Exploring the diversity of leaf beetles (coleoptera: chrysomelidae) on the islands of Vietnam: a survey of Phu Quoc Island, South of Vietnam

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Abstract
Chrysomelidae on the islands of Vietnam has been poorly known. In this study, we investigated the diversity of Chrysomelidae on Phu Quoc Island in Kien Giang province, Southern Vietnam. Specimens were collected from Phu Quoc national park and the buffer zone forest. First, all specimens were ordered into a morph-species operational taxonomic unit (OTU). We collected 52 morphological OTUs of 31 genera and 5 subfamilies, 20 of which were identified as level species. Then, all morphological OTUs were extracted, amplified, and sequenced from the 658 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. A total of 63 DNA barcode sequences of 13 species and 27 morphological OTUs were successfully sequenced and assigned to 32 Barcode Index Numbers (BIN) in Bold. In the comparison, between morphological OTUs and BINs, the number of OTUs was reduced to 20 species and 24 OTUs (a total of 44 OTUs). The number of species on Phu Quoc Island is estimated to be 1.38x – 1.96x greater. A total of 32 BINs were generated from this study, 30 of which were new to Vietnam and 28 of which were new to BOLD. The results of this study provide the first document of the leaf beetle fauna on Phu Quoc island and the first DNA Barcoding data for Chrysomelidae from this region in Vietnam, as well as additional documents of leaf beetles on islands from another world.

Keywords Asia · Biodiversity · COI · DNA barcoding · Entomology · Insect · System

Introduction
Chrysomelidae is one of the largest families of Coleoptera, with 35,000–60,000 species in the world (Schmitt 1996; Futuyma 2004; Splipnski et al. 2011; Jolivet 2015). In Vietnam, Chrysomelidae fauna have been studied only in the mainland area of North Vietnam (Tam Dao, Hoa Binh, Ha Nam, and Ninh Binh), several provinces in Middle Vietnam (Quang Binh, Quang Tri, and Thua Thien Hue provinces), and some provinces in the Central Highlands of Vietnam (Medvedev 1987, 2009a, b; Dang and Medvedev 1982, 1983; Dang 1989; Tran and Dang 2005a, b; Tran et al. 2006, 2007, 2008). Recently, Nguyen and Gómez-Zurita (2016, 2017) used molecular biology tools to identify 155 species in Nui Chua National Park and described 13 new species from this region. Documents for the family Chrysomelidae on islands in Vietnam are poor. The first record of Chrysomelidae on the islands of Vietnam was reported by Medvedev (1992), with ten species from the Con Dao and Cham Islands. Delobel (2008) described two new species on the Phu Quoc island. Skomorokhov (2011) described two new species from the Con Dao and Phu Quoc islands, and he expected that there would be 25 leaf beetle species there, but a checklist was unpublished. Most recently, Nguyen & Bezděk (2021), and Nguyen et al. (2021) described two new species on Phu Quoc island.

To date, 661 DNA barcode sequences for Chrysomelidae in Vietnam have been recorded in BOLD. All the data from Nguyen and Gómez-Zurita (2016) submitted 520 DNA barcode sequences of 829 bp fragments of the COI gene for 155 species in Nui Chua National Park. Nguyen (2020) submitted 16 DNA barcode sequences of 658 bp fragments of the COI gene from 9 species of Chrysomelidae in Vietnam. In addition, several species of Vietnamese flea beetles have been found in Senthil and Srinivasan (2021).
Previous results indicate that the Chrysomelidae fauna on the islands of Vietnam are poorly known, and many Chrysomalid species in Vietnam lack publicly available DNA barcode sequences. Therefore, the objectives of this research were to (1) document the species richness of leaf beetles from the tropical forest on Phu Quoc island and (2) generate DNA barcode data for Chrysomelidae species. The results will be added to the database of Chrysomelidae fauna, including known and unknown species from Vietnam.

