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TECHNICAL NOTE

PATHOLOGY/BIOLOGY

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Eristalinus arvorum (Fabricius, 1787) (Diptera: Syrphidae) in Human Skull: A New Fly Species of Forensic Importance†

ABSTRACT: A body of an unknown adult female was found within a shallow burial ground in Malaysia whereas the skull was exposed and visible on the ground. During autopsy examination, nine insect larvae were recovered from the interior of the human skull and subsequently preserved in 70% ethanol. The larvae were greyish in appearance, each with a posterior elongated breathing tube. A week after the autopsy, more larvae were collected at the burial site, and some of them were reared into adults. Adult specimens and larvae from the skull and from the burial site were sequenced to obtain DNA barcodes. Results showed all adult flies reared from the burial site, as well as the larvae collected from the skull were identified as *Eristalinus arvorum* (Fabricius, 1787) (Diptera: Syrphidae). Here, we report the colonization of *E. arvorum* larvae on a human corpse for the first time.

KEYWORDS: forensic science, forensic entomology, decomposition, *Eristalinus arvorum*, hover flies, Syrphidae, rat-tailed maggot, Malaysia

The family Syrphidae (Insecta: Diptera), commonly known as hoverflies or flower flies, is one of the largest dipteran families with over 6000 described species widely distributed (1,2). Adult flies have a rather uniform biology, frequently visiting flowers to feed on pollen and nectar (3). They are declared as important pollinators in agricultural and natural habitats (4,5), and some species have been used as bioindicators in biodiversity loss assessments (6–8). Larvae, on the other hand, have a large array of natural histories and feeding modes, including saprophagous, phytophagous, fungivores, parasitoids, and predators (9–12). Due to their feeding mode, some syrphid species are considered important biological control agents of pests (13–15) and as decomposers of organic matter (16–18).

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Knutson et al. (19) recorded 84 genera and 771 species of Syrphidae from the Oriental Region. Of these, 201 species and 53 genera were listed from Peninsular Malaysia and Borneo. More recently, a few new species have been described or reported from Malaysia (20–25). The most comprehensive work on Malaysian Syrphidae is still the series of Curran (26–29); unfortunately, they are incomplete in the species treated and obsolete in the classification used. *Eristalinus* Rondani, 1845 is listed among the genera occurring in Malaysia, with ten species recorded (2,19). Adults of *Eristalinus* play an important role in ecosystem services and have been reported as efficient pollinators and frequent flower visitors in onion (30,31), fennel (32), carrot (33), radish (34), mango (35), ber or jujube (36), brown or Indian mustard (37), bahera or beleric tree (38), Indian sandalwood (39), and Bengal quince (40), among others (41–43).

The genus *Eristalinus* is a member of the tribe Eristalini, whose saprophagous larvae exploit a wide range of aquatic and semi-aquatic habitats rich in decaying plant materials including wet manure, sewage, and hydrothermal springs (44,45). These filter-feeding larvae are characterized by long posterior breathing tubes that are extended to breathe from the surface when are at depth in the water body in which they are developing, hence known as long- or rat-tailed maggots (11). Although the morphological characters of the immature stages have been proven informative for the taxonomy of Syrphidae, less than 15% of the larvae of the tribe Eristalini are known (44,46,47).

Immature stages of the Eristalinae are associated with human activities, for example, among sewage of farms (pig farming), sewage of olive oil factories, carcasses of animals (44,48). A few eristaline species have been documented as causative agents in accidental myiasis in humans, mostly identified as *Eristalis* sp. or

Eristalis tenax (Linnaeus, 1758). Reported intestinal myiases may be due to the ingestion of water with some eggs or very small larvae of these eristalines (49–52), but there are also cases of human urogenital myiasis (53,54), vaginal myiasis in a cow (55), and also myiasis caused by non-eristalines, such as intestinal myiasis due to *Ornidia obesa* (Fabricius, 1775) (tribe Volucellini) (56).

