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## A new species of clearwing moth (Lepidoptera: Sesiidae: Osminiini) from Peninsular Malaysia, exhibiting bee-like morphology and behaviour

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### Abstract

A new species of Sesiidae, tribe Osminiini from Peninsular Malaysia, *Heterosphecia pahangensis* Skowron, displaying numerous bee-mimicking features, is described. DNA barcodes showed significant differences with related taxa. However, the paucity of Sesiidae barcodes from Southeast Asia prevents meaningful taxonomic comparisons. The closest match out of published data on Sesiidae barcodes is *Heterosphecia bantanakai*, Arita & Gorbunov (2000a) from the tribe Osminiini, which has 9.98% sequence divergence from *Heterosphecia pahangensis*. Photographs of the moth in its natural habitat are shown. Behavioural aspects, such as mud-puddling and mode of flight, are described and presented in a video.

**Key words:** *Heterosphecia*, mimicry, new species, *pahangensis*, Malaysia

**Supplementary data:** link to video: <https://vimeo.com/136088402> password: pahangensis

### Introduction

Representatives of the family Sesiidae are known for their excellent mimicry of wasps and bees (Robinson *et al.* 1994) which is widespread across different genera. For example, members of *Dasyphecia* are thought to be mimics of the genus *Bombus* (Kallies & Arita 2005) whereas *Podosesia syringae* is a wasp mimic (Webster 1897). The mimicking characteristics include partially hyaline, narrow wings, the presence of hair-like scales on the legs and other parts of the body, simple and often clavate antennae, and brightly coloured bands on the abdomen.

A new species of clearwing moth exhibiting bee-like morphology and behaviour was discovered in Peninsular Malaysia and is described here. DNA sequencing of the COI gene indicated that it belongs to the tribe Osminiini Duckworth and Eichlin (1977) and is closely related to *Heterosphecia* Le Cerf (1916), *Aschistophleps* Hampson (1892), and *Pyrophleps* Arita and Gorbunov (2000a). Morphological similarity to the mentioned genera confirms these results. Taking into consideration the work of Arita and Gorbunov (1995, 2000a, 2000b) and Kallies (2003) the new species is placed in the genus *Heterosphecia*. It is the eighth species belonging to this genus and first from Peninsular Malaysia.

### Material and methods

Field observations involved temperature and humidity measurements, pheromone, honey and salt lure testing as well as advanced photographic documentation. Pheromone lures for the following species were tested: *Sesia apiformis*, *Paranthrene tabaniformis*, *Pennisetia hylaeiformis*, *Synanthedon tipuliformis*, *S. vespiformis*, *S. myopaeformis* (Pherobank, Wageningen, Netherlands). Each lure was placed at the type locality on a different day and observed from around 10 am to 5 pm, however they failed to attract any sesiids.

Individuals were netted without the use of pheromones and kept in a cage until natural death. They were then immediately pinned. Male genitalia were dissected, stained with mercurochrome, and mounted in Euparal medium. Wings were stained with eosin and mounted in Euparal. A photographic documentation of morphological details, genitalia and wing preparations was done using high-resolution Olympus SZX10 and SZX16 stereomicroscopes and an Olympus DSX100 opto-digital microscope.

DNA sequence analyses were conducted on total DNA isolated from a specimen's leg. The tissue was lysed and DNA purified with the use of a silica-based purification method, involving binding DNA to a glass fiber membrane in the presence of chaotropic salts (Ivanova *et al.* 2006). Using isolated DNA as a template, the cytochrome c oxidase subunit I gene was PCR amplified with the use of primers:

5'-ATTCAACCAATCATAAAGATATTGG-3' and

5'-TAAACTTCTGGATGTCCAAAAATCA-3'. DNA was sequenced using an ABI Prism 310 automated sequencer with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The resulting sequencing data was analysed through the Basic Local Alignment Search Tool (BLAST) and DNASIS MAX software (Hitachi Software, San Bruno, CA, USA).