Materials and methods

Study location and sampling strategy

Specimens of the Chrysomelidae family were collected in July and November 2019, along the sampling paths in the forest Phu Quoc island in two regions: the buffer zone and Phu Quoc national park (along the Bien Phong road). Specimens were collected by three methods: caught directly by hand without collection tools; by sweeping trees by bug-catching net randomly along roads and by beating from low branches and vegetation, reaching as high as the arm’s reach, and using sticks to catch beetles that have fallen from the threshing tray. The coordinates of the sampling sites are in Table 1 and Fig. 1. The obtained specimens were immediately placed in vials containing absolute ethanol to preserve the DNA, and these vials were labeled with locality, temporal, and collector information for future research.

Morphospecies identification: Specimens were sorted into morphological species and used to identify the species levels if possible (Beenen 2010; Bezděk 2009, 2012, 2017, 2019; Borowiec and Świętojańska 2021; Hazmi 2012; Kimoto and Gressitt 1979, 1981, 1982; Kimoto 1989, 1998; Konstantinov et al. 2011; Medvedev 1998, 2000, 2003, 2009a, b, 2015; Moseyko 2020; Moseyko and Medvedev 2017; Romantsov 2018, 2020). The morphological species were compared with the identified BINs in BOLD. The taxonomic nomenclature at the family and subfamily levels follows Bourchard et al. (2011), the genus and species levels as in Seeno and Wilcox (1982).

Photographs of the species were taken with a Nikon Ds–Fi3 camera mounted on a Nikon SMZ800N stereo microscope and processed using the NIS–Element imaging software. Helicon Focus 7 software was used to combine the images, which are the same objects at different focal planes.

After being assigned to morphospecies the specimens were stored in 96% alcohol at 4°C until DNA extraction. Non-destructive DNA extraction was performed on whole specimens using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The mitochondrial cytochrome c oxidase subunit 1 (cox1) gene was amplified using the primers LepF1 (forward direction) (5’-ATTCAAACCAATCTAGAATTGG-3’) and LepR1 (reverse direction) (5’- TAAACTTCTTATTCTAACTCA-3’) (Hebert et al. 2004) to amplify a 658 base pair (bp) fragment of the COI gene. Each PCR reaction mixture contained 2.5 µl of 10× reaction buffer (Evrogen, Russia), 0.5 µl of 10 mM dNTPs, 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 1 µl of 25 mM Mg, 2 µl of template DNA, 0.2 µl of thermostable

Table 1 Sampling localities on Phu Quoc Island

| Locality | Latitude | Longitude | Elevation (m) | Number of specimens |
|----------|----------|-----------|---------------|---------------------|
| 1        | 10.2396  | 103.97051 | 101           | 14                  |
| 2        | 10.25105 | 103.96997 | 100           | 7                   |
| 3        | 10.25313 | 103.95040 | 50            | 3                   |
| 4        | 10.20949 | 103.96754 | 41            | 8                   |
| 5        | 10.19024 | 104.00132 | 40            | 7                   |
| 6        | 10.21635 | 104.01391 | 40            | 10                  |
| 7        | 10.36534 | 103.99071 | 33            | 6                   |
| 8        | 10.37155 | 103.988012| 35            | 4                   |
| 9        | 10.37697 | 103.98254 | 35            | 10                  |
| 10       | 10.38655 | 103.978531| 38            | 15                  |
| 11       | 10.33999 | 103.973889| 40            | 3                   |
| 12       | 10.33229 | 103.97779 | 38            | 52                  |
| 13       | 10.33267 | 103.98548 | 33            | 13                  |
| 14       | 10.33123 | 103.98812 | 40            | 31                  |
| 15       | 10.33003 | 103.99389 | 50            | 69                  |
| 16       | 10.3266  | 104.011102| 50            | 5                   |
| 17       | 10.33032 | 104.023185| 56            | 20                  |
| 18       | 10.33572 | 104.04445 | 58            | 15                  |
| 19       | 10.37222 | 104.04696 | 40            | 19                  |

