identified morphologically based on external morphological characteristics. This study reconfirmed the host plant record for only one species, *Silana farinosa*, which infests the curry leaf, *Murraya koenigii*. Notably, a total of ten species were morphologically identified, including those belonging to the tribe Aspidimorphini (*Aspidimorpha assimilis*, *Aspidimorpha elevata*, *Aspidimorpha malaccana*, *Aspidimorpha miliaris*, and *Laccoptera nepalensis*) and tribe Cassidini (*Chiridopsis punctata*, *Cassida circumdata*, *Chiridopsis scalaris*, *Notosacantha taeniata*, and *Silana farinosa*). In this study, only seven species were successfully barcoded, and the resulting data have been deposited in GenBank. Remarkably, the separation of species is clearly delineated within their respective lineages on the Neighbor-Joining tree, with the exception of several species that predominantly belong to the genus *Aspidimorpha*. The data gathered in this study are significant and contribute valuable information for genetic conservation and the preservation of plant species.

Keywords: Cassidines, *COI*, genetic information, leaf beetle, Malaysia

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INTRODUCTION

The tortoise beetle (Coleoptera: Cassidinae) comprises a group of coleopteran species that are notable for their unique and striking external morphology. These beetles exhibit a shining body pattern and possess a transparent body structure that covers the pronotum and elytral parts. In addition to their uniqueness and peculiar body features, tortoise beetles can sometimes be mistaken for ladybirds. The cassidines are herbivorous and can become significant pests of crops, particularly those from various plant families. They have been documented feeding on Poaceae, Convolvulaceae, Cyperaceae, and Rosaceae (Yang et al., 2023). Several studies conducted by Pathourm et al. (2021), Mohamedsaid and Sajap (1996), and Sajap and Mohamedsaid (1997) documented that the species *Silana farinosa* consumes the curry leaf, *Murraya koenigii* Thw. (Rutaceae). However, none of the cassidines have been recorded as pests or causing outbreaks in Malaysia. The Cassidinae species is also known to have very specific host preferences, often exhibiting monophagous feeding habits on several plant species, while others are polyphagous across various plant genera (Chaaboo, 2007). This particular species is also recognized as one of the more challenging species to collect and sample due to its solitary behavior.

In Malaysia, specifically in Peninsular Malaysia, research on Cassidinae species is quite limited. However, several studies dating back to 2010 focused on samples collected from Borneo, resulting in the description of new species, such as *Cassida malaysiana* (Borowiec, 2010). The taxonomic study of Cassidinae in Peninsular Malaysia concluded in 1993 with the work of Mohamedsaid (1993). Despite this, multiple studies were conducted by Borowiec (1998, 1999, 2010), primarily concentrating on Bornean species. However, research on this group of species remains sparse and underexplored (Buzzi, 1988). According to Mohamedsaid (2004), a total of 40 species under 10 genera of Cassidinae from Malaysia have been recorded, while the total numbers collected globally are relatively higher, with approximately 6,200 described species across 339 genera and 43 tribes (Borowiec & Świętojańska, 2024). This number represents a relatively low proportion of the total number of species recorded worldwide. The subfamily Cassidinae is derived from the family Chrysomelidae (Chaboo, 2007) and is categorized into several tribes based on their morphological structure (López-Pérez et al., 2018).

So far, none of the Cassidinae species has been barcoded in Malaysia to understand the diversity and genetics of each species, particularly for conservation purposes. Consequently, barcoding information is very useful for confirming species status due to the high variation observed in both interspecies and intraspecies relationships (López-Pérez et al., 2018). Several studies, such as those by Nie et al. (2020) and Leocádio et al. (2020) on the Chrysomelidae family, have also demonstrated that genetic information can address issues related to taxonomy and species classification.

In this study, the barcode information of the collected species is very crucial and valuable for precise species determination. The objectives of the study are to barcode the Cassidinae species collected from Peninsular Malaysia and to investigate the host preference of these species. The findings will be beneficial for the implementation of plant conservation efforts and will provide the first data or record on Cassidinae species' barcoding information from Peninsular Malaysia.

