RESEARCH ARTICLE

First observation of Afromorgus chinensis (boheman, 1858) (Coleoptera: Trogidae) on a rabbit (Oryctolagus cuniculus L., 1758) carcass and its implications in forensic entomology

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ARTICLE HISTORY

ABSTRACT

Received: 3 May 2023 Revised: 25 July 2023 Accepted: 25 July 2023 Published: 30 September 2023 Beetles (Coleoptera) are known to constitute forensic evidence in medico-legal investigations as their presence can be used to date human remains in almost all decomposition stages. Many forensic studies focus on the successional colonization pattern of flies (Diptera); however, beetles have not so far been studied extensively for this aspect. A beetle of the genus Afromorgus Scholtz, 1986, A. chinensis (Boheman, 1858) (Scarabaeoidea: Trogidae), was found beneath a late decaying rabbit carcass at Paya Indah Wetland, Dengkil, Malaysia, for the first time. Both genus and species are already known to occur in Malaysia from literature.

Keywords: Forensic entomology; Malaysia; Afromorgus chinensis; rabbit carcass; Trogidae.

INTRODUCTION

Studies on insects and arthropods for investigation in legal matters are called forensic entomology (Hall & Doisy, 1993). Carrion decomposition starts immediately after death, and internal decomposition (autolysis) represents the first stage of this process. Insects arrive on the corpse within a few seconds and contribute significantly to the decaying process (Mann et al., 1990). Necrophagous flies arrive abundantly during the first stages of decomposition and deposit their eggs on fresh and/ or bloated carcasses. Forensically important coleoptera are increasingly attracted in numbers from the beginning to the more advanced and dry stages of the decomposition process (Zhuang et al., 2011), either due to an exclusive necrophagous behaviour on carrion (predominant) or predation on larvae and adults of other necrophagous arthropods, and/or both feeding strategies. To date, the majority of forensic entomological studies were conducted on flies, while a few were carried out specifically on necrophagous beetles (Cai et al., 2011).

The minimum post-mortem interval (mPMI), which could constitute entomological evidence (Kulshrestha & Satpathy, 2001), can be estimated successfully by means of beetles collected from human remains. Trogid beetles (Coleoptera: Scarabaeoidea: Trogidae: Hide beetles), among others, have a considerable forensic importance (Strümpher et al., 2014), as they consist of six extant genera with about 300 species worldwide. All their representatives,

both adults and larvae, feed prevalently on various sources of keratin in different kinds of shed, nests, animal remains and excretions (dry skin, feathers, hairs, bones, old wool remnants, dried faeces etc.) after the soft tissue has been almost entirely consumed. Both adults and larvae are usually found on animal carcasses during the late skeletal stage of decomposition (Vaurie, 1962). Several studies, mainly related to the consequences of environmental alterations, have considered trogid beetles as economic and ecosystem health indicators (Spector, 2006; Gardner et al., 2008). Some species of the genus Omorgus Erichson, 1847 are found obligatorily in nests of owls (Vaurie, 1962), others occur in animal excavations (Scholtz, 1990), and in nests of mammals and sea turtles (Vaurie, 1962; Moron & Deloya, 1991).

The genus Afromorgus Scholtz, 1986 is widespread throughout Africa (Scholtz, 1982) as well as Oriental and South-Eastern Palaearctic Regions (Zidek, 2013; Pittino & Bezdk, 2016). A. chinensis is known to occur throughout China, Taiwan and Japan in the Palaearctic Region; Indochina: Vietnam (Annam, Cochinchina, Tonkin), Cambodia, Laos; Thailand, Myanmar, Indonesia (Java, Sumatra), and Malaysia in the Oriental Region (Harold, 1872; Waterhouse, 1875; Balthasar, 1936; Paulian, 1945; Haaf, 1954; Scholtz, 1982; Masumoto et al., 2005; Zidek, 2013; Pittino, 2006; Pittino & Bezdk, 2016; Pittino, unpublished data). However, the finding of a single female specimen of A.chinensis (Boheman, 1858) beneath a rabbit carcass in Malaysia is hereby recorded for the first time.