The localities from 1 to 10 are ecological restoration forest areas on Phu Quoc Island (buffer zone), and the localities from 11 to 19 are strictly protected forest areas in Phu Quoc national park.
| Taxon                                      | Buffer zone | Phu Quoc National park | BIN id  | Figure |
|-------------------------------------------|-------------|------------------------|---------|--------|
| **I  CASSIDINAE**                         |             |                        |         |        |
| Tribe ASPIDIMORPHINI Chapuis, 1875        |             |                        |         |        |
| 1  Aspidimorpha sp.                       | BOLD: ADHB705 2a-c |                      |         |        |
| 2  Laccoprota (Sindola) vigintisexnotata Boheman, 1855 | BOLD: AEI4065 |                      |         |        |
| Tribe NOTOSACANTHINI Hincks, 1952         |             |                        |         |        |
| 3  Notosacantha rufa (Wagener, 1881)      | BOLD: ADJ1495 3a, b |                      |         |        |
| **II  CRIOCERINAE**                       |             |                        |         |        |
| Tribe CRIOCERINI Latreille, 1804          |             |                        |         |        |
| 4  Lilloceris egena (Weise, 1922)         | BOLD: ADJ1495 3a, b |                      |         |        |
| **III  CRYPTOCEPHALINAE**                 |             |                        |         |        |
| Tribe CRYPTOCEPHALINI Gyllenhal, 1813     |             |                        |         |        |
| 5  Cryptocephalus yoshimotoi Kimoto & Gressitt, 1981 | BOLD: ADJ1495 3c, d |                      |         |        |
| Tribe CLYTRINI Kirby, 1837                |             |                        |         |        |
| 6  Tituboea laportei Baly, 1865           | BOLD: AEI6744 3e, f |                      |         |        |
| Tribe FULCIDACINA Jakobson, 1924          |             |                        |         |        |
| 7  Chlamisus sp.                          |             |                        |         |        |
| **IV  EUMOLPINAE**                        |             |                        |         |        |
| Tribe BROMIINI Baly, 1865 (1863)          |             |                        |         |        |
| 8  Aulacolepis mouhoti Baly, 1863         | BOLD: AEH9778 3i, k |                      |         |        |
| 9  Aulexis unispinosa Pii, 1935            |             |                        |         |        |
| 10 Hemiplatys pascoei Baly, 1863          | BOLD: AEH9779 4a, b |                      |         |        |
| 11 Hyperaxids sp.                         | BOLD: AEH1987 4c-f |                      |         |        |
| 12 Pseudometaxis serraticollis (Baly, 1867) | BOLD: AEI5492 4g, h |                      |         |        |
| 13 Scelodonta granulosa Baly, 1867        |             |                        |         |        |
| 14 Lepina sp.                             |             |                        |         |        |
| Tribe EUMOLPINI Hope, 1840                |             |                        |         |        |
| 15 Colaspoides rugulosus Lefevre, 1889    | BOLD: AEI7733 5a, b |                      |         |        |
| 16 Platycorynus igneofasciatus (Baly, 1860) | BOLD: AEI5570 5i, k |                      |         |        |
| Tribe EURYOPINI Chapuis, 1874             |             |                        |         |        |
| 17 Colasposoma sp.                        |             |                        |         |        |
| Tribe TYPOPHORINI Baly, 1865              |             |                        |         |        |
| 18 Basilepta sp.                          | BOLD: AEI3774 5l, m |                      |         |        |