### ***Heterosphecia pahangensis* Skowron, sp. nov.**

(Figs 1–5, 8)

**Type material.** *Holotype*, ♂, (Fig. 2) MALAYSIA: Peninsular Malaysia, Pahang, near Taman Negara National Park, N 04.38314 E 102.39983, collected 07.VIII.2014 by Marta Skowron, Deposited in Natural History Museum, Putrajaya, Malaysia; *Paratypes*, 2 ♂, same data as holotype, Natural History Museum, Putrajaya, Malaysia; *Paratypes*, 4 ♂, same data as holotype, private collection of Marta Skowron, Kuala Lumpur, Malaysia.

**Differential diagnosis.** A wingspan measuring 9.8–13.9 mm makes *H. pahangensis* the smallest described species of *Heterosphecia*. The body is also very slender. Due to the wing transparency, *H. pahangensis* may resemble *H. hyaloptera* Hampson (1919) and *H. bantanakai* Arita & Gorbunov (2000a). It differs from *H. hyaloptera* by the following characteristics: the colouration of the pericephalic hairs (black in *H. hyaloptera*), the presence of dirty yellow scales on thorax, hind tibia tuft and spur coloration, the presence of narrow white bands on the abdomen, and the presence of black to dark brown wing scales (red-orange scales in *H. hyaloptera*). From *H. bantanakai* it can be distinguished by the abdomen and leg coloration, and longitudinal division of distal part of ATA and shape of valva. Male genitalia are similar to *H. robinsoni*, Kallies (2003) and *H. tawonoides*, Kallies (2003), however they differ in the pointed gnathos and also from *H. robinsoni* in the shape of the valva and from *H. tawonoides* in the narrower tegumen. This confirms the observation of Kallies (2003) that the conformation of male genitalia in *Heterosphecia* is highly diversified. Moreover, this new species also differs in wing shape and venation from other *Heterosphecia*, i.e. the forewing is less quadrangular, veins CuA1 and CuA2 arise from a common point and the cross vein seems more rugged, whereas the hindwing is narrower and the distance between origin of vein CuA1 and M3 is more than seven times that between M3 and cross-vein (these distances are equal in other *Heterosphecia* species). Hind tibia and tarsus appear to be proportionally longer than in other species of this genus. Due to these unique characteristics and the relatively high COI gene sequence difference from related species, *H. pahangensis* could be assigned to a distinct genus in the future, however, more specimens would need to be examined and more barcode data provided.

**Description.** Alar expanse: 9.8–13.9 mm; body length: 5.4–8.0 mm; antenna 3.0–4.3 mm.

**Head:** antenna clavate, dorsally black with a few yellow scales at apex, gradually turning into yellow ventrally, tuft of black setae at tip of club; frons greyish brown; vertex covered with black and a few dirty yellow elongate scales, tiny patch of white scales adjacent to each complex eye just above antenna; complex eyes and ocelli brown; proboscis orange, well-developed, functional; labial palpus long and upturned, with elongate scales ventrally and apically, white with dark brown to black apex; pericephalic hairs white with a few yellow hairs dorsally and a few black hairs ventrally.

**Thorax:** patagia black; mesothorax anteriorly black with thin dirty yellow and a few white scales, mesopleura black with white stripe on upper margin; metathorax black; white hair-like scales protruding from close proximity to hind coxa and covering 1<sup>st</sup> abdominal segment.



**FIGURE 1.** *Heterosphecia pahangensis* in its typical resting position. Evident mimicry characteristics include: narrow, transparent wings, strongly tufted hind tibia, bright bands on the abdomen, clavate antenna.