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MATERIALS AND METHODS

A forensic entomological study was conducted at Paya Indah Wetland, Dengkil, Malaysia (Figure 1; 2.86°N 101.62°E; 266 m above sea level), site dominated by a tropical bamboo species, Bambusa multiplex (buluh pagar in Malay). The mean temperature and precipitation of the site were measured with a data logger Elitech 32000 (UK), and a rain gauge, respectively. Three rabbit (Oryctolagus cuniculus Linnaeus, 1758) carcasses were placed on the ground surface on 9 August 2019 for entomological and soil chemical studies (Figure 2). Each carcass was kept in a cage 90 cm long \times 90 cm wide \times 45 cm high to prevent scavenging animals, such as monitor lizards and civet cats. 15 field observations were made every two days for 30 days in all: insects and soil samples associated with rabbit carcasses were taken on each sampling day.

We documented the presence of *Afromorgus chinensis* during the decomposition of a rabbit carcass (Figure 1). The mean temperature of the study site was $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and the average rainfall recorded was 45 mm throughout the study period. The single female specimen (n = 1) was collected by a forceps beneath a carcass in an advanced stage of decay on the day 18 post-mortem, preserved in 70% ethanol and transferred for identification to the Parasitology Laboratory, Institute of Medical Molecular Biotechnology (IMMB), Universiti Teknologi MARA (UiTM). It was first identified with a stereomicroscope Olympus SZ51 at 5.6x magnification, then the coauthor (Pittino, R.) performed dissection of the genitalia confirming sex and determination with a Zeiss DR at 16x / 63x magnification.

Two legs of A. chinensis were used for DNA extraction. Genomic DNA was extracted using the i-genomic CTB DNA Extraction Mini Kit

(iNtron Biotechnology Inc., Seongnam, South Korea) following the manufacturer's instructions. The COI gene region of the beetle was amplified using generic invertebrate amplification primers (Folmer $et~al.,\,1994$). The polymerase chain reaction (PCR) amplification was undertaken in a final volume of 25 μL containing 25–50 ng genomic DNA, 12.5 μL of MyTaq Red Mix (BioLine, Australia), and 10 pmol of each forward and reverse primer. The PCR cycling condition was 1 min at 96°C, followed by 35 cycles of denaturation at 94°C for 1 min; annealing at 55°C for 1 min; extension at 72°C for 1 min 30 s, and a final extension at 72°C for 7 min.

The COI sequence of A chinensis (OL423437) generated from this study was deposited at the National Center for Biotechnology Information (NCBI) GenBank. The COI sequences of Omorgus spp., namely O. punctatus (Germat, 1824), O. monachus (Herbst, 1790), O. suberosus (F.,1775) and Afromorgus chinensis were retrieved from the GenBank for phylogenetic tree reconstruction. Trox aequalis Say, 1831 (HM433166) was used as an outgroup. A neighbor-joining (NJ) phylogenetic tree of Omorgus/Afromorgus was traced using MEGA X (Kumar et al., 2018). The NJ bootstrap values were estimated using 1,000 replicates with Kimura's two-parameter model of substitution (K2P distance). To determine interspecific variation among species, uncorrected (p) pairwise genetic distances were calculated using PAUP 4.0B10 (Swofford, 2002). Unfortunately, additional COI sequences of Afromorgus spp. were not available at GenBank, thus a complete genetical analysis could not be carried out, however, we can equally retain the phylogenetic tree resulting from the present study by virtue of the close phyletically between the two genera.