| 19 Cleoporus sp.1                         |             |                        |         |        |
| 20 Cleoporus sp.2                         |             |                        |         |        |
| Taxon | Buffer zone | Phu Quoc National park | BIN id | Figure |
|-------|-------------|------------------------|--------|--------|
|       | 1 2 3 4 5 6 7 8 9 10 | 11 12 13 14 15 16 17 18 19 |        |        |
| 21    | Cleoporus sp.3 | 1 | | 5n, o |
| 22    | Nodina sp. | 8 5 2 1 1 10 15 | 5 1 6 2 1 1 5 | BOLD: AEI2513 5p, q |
| 23    | Pagria sp.1 | 6 1 | 1 | BOLD: AEI5569 6a-d |
| 24    | Pagria sp.2 | 1 | | BOLD: AEI2512 6c, f |
| 25    | Nonarthra variabilis Baly, 1862 | 1 1 | | BOLD: AEK9752 6g, h |
| 26    | Nonarthra sp. | | | BOLD: AEK501 6i, k |
| V     | GALERUCINAE | | | |
| 27    | V GALERUCINAE Tribe ALTICINI Newman, 1834 | | | |
| 28    | Aulacophora indica (Gmelin, 1790) | 1 | | |
| 29    | Aulacophora lewisii Baly, 1886 | 5 | | |
| 30    | Charaea dinhuongi Nguyen, 2021 | | | |
| 31    | Hoploxooides curtipes Medvedev, 2000 | 1 1 | 1 4 | BOLD: AEI0765 7a, b |
| 32    | Hoploxooides sp.1 | 3 3 | | BOLD: AEI0766 7c, d |
| 33    | Hoploxooides sp.2 | | | BOLD: AEK5218 7e, f |
| 34    | Mimastra submetallica Jacoby, 1884 | 3 1 1 40 5 | | BOLD: AEK9751 7g, h |
| 35    | Monolepta wilsoni Kimoto, 1989 | 1 | | |
| 36    | Monolepta sp.1 | 1 1 | 1 | BOLD: AEI0887 8c, d |
| 37    | Monolepta sp.2 | 1 | | BOLD: AEI1997 8e, f |
| 38    | Monolepta sp.3 | 1 | | |
| 39    | Paleosepharia sp. | 2 2 | | BOLD: AEK8376 8i, k |
| 40    | Taumaceria phuquoca Nguyen and Bezdék, 2021 | 2 2 | | BOLD: AEH0379 8l, m |
| 41    | Taumaceria sp. | 1 | | BOLD: AEI0764 8n, o |
| 42    | Tribe LUPERINI Gistel, 1848 | | | |
| 43    | Aulacophora indica (Gmelin, 1790) | 1 | | |
| 44    | Aulacophora lewisii Baly, 1886 | 5 | | |
| 45    | Charaea dinhuongi Nguyen, 2021 | | | |
| 46    | Hoploxooides curtipes Medvedev, 2000 | 1 1 | 1 4 | BOLD: AEI0765 7a, b |
| 47    | Hoploxooides sp.1 | 3 3 | | BOLD: AEI0766 7c, d |
| 48    | Hoploxooides sp.2 | | | BOLD: AEK5218 7e, f |
| 49    | Mimastra submetallica Jacoby, 1884 | 3 1 1 40 5 | | BOLD: AEK9751 7g, h |
| 50    | Monolepta wilsoni Kimoto, 1989 | 1 | | |
| 51    | Monolepta sp.1 | 1 1 | 1 | BOLD: AEI0887 8c, d |
| 52    | Monolepta sp.2 | 1 | | BOLD: AEI1997 8e, f |
| 53    | Monolepta sp.3 | 1 | | |
| 54    | Paleosepharia sp. | 2 2 | | BOLD: AEK8376 8i, k |
| 55    | Taumaceria phuquoca Nguyen and Bezdék, 2021 | 2 2 | | BOLD: AEH0379 8l, m |
| 56    | Taumaceria sp. | 1 | | BOLD: AEI0764 8n, o |
| 57    | Tribe SERMYLINI Wilcox 1965 | | | |
| 58    | Dercetina sp.1 | 2 2 | 3 2 | BOLD: AEI0763 9a-h |
| 59    | Dercetina sp.2 | 2 1 7 | 4 2 2 | BOLD: AEI5981 9i-m |
| 60    | Dercetina sp.3 | 1 | | BOLD: AEI5115 9n, o |
| 61    | Dercetina sp.4 | 1 1 | | BOLD: AEI7352 9p, q |
| Total individuals | 14 7 3 8 7 10 6 4 10 15 3 52 13 31 69 5 20 15 19 | | |
| Total species | 6 3 3 3 6 3 3 1 1 1 1 12 7 14 14 3 5 7 5 | | |
| Total | 84 individuals, 19 species | 227 individuals, 33 species | | 32 |
Taq DNA polymerase (Evrogen, Russia), and 17.8 µl deionized water. The PCR protocol used is as follows: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s; annealing at 42 °C for 40 s; elongation at 72 °C for 60 s; and final elongation at 72 °C for 5 min. PCR products were visualized via electrophoresis using a 1.5% agarose gel and then purified using ammonium acetate and cold isopropanol. They were sequenced in both directions using the BigDye Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) with the same PCR primers. The DNA-extracted specimens were mounted dry and labeled with a voucher number for future reference at the Institute of Ecology and Biological Resources (IEBR).