MATERIALS AND METHODS

Insect Sampling

The sampling of beetles was conducted across several states in Peninsular Malaysia. The locations were randomly selected based on records of the availability of host plants, and sampling was performed using active sampling (i.e. sweep netting) through observation of the potential host plant species, such as legumes and shrubs, following the methods of Muhaimin et al. (2017, 2019). The Cassidinae were collected either with a net or handpicked once the insects were spotted. Sampling took place on a clear day between 10:00 a.m. and 12:00 p.m. with several time intervals (every 10 min). The specimens were subsequently collected and preserved in 70% alcohol for morphological identification and molecular work.

Host Plant Records and Insect Rearing

The host plants or visited plant species of Cassidinae were recorded based on the locations where the beetles (both adult and larval stages) were spotted and captured. Information was collected regarding locality, altitude, collector, and date. The larvae that fed on the plants through observation in the field were reared in the laboratory until the emergence of the adult stages. Identification was also conducted based on the photographs of leaf samples by a plant taxonomist from the Herbarium of Universiti Kebangsaan Malaysia. After that, the specimens were collected and preserved in 70% alcohol for morphological identification and molecular work.

Morphological Identification

The specimens were identified based on morphological characteristics up to the species level, when possible, and categorized into morphospecies using a microscope (Stereomicroscope Stemi D4). This identification process referenced the works of Mohamedsaid (1993), Borowiec (1998, 1999, 2010), and a picture available at https://www.nickybay.com/cassidinae-checklist-tortoise-beetles. Additionally, assistance was obtained from Dr. Lukáš Sekerka, a Cassidinae taxonomist at the National History Museum in the Czech Republic. During morphological identification, the features on the pronotum, spots, and patterns (markings) on the elytra, and also coloration were observed and documented for subsequent analysis. The specimens were also microscopically photographed using the stereomicroscope equipped with a DSLR camera and analyzed using the Image Analyser with ToupTek microscope camera and software.

DNA Barcode

DNA extraction, PCR Amplification and Sequencing Analysis

DNA samples were extracted from each species of all collected specimens using the DNeasy Blood and Tissue Kit (Qiagen, Germany). The extraction process adhered to the manufacturer's protocol, which involved initial soaking in Proteinase K and ATL buffer for the lysis process, followed by the remaining steps as outlined by the manufacturer. For several species with low collection numbers, a modified freezing method was employed, with slight adjustments to several steps as described by Yaakop et al. (2013). Then, PCR amplification was performed using the cytochrome *c* oxidase subunit I (*COI*) gene with primers developed by Folmer (1994), as well as the PCR conditions proposed by Halim et al. (2017) and Musa, Halim et al. (2024). The resulting PCR product was verified through gel electrophoresis using a 1.5% agarose gel and subsequently sent to Apical Sdn. Bhd., Selangor, Malaysia, for sequencing analysis.

DNA Editing and Alignment, and BLAST Analyses

The sequences obtained were edited manually using Sequencher 5.4.6 (Gene Codes Corporation, Ann Arbor, MI USA). Additionally, the Basic Local Alignment Search (BLAST) was employed for species confirmation and comparison based on several parameters, including the total score, expected value, maximum identical value, maximum score, and query coverage.

Genetic Distance

Genetic distance analysis was conducted using PAUP* version 4.0 (Sinauer Associates, Sunderland, Massachusetts) software, employing the Kimura-2-parameter (K2P) model (Zainudin et al., 2010). This analysis aims to investigate the genetic distance of the Cassidinae samples and the GenBank sequences utilized in this research.

Tree Reconstruction

The separation of species was visualized through tree reconstruction using distance criteria, specifically the Neighbor-Joining (NJ) method via phylogenetic analysis. The NJ tree was constructed using PAUP* version 4.0 software and employed using the K2P algorithm model with bootstrap analysis (1,000 replications). The outgroup species selected for this analysis belong to the subfamily Hispinae, and the ingroups are Cassidinae (Table 1).