Syrphid flies have been also reported in forensic entomology cases. In the specific case of Malaysia, Lee et al. (57) reviewed forensic entomology cases in this country from 1972 to 2002 and found only one case where larvae of *Eristalis* sp. were found in a corpse. The authors stated that the presence of the eristaline larvae may indicate that the corpse was in an environment associated with water, as these larvae are usually aquatic in nature. Salleh et al. (58) reported third instar of *Eristalis tenax* in a body found in a river, and more recently, Syamsa et al. (59) also reported *Eristalis* spp. in two outdoor forensic cases in Malaysia. Other than *Eristalis*, *Syritta pipiens* (Linnaeus, 1758) in human cadavers in Italy (60) and *O. obesa* from pig carcasses in Brazil (61) have been recorded in forensic entomology. Hardly any previous work where flower flies were found associated with forensic cases has identified the syrphid to species level, and due to the overall morphological appearance, most of these eristaline immatures were identified as *Eristalis* sp., although this might not be the case due to misidentification. The absence of taxonomic work on eristaline immature to help with identification in forensic cases and the lack of biological information of syrphid species, especially in the forensic context, prompted us to study a new reported species of forensic relevance using morphological and molecular data. Here, we reported larval colonization on a human corpse by *Eristalinus arvorum* (Fabricius, 1787) (Diptera: Syrphidae) for the first time.

Case Description

A body of an unknown adult female was discovered in May 2017 at Bukit Beruntung, Selangor, Malaysia (3°24′17″N 101°32′54″E, 42 m.a.s.l.). The body was partially buried with the skull exposed. The depth of burial was estimated at 0.5 m from the ground surface. The burial site was an open area in a secondary forest approximately 56 m from the nearest driveway. The dominant vegetation found at the burial site was *Imperata cylindrica* Cirillo, and the soil was moderately wet probably due to previous rains. The remains were sent to the Forensic Department at the Sungai Buloh Hospital for medico-legal autopsy examination. General examination showed that the deceased was in an advanced stage of decomposition. The outer aspect of the skull was completely void of soft tissues. The decomposing brain matter appeared greyish and semi-fluid in consistency. Larvae were present inside the skull. The upper part of the torso was mostly skeletonized with intermingled ribs. The decomposing skin and muscle of the abdomen were present; however, the internal organs had completely turned into a mass of brownish clay-like substance. The upper and lower limbs were disarticulated and decomposing soft tissues were present covering the skeletal structure. Autopsy and anthropology examinations concluded the skeletal remains belonged to a female, estimated age of mid-thirties to mid-forties, having features of Mongoloid (Asian) in ancestry. The cause of death was multiple blunt traumas. The manner of death was homicide.

Materials and Methods

During autopsy, larvae were collected from the skull interior using forceps and were preserved in 70% ethanol. The larvae were then weighted using a digital scale (A&D FX-300i, Japan) and measured using a ruler, photographed, and examined under a digital stereoscope (Olympus SZX7, Japan) with maximum magnification 5.6×. General characteristics of larval morphology were determined using Rotheray (9), Pérez-Bañón et al. (44), Thyssen (62), and Dixon (63).

As all the larvae collected from the skull were dead, there was no larva available for rearing purposes. To solve this problem, we aimed to collect from the death scene adult syrphid flies or larvae for rearing purposes. Therefore, a visit to the death scene at Bukit Beruntung, Selangor was conducted a week after the autopsy where two fresh cattle liver baits were setup to attract syrphid flies. The baits were placed in plastic containers half-filled with rainwater to imitate the natural oviposition site (60). The cattle liver baits were left at the scene (approximately 7.4 m from the burial site) for five days to initiate fly oviposition, but after this time no syrphid larva was found in the liver baits. However, nine live rat-tailed maggots were observed moving in the trapped water of the burial site, which were collected and brought back alive to the Institute for Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA (UiTM) for rearing. One maggot was preserved in 90% ethanol while the rest (n = 8) were reared in plastic containers containing cattle liver (~50 g) and water (100 mL, added *ad libidum*) under mean temperature 28.4°C from 29 May to 3 July 2017. Emerged adult flies were provided with water and sugar in order to maintain the colony in the laboratory. Cattle liver was also supplied to initiate oviposition after a week of eclosion. Daily observations were made and dead adults along the rearing process were pinned, photographed, labeled, and dried in an oven at 40°C for three days. Both preserved larvae and pinned adult specimens were sent to the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) in Bonn, Germany, for morphological and molecular identification of the species.