Forward and reverse Sanger sequences were assembled, edited, and aligned in Geneious Prime 2019.0.4 (https://www.geneious.com). Sequences were deposited in Genbank (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers MW810795-MW810854 and BOLD v. 4 (www.barcodingfife.com, Ratnasingham and Hebert 2007). Sequences were assigned automatically to Barcode Index Numbers (BINs) in BOLD (Ratnasingham and Hebert 2013). The inter-species and intra-species distances were computed in the MEGA-X software v. 10.2.4. using the p-distance (Kumar et al. 2018).

Species richness estimation: Our leaf beetle survey in Phu Quoc Island is the first in this locality, and there is no reference catalog for the total expected diversity of Chrysomelidae in this region of Southern Vietnam. To assess, even in exploratory terms, our degree of success in sampling local diversity, we applied nonparametric and rarefaction methods (factor 11x) based on incidence data to investigate expected species richness in different localities (in the national park

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**Fig. 2** Dorsal and ventral habitus of leaf beetle species. (a-c) *Aspidimorpha* sp.; (d-f) *Notosacantha rufa*
and buffer zone) of the data. A range of species richness estimators was calculated using 100 sample order randomization in EstimateS 9.1 (Colwell 2013). The Chao2 indexes were estimated with bias correction, except when the estimated coefficient of variation in abundance and/or incidence distributions was above a certain threshold (CV > 0.5), and the classic Chao2 index was used instead (Chao 1987).

Results

A total of 311 leaf beetle individuals were collected in this study, belonging to 44 OTUs (20 OTUs were identified to species level based on morphological characters) in 31 genera and five subfamilies after comparison with the DNA barcode. Of which 84 individuals of 19 OTUs

![Dorsal and ventral habitus of leaf beetle species.](image)
were obtained in the buffer zone and 227 individuals of 33 OTUs were obtained in Phu Quoc National Park (Table 2, Figs. 2, 3, 4, 5, 6, 7, 8 and 9). Most specimens belonged to the Chrysomelidae subfamilies dominant on Phu Quoc island, namely, Eumolpinae (177 specimens, 14 genera, 17 OTUs) and the assemblage of Galerucines (120 specimens, 10 genera, 20 OTUs). Other subfamilies were less frequent: Criocerinae (6 specimens, one genus, one OTU), Cassidinae (4 specimens, 3 genera, and 3 OTUs), Cryptocephalinae (4 specimens, 3 genera, 3 OTUs) (Table 3). In all collected genera, Dercetina and Monolepta have the highest number of OTU (four OTUs), Cleoporus with 3 OTUs; Nonarthra, Pagria, Aulacophora, Hoplosaenidea, and Taumacera, with two OTUs; other genera only recorded one OUT. Expected species diversity in Phu Quoc Island using the available samples and different partitions of data, with total species richness estimates ranging from 1.38x (ACE) to 1.96x (ICE and Chao2) higher than the number of obtained species (Table 4).