Table 1
List of specimens along with the results obtained from the morphological and molecular identification of Cassidinae species in the current study and the GenBank information used for tree reconstruction, with the value of maximum score, total score, query coverage, E-value, similarity percentage, and accession number, and the additional Genbank sequences

No.	Code/ voucher no.	Locality	Tribe	Maximum score	Total score	Query cover (%)	E-value	Similarity percentage (%)	Species closely related to Genbank accession no.	Genbank accession no.	Species identified
1	425	Malaysia: Pahang, Fraser Hill	Hispinii	-	-	-	-	-	-	MW175466	Hispini sp.
2	USNM:ENT: 01117140	Myanmar	Aspidimorphini	-	-	-	-	-	-	MF804568	*Aspidimorpha* sp.
3	J	Vietnam	Aspidimorphini	-	-	-	-	-	-	MN845123	*Aspidimorpha furcata*
4	Cas-36-2019	Vietnam	Aspidimorphini	-	-	-	-	-	-	MZ303504	*Aspidimorpha furcata*
5	H	Vietnam	Aspidimorphini	-	-	-	-	-	-	MN845120	*Aspidimorpha dorsata*
6	13	Malaysia: Pahang, Cameron Highlands	Aspidimorphini	1050	1050	100	0.0	98.67	MN845123 *Aspidimorpha furcata*	PQ187448	*Aspidimorpha assimilis*
7	404	Malaysia: Pahang: Fraser Hill	Aspidimorphini	1077	1077	100	0.0	99.67	MF804566 *Aspidimorpha* sp.	MN897085	*Aspidimorpha assimilis*
8	479a	Malaysia: Pahang: Fraser Hill	Aspidimorphini	1077	1077	100	0.0	99.67	MF804566 *Aspidimorpha* sp.	MN897084	*Aspidimorpha assimilis*
9	22	Malaysia: Perak, Batu Kurau	Aspidimorphini	1011	1011	100	0.0	97.33	MN845121 *Aspidimorpha furcata*	PQ524205	*Aspidimorpha assimilis*
10	515d	Malaysia: Pahang, Fraser Hill	Aspidimorphini	902	902	100	0.0	93.19	KJ195294 *Aspidimorpha sanctaecrucis*	MN934809	*Aspidimorpha elevata*
11	7	Malaysia: Pahang, Cameron Highlands	Aspidimorphini	974	974	100	0.0	95.85	KJ195294 *Aspidimorpha sanctaecrucis*	PQ203305	*Aspidimorpha elevata*

Table 1 *(continue)*

No.	Code/ voucher no.	Locality	Tribe	Maximum score	Total score	Query cover (%)	E-value	Similarity percentage (%)	Species closely related to Genbank accession no.	Genbank accession no.	Species identified
12	531c	Malaysia: Pahang, Fraser Hill	Aspidimorphini	902	902	100	0.0	93.19	KJ195294 *Aspidimorpha sanctaecrucis*	MN934808	*Aspidimorpha elevata*
13	363b	Malaysia: Pahang, Fraser Hill	Aspidimorphini	902	902	100	0.0	93.19	KJ195294 *Aspidimorpha sanctaecrucis*	MN934810	*Aspidimorpha elevata*
14	1	Malaysia: Pahang, Cameron Highlands	Aspidimorphini	-	-	-	-	-	-	-	*Aspidimorpha elevata*
15	29	Malaysia: Perak, Zoo Taiping	Aspidimorphini	-	-	-	-	-	-	-	*Aspidimorpha elevata*
16	15	Malaysia: Selangor, Serdang, Ladang UPM	Aspidimorphini	1016	1016	100	0.0	97.01	KJ195294 *Aspidimorpha sanctaecrucis*	PQ203270	*Aspidimorpha malaccana*
17	16	Malaysia: Selangor, Serdang, Ladang UPM	Aspidimorphini	986	986	100	0.0	95.85	KJ195294 *Aspidimorpha sanctaecrucis*	PQ203271	*Aspidimorpha malaccana*
18	5	Malaysia: Pahang, Fraser Hill	Aspidimorphini	-	-	-	-	-	-	-	*Aspidimorpha miliaris*
19	19	Malaysia: Perak, Batu Kurau	Aspidimorphini	-	-	-	-	-	-	-	*Aspidimorpha miliaris*
20	RoLn1	India	Aspidimorphini		-	-	-	-	-	KM226876	*Laccoptera nepalensis*
21	F2	Malaysia: Pahang, Fraser Hill	Aspidimorphini	1028	1028	100	0.0	97.84	KM226876 *Laccoptera nepalensis*	MW168661	*Laccoptera nepalensis*
22	TB	India	Cassidini	-	-	-	-	-	-	OR416859	*Chiridopsis* sp.
23	L	Vietnam	Cassidini	-	-	-	-	-	-	MN845124	*Chiridopsis bowringii*