Species Identification

Adults reared from larvae collected at the burial site were identified to species using available identification keys, for example, Curran (64) and Thompson et al. (65). At the same time, reared adults and larvae collected from the skull and at the burial site were used to sequence the 5′ region of the cytochrome c oxidase subunit I (COI) gene, also known as the DNA barcode (66,67). One leg, in the case of adults, and part of the posterior breathing tube or the entire specimen, in the case of immature stages, were used for DNA extraction. Extractions were carried out using the NucleoSpin Tissue DNA Extraction kit (Machery-Nagel, Düren, Germany) following the manufacturer's instructions and samples were resuspended in 100 μL ultrapure water. Entire specimens or remnants of specimens were preserved and labeled as DNA voucher specimens for the purpose of morphological studies and deposited in the collections of the ZFMK, as listed in Table 2.

The COI fragment was amplified using the forward primer LCO1-1490 (68) and the reverse primer COI-Dipt-2183R, also known as COI-780R (69). PCR amplification protocols were the same as described in Rozo-Lopez and Mengual (70). All new sequences were submitted to GenBank (see Table 2 for accession numbers).

Results

From the adult syrphid flies reared from larvae collected at the burial site, only five adults managed to eclose from pupae

and they survived for 2–3 weeks in an insect cage. These adults were identified as *Eristalinus arvorum* (Fabricius, 1787) (Diptera: Syrphidae) based on morphological characters (Fig. 1) using the above-mentioned identification keys and comparing them with collection material at the ZFMK.

As for larvae collected during the autopsy, the mean larval body length was 29.77 ± 7.24 mm, mean width 3.51 ± 0.80 mm, and mean weight 0.09 ± 0.03 g (Fig. 2, Table 1). The larvae were greyish in color, each with an elongated breathing tube (Fig. 3). Morphological and ecological characteristics suggested that these larvae were immature stages of the family Syrphidae, most likely of the tribe Eristalini, using the available identification keys and biological information (3,9,46,63).

A total of nine COI sequences, at least 652 bp long, were obtained from two adult males, three adult females, two-third-instar larvae from the skull, and two other immatures (one-third-instar and another smaller larva) from the burial site. The uncorrected pairwise distance of the sequences obtained from the adults and from the larvae range from 0 to 0.613%, except for the sequence of the small larva (specimen ZFMK-DIP-00057717) that differed a bit more (2.837–3.144%). The identical COI sequences of adults (identified using morphological characters) and third-instar larvae from the skull and burial site confirm the identity of the larvae found in the human skull as *Eristalinus arvorum* (Table 2). In order to corroborate our molecular identification of the immature stages and test the intraspecific variability of *E. arvorum*, we used the BOLD Identification System (IDS; http://www.boldsystems.org/index.php/IDS_OpenIdEngine). Our newly obtained sequences are identical or extremely similar (0–0.311%) to another COI sequence of *E. arvorum* (Process ID: SYCNC195-16), provided by Jeff H. Skevington (CNC, Ottawa, Canada). The reported uncorrected pairwise distances are due to common molecular variability among individuals of the same species.