Fig. 4 Dorsal and ventral habitus of leaf beetle species. (a, b) Hemiplatys pascoei; (c-f) Hyperaxis sp.; (g, h) Pseudometaxis serraticolis; (i, j) Scelodonta granulosa; (k, l) Lepina sp
DNA barcoding

A total of 52 morphological OTUs (20 species and 32 OTUs) were extracted DNA, but only 40 of these were successfully sequenced (13 species and 27 OTUs) and generated 62 barcode compliant sequences and one non-barcode compliant sequence. These 63 sequences were uploaded and assigned to 32 BINs (13 species and 19 of 27 OTUs) (in BOLD, Table S1). The differences between the number of successfully sequenced OTUs and the number of BINs showed the polymorphic species, which was confirmed by the analysis of DNA Barcoding and reduced from 52 morphological OTUs to 44 OTUs. The number of OTUs agreed upon between the morphological identification and the DNA barcode method is 28 OTUs (63.6%).

Analysing the sequence composition in BOLD showed that the average percentage of the sequences G, C, A, and T was 16.47% (14.21%-18.28%), 18.13% (14.71%-23.2%), 29.78% (27.67%-32.53%), and 35.61% (30.72%-40.25%), respectively. The average percentage of GC content was 34.61% (29.97%-41.47%) and GC was biased at the first codon position with a mean GC content of 44.23% (38.99%-48.80%) (Table S2). Intraspecific P-distances ranged from 0 to 0.02 with a mean of 0.011 and interspecific P-distances were arranged from 0.23 (Tables S3 and S4).

Discussion

Species-richness of chrysomelidae on Phu Quoc Island

There have been no previous studies on the species richness of Chrysomelidae from the islands in Vietnam to compare with the current study, but this study can be compared with previous studies in Vietnam in the mainland forest, as Nguyen and Gómez – Zurita (2016) Report 155 species based on DNA barcode sequences in Nui Chua National Park by beating canopy trees; Tran and Dang (2005a) use sweeping method to collect 189 species in Tam Dao National Park; 96 species in Dakrong nature reserve (Tran and Dang 2005b); 115 species in the Muong Phang nature reserve, 86 species in Hang Kia – Pa Co nature reserve, and 45 species in Ba Be national park (Dang and Tran 2004). The low species richness obtained in Phu Quoc Island was due to the difference in environmental conditions and forest type on the island, sampling strategy, and sampling method compared with previous studies (Wagner 2000, Whittaker and Fernández 2007). The results of this study can be compared with several studies on Islands from another world as 68 OTUs on the west coast Island of Sabah in Malaysia (Yeong et al. 2018) and 47 species on the island of St. Vincent (Peck 2010). The subfamilies Galerucinae and Eumolpinae are dominant on Phu Quoc Island, which is consistent with recent research in the Oriental region (Yeong et al. 2018; Nguyen and Gómez-Zurita 2016; Kimoto 1989). Two species, Aulacophora indica, and Aulacophora lewisi are agricultural pest species (pumpkin beetle) and were first recorded on the island of Vietnam.

Expected species richness estimators showed that we would have succeeded in sampling 51–72. 5% (depending on the estimator) of the total diversity in the tropical forest on Phu Quoc Island. This is in the same range as that achieved in similar studies of tropical leaf beetle communities, even those using more varied or systematic collection techniques (Sánchez-Reyes et al. 2014; Nguyen and Gomez-Zurita 2016).
DNA barcoding