Table 1 *(continue)*

No.	Code/ voucher no.	Locality	Tribe	Maximum score	Total score	Query cover (%)	E-value	Similarity percentage (%)	Species closely related to Genbank accession no.	Genbank accession no.	Species identified
24	SSG-2014 C83	India	Cassidini	-	-	-	-	-	-	KJ195316	*Chiridopsis bipunctata*
25	C70	India	Cassidini	-	-	-	-	-	-	KJ195312	*Chiridopsis undecimnotata*
26	190	Malaysia: Pahang: Fraser Hill	Cassidini	938	938	100	0.0	94.52	OR416859 *Chiridopsis* sp.	MN955586	*Chiridopsis punctata*
27	21	Malaysia: Perak, Batu Kurau	Cassidini	762	762	100	0.0	88.04	OR416859 *Chiridopsis* sp.	PQ524206	*Chiridopsis scalaris*
28	30	Malaysia: Perak, Zoo Taiping	Cassidini	-	-	-	-	-	-	-	*Chiridopsis punctata*
29	SFV 1	India	Cassidini	-	-	-	-	-	-	PP373131	*Silana farinosa*
30	28	Malaysia: Kedah	Cassidini	1046	1046	100	0.0	98.83	PP373131 *Salina farinosa*	PQ549944	*Silana farinosa*
31	11	Malaysia: Johor, Benot Pontian	Cassidini	1055	1055	100	0.0	98.84	PP373131 *Salina farinosa*	PQ203298	*Silana farinosa*
32	32	Malaysia: Perak: Manong, Kpg. Bekor	Cassidini	1055	1055	100	0.0	98.84	PP373131 *Salina farinosa*	PQ549945	*Silana farinosa*
33	33	Malaysia: Perak, Manong, Kpg. Bekor	Cassidini	1046	1046	100	0.0	98.50	PP373131 *Salina farinosa*	PQ549946	*Silana farinosa*
34	10	Malaysia: Pahang, Cameron Highland	Cassidini	-	-	-	-	-	-	-	*Cassida circumdata*
35	43	Malaysia: Selangor: Serdang, Ladang UPM	Notosacanthini	-	-	-	-	-	-	-	*Notosacantha taeniata*

RESULTS

Morphological Identification

A total of ten species belonging to the Cassidinae subfamily, which is divided into two tribes namely Cassidini and Aspidimorphini, were successfully identified morphologically up to the genus and species levels. The species included in the Cassidini tribe were *Silana farinosa*, *Chiridopsis punctata*, *Cassida circumdata*, *Chiridopsis scalaris*, and *Notosacantha taeniata*. The Aspidimorphini tribe included *Aspidimorpha miliaris*, *Aspidimorpha assimilis*, *Aspidimorpha malaccana*, *Aspidimorpha elevata*, and *Laccoptera nepalensis*. Several species exhibited variation in characteristics, particularly in the genus *Aspidimorpha* (*A. malaccana* and *A. elevata*), which were collected from multiple locations.

Records on Host Plant Species

Based on observations made in the field and subsequently confirmed through laboratory investigations, the leaves of the curry plant (*M. koenigii*) were consumed by the larval stage of *S. farinosa*.

Molecular Identification

The barcoding analysis identified seven distinct species. All the sequences submitted to GenBank, NCBI, are listed in Table 1 with corresponding accession numbers. This table includes a list of specimens together with the results from the morphological and molecular identification of Cassidinae species in this study.

Genetic Distance

The genetic distances among the Cassidinae species are presented in Tables 2 and 3. In this study, the genetic separation between *A. malaccana* and *A. elevata* was observed to be between 0.053, with the range between the same species of *A. malaccana* of 0.007 and *A. elevata* of 0.04. The genetic separation between *A. assimilis* and *A. furcata* ranges between 0.083, with the range between the same species of *A. furcata* of 0.028 and *A. assimilis* of 0.10 (Tables 2 and 3).