The small larva from the burial site (specimen ZFMK-DIP-00057717) might not be the same species based on the

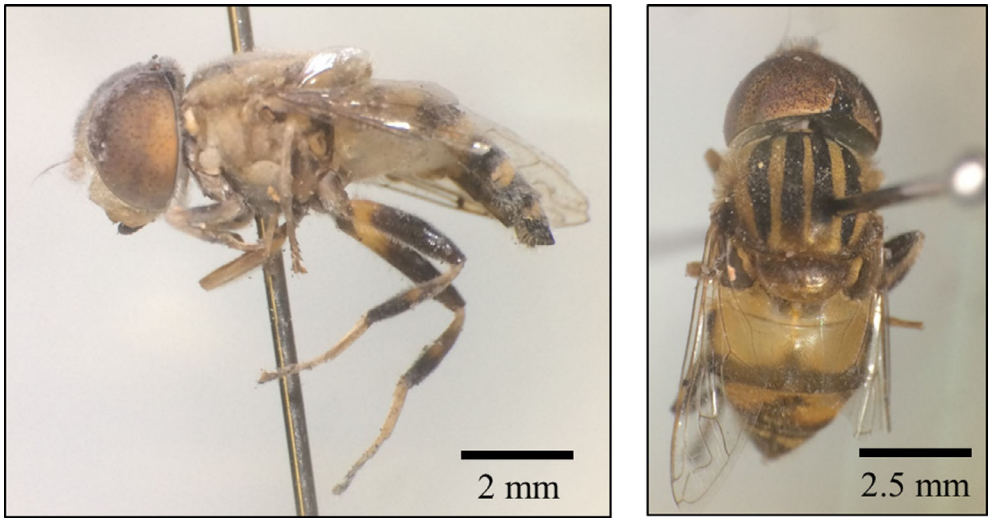

FIG. 1—*Adult of* Eristalinus arvorum *(Diptera: Syrphidae) reared from larvae collected at the burial site in Bukit Beruntung, Selangor, Malaysia. Habitus view of the female adult fly, ZFMK-DIP-00057713 (Right); Dorsal view of the female adult fly, ZFMK-DIP-00057713 (Left).*

FIG. 2—Eristalinus arvorum *(Diptera: Syrphidae) larvae collected from the human skull interior in the present case during autopsy. Note the long telescopic respiratory tubes present on each larva. Among these, two-third-instar specimens were sent to Germany (ZFMK) for molecular identification.*

differences in the COI sequence and, without any further evidence, we identified it as *Eristalinus* sp.

Discussion

Larvae found in the skull, adults reared from larvae collected later at the burial site and those larvae sampled later that did not develop into adults were identified as *Eristalinus arvorum,* either via morphological characters or DNA barcodes. *Eristalinus arvorum* is one of the most common pollinators for a variety of fruit trees in Asia (31,39,71) and is widely distributed in the Oriental, Australasian, and Palaearctic Regions, ranging from Hawaii to Japan, south to Australia and west to India (72). But, as in many other species, the natural history of its immature stages is completely unknown.

Flower fly larvae pass through three instars or stages before developing into pupae (9). The first two stages are relatively short, usually lasting a few days each. The third instar may last from several days to many months, even years depending on species and abiotic condition (9). Knowing the development of a species and understanding the influence of environmental factors may help to estimate the time since death (the Post-Mortem Interval, or PMI), a critical value in forensic investigations to narrow the "window of time" during which the death may have occurred (73). Due to the lack of local developmental information for *E. arvorum*, larvae found in the skull were not used to estimate the minimum Post-Mortem Interval (mPMI).

Available information regarding the development of *Eristalinus* immatures was obtained in controlled laboratory conditions using artificial rearing media of nonhuman origin (74). Although Hurtado Asencio (74) studied two different *Eristalinus* species, the larval development had several variables to study, such as temperature, larval sex, species, crowdedness, light intensity and rearing diet, among others. Thus, a grounded estimation of the mPMI cannot be based on studies conducted using different species of fly, at different geographical region or using different types of rearing diet.