This study generated 32 BINs in BOLD (19 BIN of unnamed species, 13 BIN of named species). One sequence was not long enough to be assigned to BIN. 18 BINs were unique, 28 BINs were new to BOLD, and 30 BINs were new to Vietnam (Table S1).
18 unique BINs of six species (*Charaea dinhcuongi* Nguyen (new species described from Vietnam in Nguyen et al. (2021)), *Nonarthra variabilis* Baly (distribution wide in Asia as Lee (2014)), *Tituboea laportei* Baly (distribution in Oriental region as Regalin (1997)), *Hemiplatys passedi* Baly (distribution in Cambodia and Vietnam (Kimoto and Gressitt 1982)), *Laccoptera (Sindiola) vigintisexnotata* Boheman (distribution in Assam Indiae orientalis as Borowiec and Świętojańska (2021)) and *Mimastra submetallica* Jacoby (distribution in Southeast Asia as Bezděk (2009)) and 12 OTUs.

Four species, *Lilioceris egena*, *Aulacophora indica*, *A. lewisi*, and *Aspidimorpha sp.*, were recorded in BOLD with a BIN of each species, but are new records in BOLD from Vietnam. Three species, *L. egena*, *A. indica*, and *A. lewisi*, were collected from the known geographic distribution. *Aspidimorpha sp.* was recorded in Myanmar. *A. lewisi* and *A. indica* are agricultural pests of Cucurbitaceae and have a wide distribution in Asia, not in Bangladesh and Pakistan (Lee and Beenen 2015). Still, in BOLD they were recorded in Bangladesh and Pakistan, which are new localities for these species. *Lilioceris egena* has a wide...
Fig. 8 Dorsal and ventral habitus of leaf beetle species. (a, b) *Monolepta wilsoni*; (c, d) *Monolepta* sp. 1; (e, f) *Monolepta* sp. 2; (g, h) *Monolepta* sp. 3; (i, j) *Paleosepharia* sp.; (k, l) *Taumacera phuquoca*; (m, n) *Taumacera* sp.
Fig. 9  Dorsal and ventral habitus of leaf beetle species. (a-h) Dercetina sp. 1; (i-l) Dercetina sp. 2; (m, n) Dercetina sp. 3; (o, p) Dercetina sp. 4
distribution in Asia (Konstantinov et al. 2011) and species recorded in BOLD were collected from their known geographic distributions.

Four OTUs are polymorphism species: *Dercetina* sp. 1 has four morphological identified OTUs (Fig. 9a-h) with mean intraspecific distances of 1.47%, *Hyperaxis* sp. has three morphological identified OTUs (Fig. 4c-f) with mean intraspecific distances of 0.82%, *Pagria* sp. 1 and *Dercetina* sp. 2 have two morphological identified OTUs (Figs. 6a-d and 9i-m, respectively) with mean intraspecific distances of 0% and 0.89% (respectively). All the intra-species distances are similar in the optimal threshold for molecular identification of Chrysomelidae, with genetic distances below 3% for the species level (Magoga et al. 2018; Papadopoulou et al. 2013). Polymorphism in Chrysomelidea is common and has been reported in previous documents (Flinte et al. 2010; Nie et al. 2012; Benkovskaya and Nikonorov 2016; Yeong et al. 2018; Nahrung et al. 2020 and Lee 2022). The polymorphism in Chrysomelidae is a result of adaptation to the environment, such as plant host, temperature, and humidity, and may be under genetic control (De Jong and Nielsen 1999; Nahrung and Allen 2005; Strickland et al. 2019).

### Conclusions

With 44 OTUs collected from Phu Quoc Island, 24 of which are unnamed species, this indicates that many species have not yet been discovered and that there is a need to expand the investigation diversity of Chrysomelidae on other islands in Vietnam. Of the 32 BINs generated from the study, 30 BINs were new to Vietnam and 28 BINs were new to BOLD, which indicates a severe lack of public sequence databases for Chrysomelidae species from Vietnam. The results of this study led to a better understanding of Chrysomelidae diversity on islands in Vietnam and contributed to the gradual building of a public reference database for Chrysomelidae fauna in Vietnam.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42690-022-00884-6.

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### Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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