**Legs:** fore, mid and hind femur smooth-scaled; fore and mid tibia and tarsus with elongate scales gradually shortening towards 5<sup>th</sup> tarsomere; fore coxa white, fore femur black distally and white basally; fore tibia black with a few white scales basally; tarsomeres proximal half white, distal half black; mid femur black externally and white-yellow internally with a few white hair-like scales basally; mid tibia black with elongate, white to yellow spot subbasally; 1<sup>st</sup> tarsomere black with a white to yellow spot centrally, tarsomeres 2–5 black; spurs black; hind femur black externally and white-yellow internally with a few white hair-like scales basally; characteristic tufts of hair-like scales on entire hind tibia and 1<sup>st</sup> tarsomere: black scales with bands of blue sheen and a few white scales subbasally, golden-brown sheen and/or admixture of white or yellow scales; distal part of tibia and 1<sup>st</sup> tarsomere yellow in most specimens; tarsomeres 2–5 with slightly elongate black scales with admixture of white or yellow scales on 2<sup>nd</sup> tarsomere; spurs black, sometimes with golden-brown sheen. Hind tibia and tarsus much longer than abdomen. All legs with blue sheen in sunlight (Supplementary video: time code 00:30–00:36).

**Abdomen:** smooth-scaled; black with blue sheen and well-defined, narrow white bands on distal margin of 2nd, 6th and 7th tergite and a white band (faint or well-defined) on distal margin of 5th tergite; sternites 1–7 black with white bands on distal margins; segment 8 black; anal tuft small, black, sometimes with a few yellow scales.

**Forewing** (Fig. 3): well-developed transparent area covered with semi-hyaline scales with blue sheen (Supplementary video: TC 00:44–00:54); vein R5 absent, R3 and R4 separate basally, M1 extended to apex, CuA1 and CuA2 arising from common point, cross vein rugged, remnant of veins 1A+2A very short; veins, costal margin, Cu-stem and discal spot covered with black to dark brown scales; anterior transparent area (ATA) divided into three transparent areas; external transparent area (ETA) divided into 6 cells, out of which four between veins R3-R4+5, R4+5-M1; M1–M2 and M2–M3 may have faint, narrow longitudinal stripes; cilia black.



**FIGURE 2.** *Heterosphecia pahangensis* sp. nov., holotype ♂

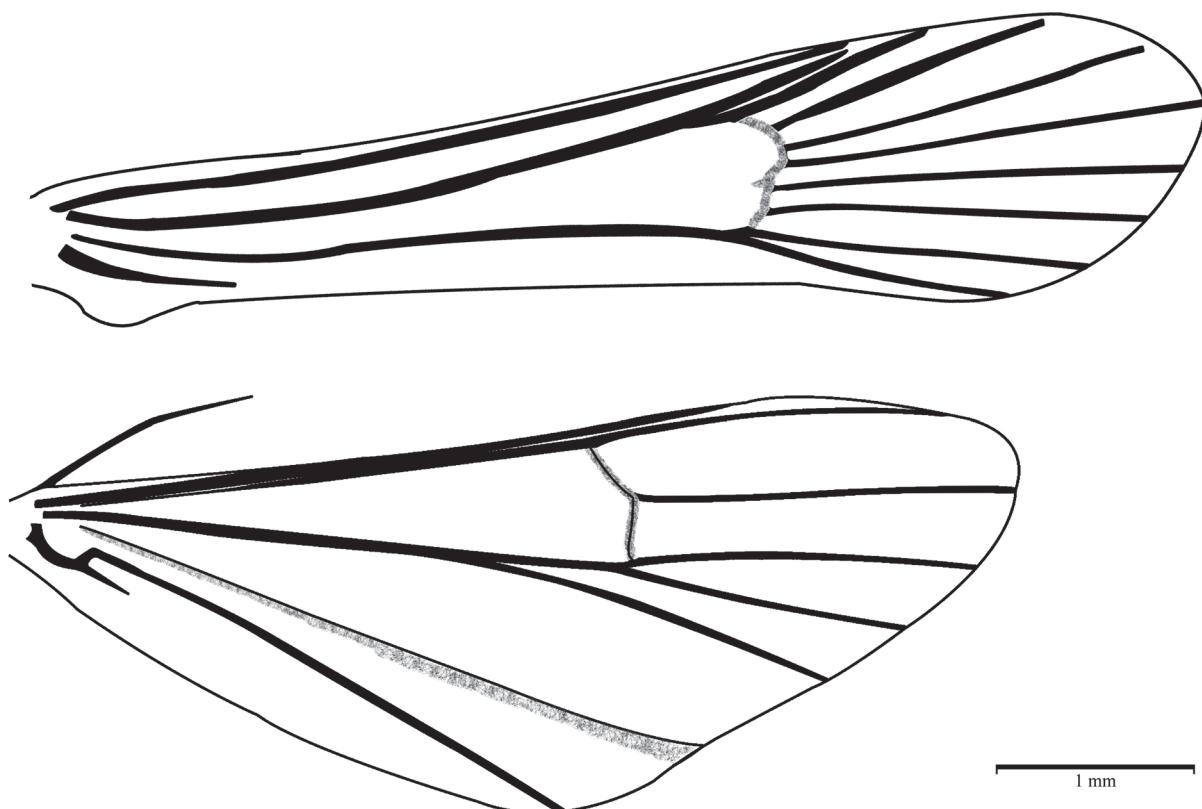
**Hindwing:** transparent, covered with semi-hyaline scales submarginally; vein M<sub>2</sub> arising from about middle of cross-vein, distance between origin of vein CuA<sub>1</sub> and M<sub>3</sub> more than 7 times that between M<sub>3</sub> and cross-vein, 1A well-developed, arising from 2A, remnant of veins 2A+3A fused distally; veins black to dark brown; discal spot very narrow, black; cilia black with a few white scales basally.