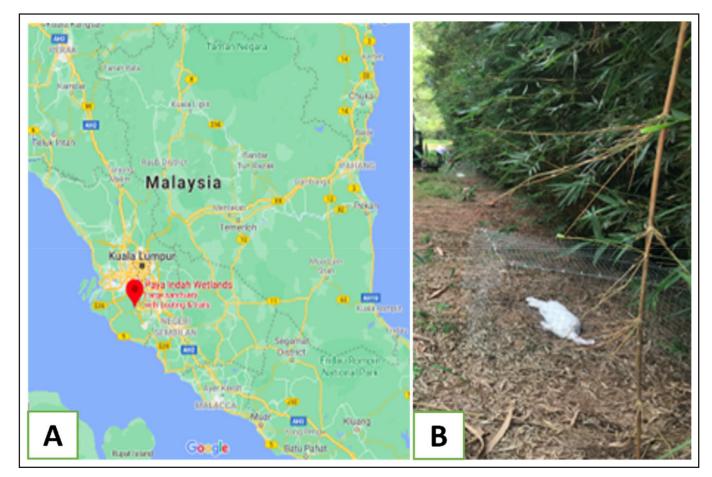


Figure 1. (A). A map showing the study location at Paya Indah Wetlands located in Dengkil, Selangor, Peninsular Malaysia (source: www.google. com.my/maps). (B). A rabbit carcass placed on the soil surface at the study site. A cage was set up around the carcass to prevent vertebrate scavengers.

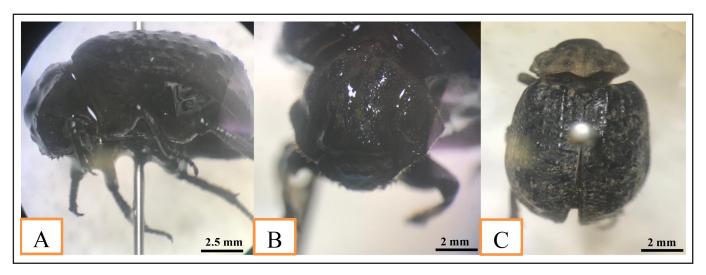


Figure 2. Habitus of the adult Afromorgus chinensis (Coleoptera: Trogidae). (A) Lateral view; (B) Forebody; (C) Dorsal view.

RESULTS AND DISCUSSION

As stated above, the determination of Afromorgus chinensis, was essentially based on external morphology of a single available female specimen, but it should be confirmed by the study of at least one additional male. The collected specimen is blackish, about 12 mm long and winged with prominent humeral umbones and smooth pronotal and elytral margins. Head and pronotum closely punctate, frons distinctly bituberculate, tubercles suboval, transverse, well separated from each other, vertex with small but distinct short and narrow shiny median area. Pronotum clearly attenuated forward and dilated backward, sides widely explanate with narrowly rounded produced posterior angles, margins slightly sinuate with rather long quite close brownish setae, pronotal disc prominent with distinct anteromedian furrow and deep median basal fovea. Elytral sides broadly explanate with fine short sparse setae along margins, odd intervals (costae) with smooth suboval weakly convex subconfluent tubercles, posterior end of tubercles tomentose with fine short pubescence, the innermost costa (3rd from the suture) more elevated forward, other costae uniformly flattish. Even intervals (intercostae) with small tubercles and intervening shiny glabrous flat sub-square irregular areas; striae ill defined with sparse indistinct coarse punctures. Female genitalia with no distinctive diagnostic characters.

As expected, in our study the genetical analysis conducted on A. chinensis proved our sample clearly differs from all examined Omorgus spp. (suberosus, monachus, punctatus) by 15.05-15.86%, 16.27%, and 18.37-19.24, respectively, but, quite unexpectedly, showed large discrepancy (by 18.11%, Figure 3) with respect to the sole representative in GenBank (MK937809). However, the origin and details of this strain are unknown, hence further comparative studies could not be carried out. It is uncertain whether this is a matter of a cryptic species complex or because of imperfect taxonomy. The verified great genetic difference would suggest them as separate species, but the morphological characters should rather suggest our sample may fall within the variability range observed in several specimens from the Asian South East (Pittino, unpublished data). Consequently, both specimens providing the respective genetic materials should be verified with certainty having further males from the study site.