Syrphidae do not have the same importance in forensic entomology as other dipteran families like Calliphoridae or Sarcophagidae (75–77), but they can be found in forensic cases around the globe (e.g., Archer and Ranson (78); Lindgren et al. (79)). Due to their larval biology and feeding modes, larvae of Syrphidae may play an important role in the decomposition. Thus, their identification needs to be precise and their study is recommended. In two reviews of forensic entomology in Malaysia (77,80) no flower fly species was included, even though Salleh et al. (58) reported third instar of *Eristalis tenax* and Lee et al. (57) mentioned *Eristalis* sp. Later, Syamsa et al. (59) also reported a larva of *Eristalis* sp. from an outdoor forensic case in Malaysia. The lack of identification keys and information about the morphology of *Eristalinus* larvae in Asia hamper the proper identification of immatures. Due to the overall similarity in the larval morphology of the entire tribe Eristalini, some immature stages of the genera of this tribe might be misidentified. The presence of syrphid immature in forensic cases deserves study to ensure an accurate identification, and this can be only done by: 1) studying the morphology of the larvae using electronic microscopy (i.e., Pérez-Bañón et al. (44); Campoy et al. (81)); 2) rearing the larvae into adults in order to base the species identification on adults, and; 3) using DNA barcodes.

So far, only one work used integrative taxonomy to study the genus *Eristalinus* in Europe based on larval morphology and molecular characters (44). However, DNA barcodes have been applied in forensic entomology for species identification with great success (82–91). This short DNA sequence of the COI gene has been also used to link stages (larva and adult) and sexes (male and female) in flower flies (92,93). Our work is another example of the use of DNA barcoding to corroborate the identification of immature stages. Moreover, the newly obtained COI sequences will help in future investigations to assess the taxonomy of eristaline larvae.

TABLE 1—*Measurements of body length, tail length, total length (body and tail), width length, and weight for each individual of* Eristalinus arvorum *(Diptera: Syrphidae) larva collected from the human skull in the present case in Selangor, Malaysia.*

No.	Body Length (mm)	Tail Length (mm)	Total Length (body + tail) (mm)	Width (mm)	Weight (g)
1	15.69	18.03	33.72	3.94	0.120
2	15.78	23.28	39.06	3.83	0.118
3	15.13	21.83	36.96	3.75	0.108
4	14.89	18.89	33.78	3.66	0.098
5	13.99	16.58	30.57	4.04	0.074
6	13.87	15.34	29.21	4.12	0.098
7	14.34	14.09	28.43	3.64	0.101
8	11.98	9.87*	21.85	3.21	0.051
9	6.06	8.32	14.38	1.36	0.050
Mean	13.53	16.25	29.77	3.51	0.090
SD	2.85	4.71	7.24	0.80	0.030

Length measurements were done using a software (CellD, Olympus, Japan) installed on a digital microscope, while weight was measured using a digital scale.

*Tail was partly broken during measurement.

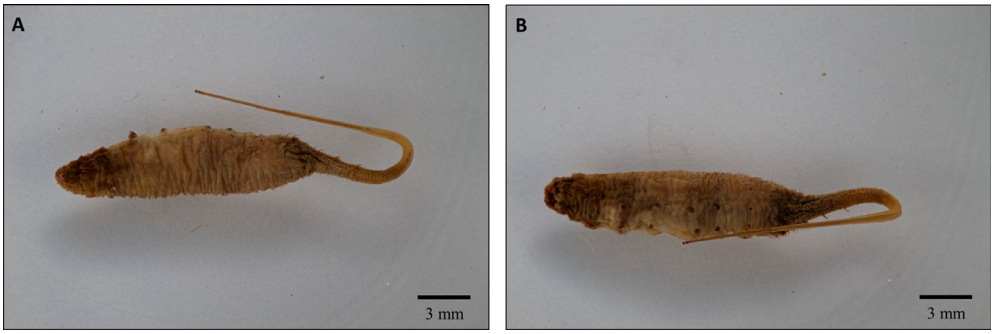

FIG. 3—*Dorsal (A) and ventral (B) view of third-instar larva of Eristalinus arvorum (Diptera: Syrphidae) collected from the present case.*