Table 2
Genetic distance of Cassidinae sample implemented in the tree reconstruction (within group mean distance)

		d
1	*Hispini* sp.	-
2	*Aspidimorpha* sp.	-
3	*Aspidimorpha assimilis*	0.100
4	*Aspidimorpha dorsata*	-
5	*Aspidimorpha elevata*	0.040
6	*Aspidimorpha furcata*	0.028
7	*Aspidimorpha malaccana*	0.007
8	*Chiridopsis* sp.	-
9	*Chiridopsis bowringii*	-
10	*Chiridopsis bipunctata*	-
11	*Chiridopsis punctata*	-
12	*Chiridopsis scalaris*	-
13	*Chiridopsis undecimnotata*	-
14	*Laccoptera nepalensis*	0.021
15	*Silana farinosa*	0.006

Table 3
Genetic distance of Cassidinae samples implemented in the tree reconstruction (between group mean distance)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*Hispini* sp.	-														
2	*Aspidimorpha* sp.	0.233	-													
3	*Aspidimorpha assimilis*	0.237	0.092	-												
4	*Aspidimorpha dorsata*	0.241	0.155	0.125	-											
5	*Aspidimorpha elevata*	0.197	0.207	0.204	0.196	-										
6	*Aspidimorpha furcata*	0.226	0.019	0.083	0.149	0.198	-									
7	*Aspidimorpha malaccana*	0.192	0.227	0.221	0.199	0.053	0.215	-								
8	*Chiridopsis* sp.	0.183	0.191	0.205	0.200	0.217	0.190	0.213	-							
9	*Chiridopsis bowringii*	0.181	0.226	0.232	0.240	0.210	0.226	0.198	0.167	-						
10	*Chiridopsis bipunctata*	0.188	0.194	0.207	0.210	0.217	0.195	0.216	0.018	0.169	-					
11	*Chiridopsis punctata*	0.199	0.198	0.207	0.194	0.232	0.198	0.227	0.052	0.177	0.059	-				
12	*Chiridopsis scalaris*	0.204	0.191	0.198	0.205	0.210	0.195	0.197	0.124	0.131	0.126	0.138	-			
13	*Chiridopsis undecimnotata*	0.220	0.233	0.232	0.233	0.226	0.226	0.213	0.170	0.175	0.172	0.161	0.179	-		
14	*Laccoptera nepalensis*	0.216	0.220	0.210	0.197	0.228	0.216	0.227	0.216	0.203	0.223	0.206	0.199	0.203	-	
15	*Silana farinosa*	0.201	0.222	0.228	0.237	0.228	0.223	0.226	0.229	0.242	0.224	0.225	0.227	0.229	0.242	-

Microscopic Figures

All the species collected in this study are presented in the microscopic figures and photographs in Figure 1.

Tree Reconstruction

The NJ tree showed a clear separation between the ingroup and outgroup, as supported by low bootstrap values. All the species were also separated from one another, as strongly