Various studies of forensic entomology recorded presence of trogid beetles on animal carcasses usually at the latest stages of decomposition across different regions, as from Algeria (Guerroudj &

Berchi, 2019; Naima, 2020), Argentina (Horenstein & Linhares, 2011), Brazil (Mise et al., 2008; Mayer & Vasconcelos, 2013; Santos et al., 2014; Zanetti et al., 2015), Ivory Coast (Koffi et al., 2017), Poland (Jarmusz, 2020), Thailand (Vitta et al., 2007), and the USA (Tabor et al., 2004, 2005; Goddard, 2012). Reports of trogids, scarabeids, and dermestids infestations on human corpses at the skeletal stage of decomposition were not uncommon (Rodriguez & Bass, 1983). Philips et al. (2003) collected 110 adults and 62 larvae of forensically important mites (Acari) on trogid beetles found on pellets of owl and eagle, and on owl, eagle, falcon, and small-mammal carcasses in different states of the USA. Correa et al. (2013) collected 24, 7, and 1 specimen, respectively, of Omorgus suberosus (F.,1775), in different traps baited with carrion, cow dung, and pig manure. Our recent observations also confirmed that O. suberosus is attracted to decaying animal carcasses. Climatic condition is an important factor affecting the seasonality of *Omorgus* spp.: Lopes et al. (2009) observed their highest abundance during the rainy season in Brazil. Trevilla-Rebollar et al. (2010) sampled 12 O. suberosus individuals from April to December 2007 with carrion-baited traps in Mexico.

Nevertheless, the use of these observations in forensic entomology is still underestimated, which would indicate the importance of documenting their occurrence on decaying carrions. This study first reports the presence of *Afromorgus chinensis* on a rabbit carcass in a late decaying stage. Further similar findings should be recorded as they could be useful, mainly in establishing the mPMI or serving as a locality indicator. Thus, we recommend new ecological and behavioural research on trogid beetles in order to better understand their actual role in the process of carrion decomposition and subsequently reveal their actual forensic significance.

Finally, further field research should be conducted in the future on the same environments as the present study in order to collect additional both male and female specimens. This would allow us to complete both morphological and genetic studies hereby started, to definitely confirm the specific identity of the sole female specimen examined, maybe also clarifying origin, details, unknowns, and uncertain phyletic ally of this strain that have emerged from the neighbour-joining tree of our genetic analysis. The apparent genetic difference from the other *A. chinensis* from GenBank (MK937809) actually calls for a valid explanation.

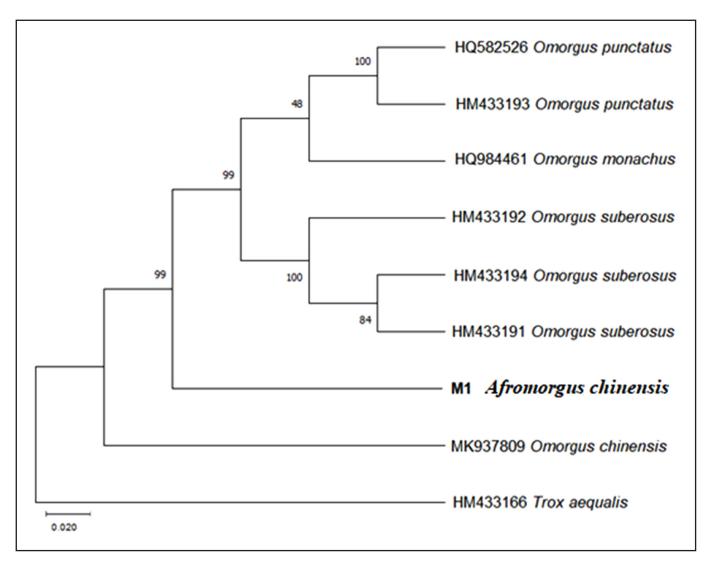


Figure 3. Neighbor-joining tree of *Omorgus* and *Afromorgus* species (Coleoptera: Trogidae) based on the mitochondrial COI gene sequences. Scale bar represents 0.020 substitutions per nucleotide position.

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Conflict of interests

The authors declare that they have no conflict of interests.

Ethical consideration

The Research Ethics Committee (UITM CARE) has approved the study protocol of Universiti Teknologi MARA [262/2019 (15/02/2019)]

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