TABLE 2—*Specimens (adults and larvae) sequenced to obtained COI sequences, including GenBank accession numbers.*

Species	Stage	Label Information	DNA Voucher Code	COI-5′
Eristalinus arvorum (Fabricius, 1787)	Adult, male	MALAYSIA: Selangor, Bukit Beruntung, 28.VI.2017, 03°24′17″ N 101°32′54″ E. Leg.: Isa M.S. Ex. larva from freshwater pond at a burial site. Reared on beef liver.	ZFMK-DIP-00057712 (laboratory code D347)	MK751015
Eristalinus arvorum (Fabricius, 1787)	Adult, female	MALAYSIA: Selangor, Bukit Beruntung, 28.VI.2017, 03°24′17″ N 101°32′54″ E. Leg.: Isa M.S. Ex. larva from freshwater pond at a burial site. Reared on beef liver.	ZFMK-DIP-00057713 (laboratory code D348)	MK751016
Eristalinus arvorum (Fabricius, 1787)	Adult, female	MALAYSIA: Selangor, Bukit Beruntung, 28.VI.2017, 03°24′17″ N 101°32′54″ E. Leg.: Isa M.S. Ex. larva from freshwater pond at a burial site. Reared on beef liver.	ZFMK-DIP-00057714 (laboratory code D349)	MK751017
Eristalinus arvorum (Fabricius, 1787)	Adult, male	MALAYSIA: Selangor, Bukit Beruntung, 28.VI.2017, 03°24′17″ N 101°32′54″ E. Leg.: Isa M.S. Ex. larva from freshwater pond at a burial site. Reared on beef liver.	ZFMK-DIP-00057715 (laboratory code D350)	MK751018
Eristalinus arvorum (Fabricius, 1787)	Adult, female	MALAYSIA: Selangor, Bukit Beruntung, 28.VI.2017, 03°24′17″ N 101°32′54″ E. Leg.: Isa M.S. Ex. larva from freshwater pond at a burial site. Reared on beef liver.	ZFMK-DIP-00057716 (laboratory code D351)	MK751019
Eristalinus sp.	Larva, possibly L1	MALAYSIA: Selangor, Bukit Beruntung, 29.V.2017, 03°24′17″ N 101°32′54″ E. Leg.: Isa M.S. Ex. freshwater pond at a burial site.	ZFMK-DIP-00057717 (laboratory code D352)	MK751020
Eristalinus arvorum (Fabricius, 1787)	Larva, L3	MALAYSIA: Selangor, Bukit Beruntung, 29.V.2017, 03°24′17″ N 101°32′54″ E. Leg.: Isa M.S. Ex. freshwater pond at a burial site.	ZFMK-DIP-00057718 (laboratory code D353)	MK751021
Eristalinus arvorum (Fabricius, 1787)	Larva, L3	MALAYSIA: Selangor, Bukit Beruntung, 17.V.2017, 03°24′17″ N 101°32′54″ E. Leg.: R. Rahimi. Ex. human skull.	ZFMK-DIP-00057719 (laboratory code D355)	MK751022
Eristalinus arvorum (Fabricius, 1787)	Larva, L3	MALAYSIA: Selangor, Bukit Beruntung, 17.V.2017, 03°24′17″ N 101°32′54″ E. Leg.: R. Rahimi. Ex. human skull.	ZFMK-DIP-00057720 (laboratory code D356)	MK751023

To our knowledge, this is the first record of the genus *Eristalinus* colonizing a human cadaver and it is the first time that larvae of *E. arvorum* are reported on human corpses. Further studies on flower fly larval developmental duration and ecological role (e.g., sequence during insect succession) during carrion decomposition is therefore warranted.

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