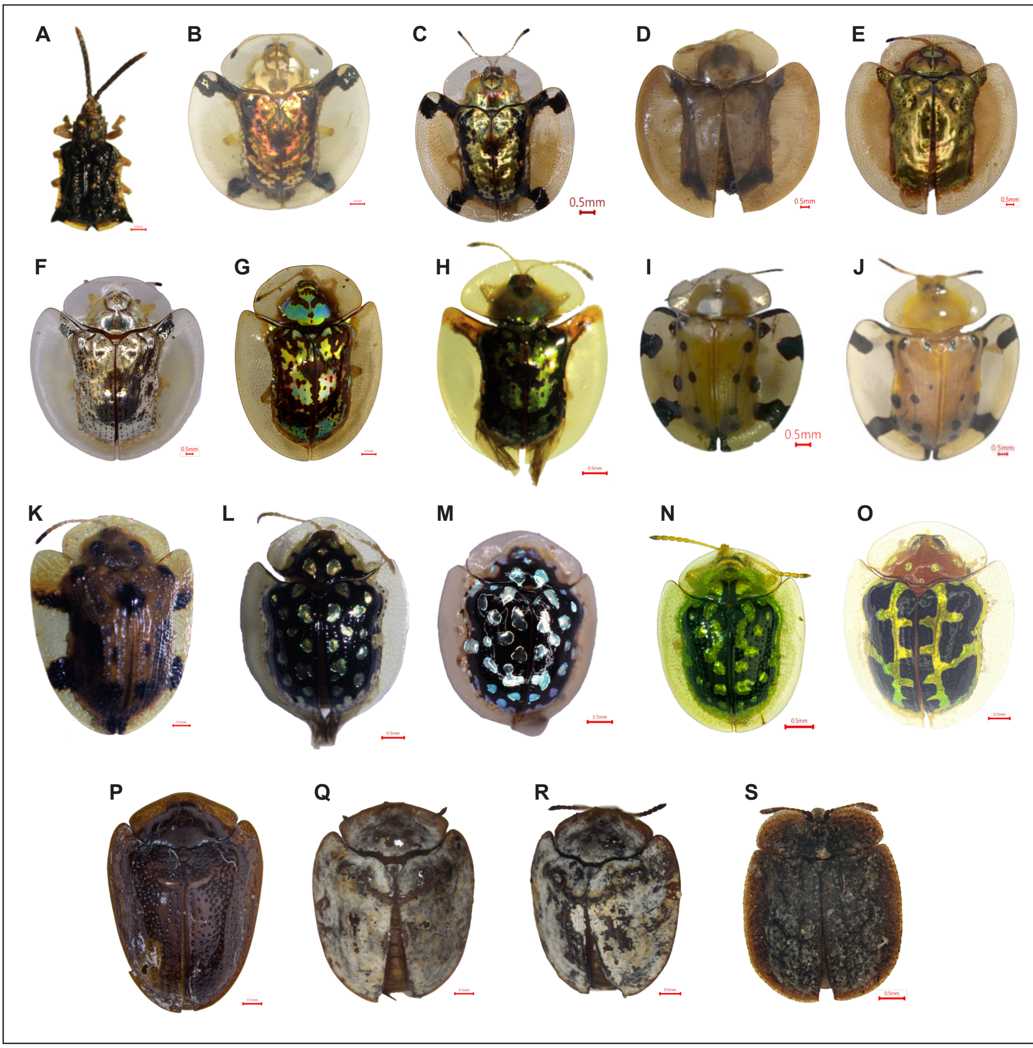

Figure 1. Cassidinae species collected in this study: A, Hispini sp. (425); B, *A. malaccana* (16); C, *A. malaccana* (15); D, *A. elevata* (7), E, *A. elevata* (1), F, *A. elevata* (29); G, *A. assimilis* (22); H, *A. assimilis* (404); I, *A. miliaris* (5); J, *A. miliaris* (19); K, *L. nepalensis* (F2); L, *C. punctata* (190); M, *C. punctata* (30); N, *C. circumdata* (10); O, *C. scalaris* (21); P, *S. farinosa* (28); Q, *S. farinosa* (33); R, *S. farinosa* (32); S, *N. taeniata* (43)

supported by the low to high bootstrap values ranging from 58% to 100%, except for several species under the genus *Aspidimorpha.* However, the separation among tribes was not distinctly evident, showing a mixture between them. The separation between Cassidinae and Hispinae was clear, but there was no clear separation between tribes, as both tribes (Cassidini and Aspidimorphini) were paraphyletic (Figure 2).

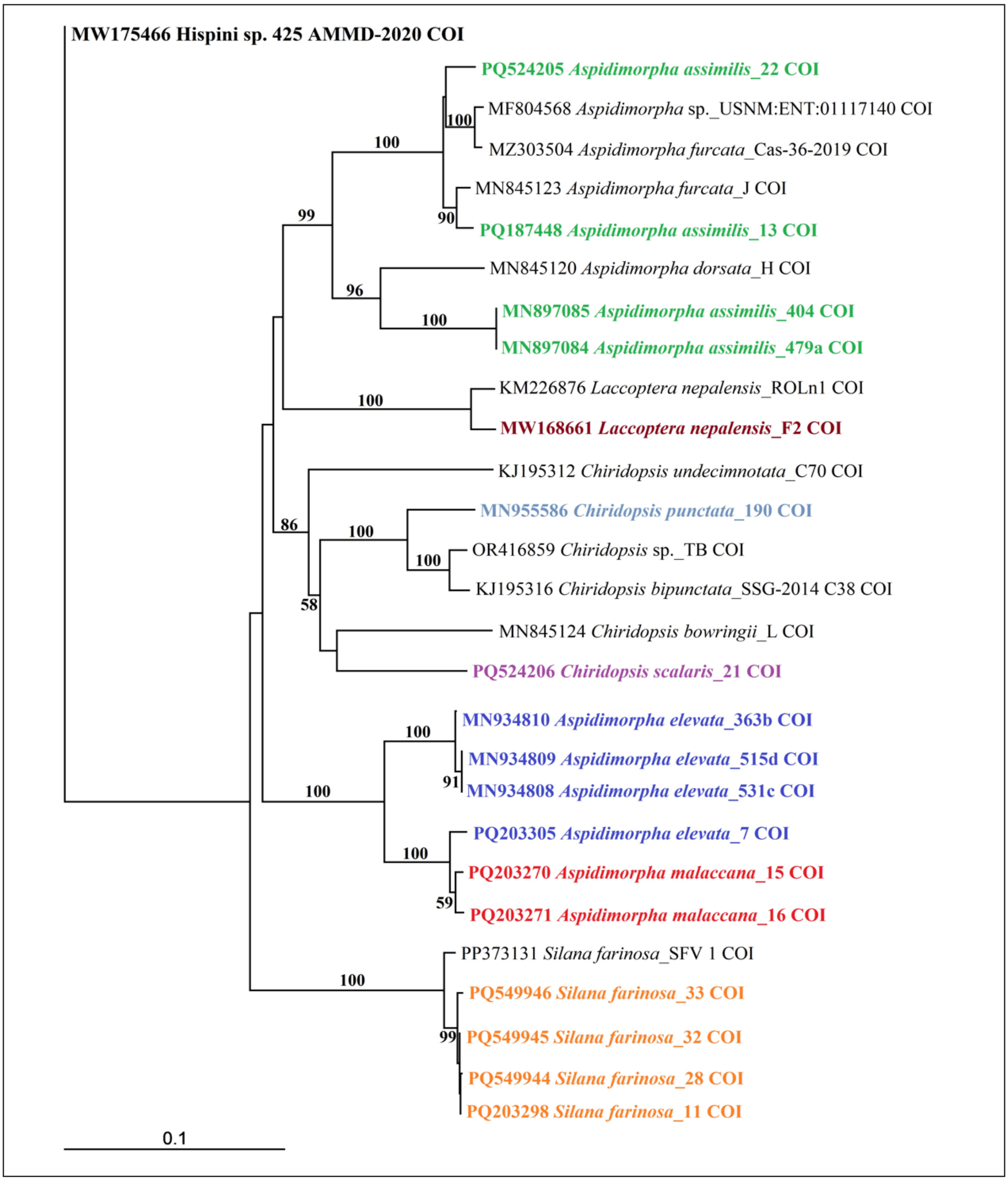

Figure 2. Neighbor-joining tree based on mitochondrial cytochrome C oxidase I (*COI)* sequences of Cassidinae species. The bootstrap values are indicated on the branches

DISCUSSION

The study of Cassidinae diversity and its interactions with plant species in Malaysia is quite limited due to the scarcity of available information and published research on these species. Therefore, it is urgently necessary to investigate the status of Cassidinae species in this geographical region. Only a few Cassidinae species have been barcoded from Malaysia, primarily through mitochondrial metagenomic studies of beetle species, particularly those from Borneo, as reported by Crampton-Platt et al. (2015). Research from neighboring countries mainly focused on new species records from specific areas, insect-plant interactions, and the taxonomy and phylogeny of Cassidinae species (López-Pérez et al., 2018; Yang et al., 2023).

This number is considered low compared to the species recorded in Malaysia, representing only 20%, comprising 10 species obtained from this study out of a total of over 50 species recorded in Malaysia by Mohamedsaid (1993), in addition to several papers published on new species from Malaysia, such as those by Borowiec and Świętojańska (2014). The numbers obtained from this study do not accurately reflect the diversity and richness of Peninsular Malaysia due to inconsistent sampling efforts in the selection of sampling sites. The sampling process employs active sampling, which is very time-consuming and requires significant human effort.

The record of host preferences or host plants of Cassidinae is important due to their functional role as leaf-eating beetles as herbivores that can potentially become pests in agriculture (Salem et al., 2020). However, information on many other species remains unexplored. In this study, the reconfirmation that *S. farinosa* infests curry leaves has been proven based on laboratory observation. A study by Begha and Oliveira (2024) also confirmed the host preference based on the immature stages of the Cassidinae species, *Hybosa acutangula* Spaeth, 1913 from Brazil. The records are very convincing as they are based on careful observation and rearing until the emergence of the adult samples. This study identified several plant families involved in the food webs of the Cassidinae, including Fabaceae, Convolvulaceae, Myristicaceae, and others, which are utilized as host plants. This finding is supported by Borowiec et al. (2013), Mohamedsaid and Sajap (1996), Sajap and Mohamedsaid (1997), and Yang et al. (2023), indicating that the same tribe shares several groups of plant families, demonstrating their role as generalist consumers.

In taxonomic contexts, the sister-species relationships between two subfamilies, Cassidinae s. str. and Hispinae s. str., are distinguished based on their distinct external morphological characteristics. Cassidinae have smooth, flat elytral edges, and a rounded shape, while Hispinae have spines, non-flattened elytral edges, and a non-rounded shape, and the separation of these subfamilies is supported in Figure 2. However, within the Cassidinae, only two tribes namely Cassidini and Aspidimorphini (Borowiec &

Świętojańska, 2014), were collected, and only seven species were barcoded in this study. Each species is located within a specific lineage, and each clade represents the same tribe and species, supported by morphological characteristics. The reconstruction of the NJ tree proved to be a viable method for clearly illustrating species separation, as corroborated by several studies on DNA barcoding (Rusinko & McPartlon, 2017).

In this study, many species were easily identified based on external morphology, except for several species that exhibit high resemblance, namely *A. malaccana* and *A. elevata*, and *A. assimilis* and *A. furcata*. These species show very low divergence between species based on genetic distance results when compared to other species included in the NJ tree. According to Borowiec (1998, 1999, 2010) and Mohamedsaid (1993), all species within the same genus possess specific species characteristics; however, *A. assimilis* was distinctly separated and also located quite far from the genus clade (*A. elevata* and *A. malaccana*). This situation may arise due to the limited number of sequences and other potential biases that affect the estimation of species divergence (Zheng et al., 2011). Additionally, the use of the single-gene *COI* as barcoding data is effective for species identification. However, it has limited resolution for closely related or recently diverged species, as evidenced by the low genetic divergence between species such as *A. elevata* and *A. malaccana*. Therefore, our results suggest that *COI* alone is unlikely to accurately delimit species. It should be combined and integrated with morphological identification for more precise species identification (Ranasinghe et al., 2022)

Interestingly, the barcode information for the *COI* of seven species of Cassidinae from Peninsular Malaysia has been firstly submitted and deposited in GenBank, NCBI. The barcode information obtained from this study is highly significant for confirming the species status of these organisms in Malaysia. Generally, these species exhibit high morphological variation, particularly in the patterns or spots on their elytra, such as on Coccinellidae in studies by Halim et al. (2017), Musa, Halim et al. (2024) and Musa, Hatta et al. (2024). By referring to their DNA barcodes, the diversity of the same species both in Malaysia and globally can be accurately identified. This identification is not limited to beetle species only, but it also extends to other insect species, as demonstrated by Nor-Atikah et al. (2019), Yaakop et al. (2020), Yaakop, Amiruddin et al. (2022) and Yaakop, Sabri et al. (2022).

CONCLUSION

The latest information on Cassidinae species in Peninsular Malaysia is urgently needed to investigate species richness, barcode information, and insect-host interactions. This information is essential for understanding the complex food webs of Cassidinae in Peninsular Malaysia, which are crucial for genetic conservation and the preservation of plant species.

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