**Male genitalia** (Fig. 4): Valva broad with upturned distal portion, covered in long, pale brown setae; gnathos small, pointed distally; uncus covered in pale brown setae; tegumen somewhat narrowed distally; saccus quite long and thin, with flat or slightly bifurcate base; basal projection of sacculus ending with a spike; aedeagus about twice as long as valva.

Female unknown.

**Variation.** Individuals vary markedly in size, as well as coloration of tufts on hind tibia (Fig. 5): the characteristic yellow patch on distal part of tibia is not present in all of the specimens and varies in colour intensity;

in all of the observed individuals most of the tuft is composed of black scales with a blue sheen here and there and a few white scales proximally, but these can be mixed with additional white or yellow scales and/or have a golden-brown sheen, possible bands of blue sheen (visible on live individuals with erected scales on the tibia). The white band on the distal margin of 5<sup>th</sup> abdominal tergite can be well-defined or faint. Narrow longitudinal stripes between the veins of the external transparent area are usually absent, and were observed only in a few specimens.

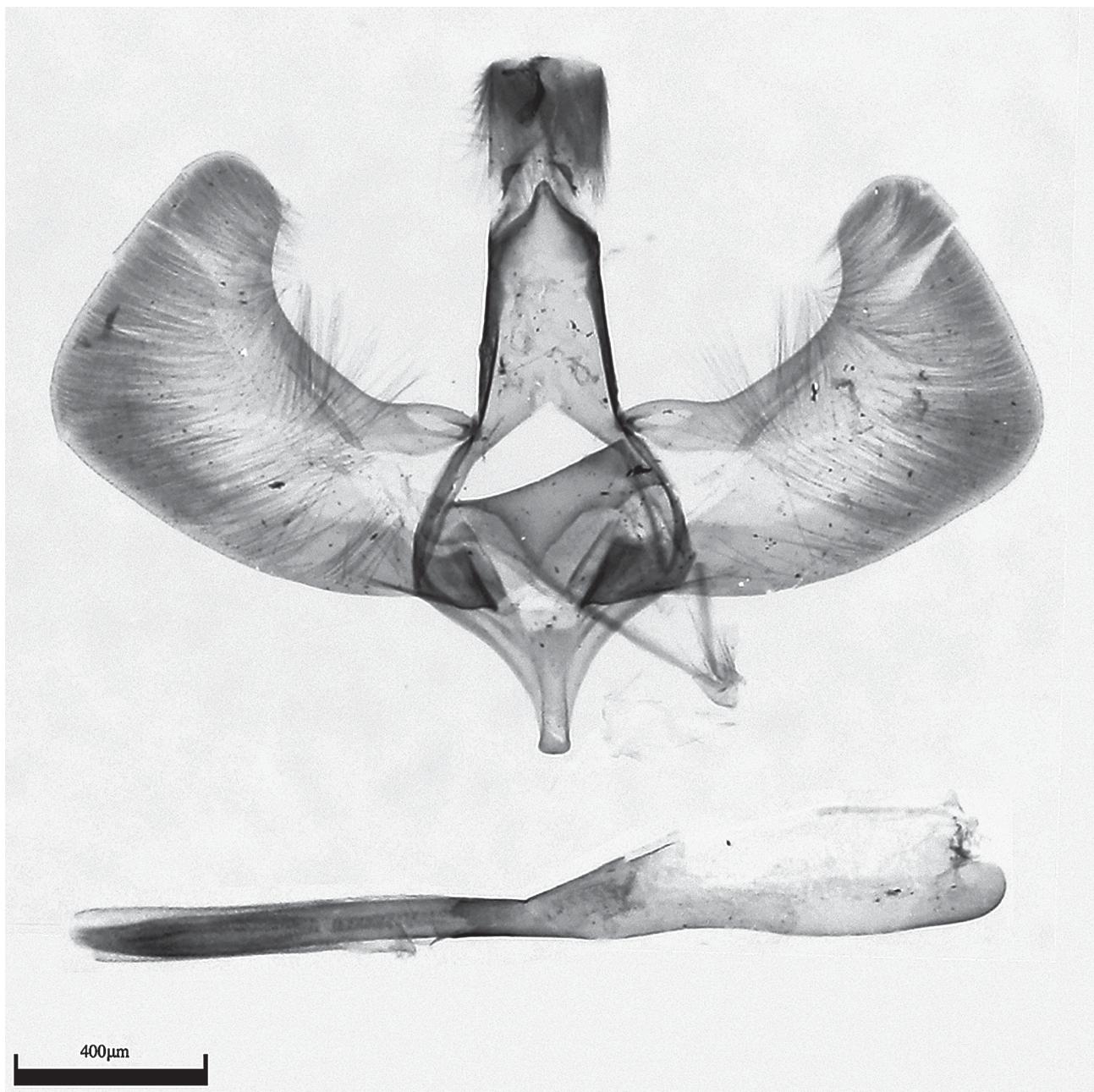


**FIGURE 3.** Wing venation of *Heterosphecia pahangensis*.

**DNA analysis.** Sequencing of the mitochondrial cytochrome c oxidase subunit I gene, which is used as a genetic marker or “barcode” for Lepidoptera, among others, showed significant differences with related genera. The closest match is *Heterosphecia bantanakai* (BOLD specimen IDs CCDB-04687 E05 and CCDB-04609 H06) from the tribe Osminiini, which has a high value of 10% sequence divergence from *Heterosphecia pahangensis*. Three individuals were barcoded (Table 1). Two of them yielded identical barcodes, whereas the third differed by nine nucleotides (Fig. 6). However, most of these substitutions were neutral, and the resulting amino acid sequence displayed only one difference.

**TABLE 1.** GenBank and Barcode of Life (Ratnasingham & Hebert 2007) accession numbers to COI sequences of three specimens of *Heterosphecia pahangensis* and their closest match—*Heterosphecia bantanakai*.

Specimen	GenBank accession number	BOLD Specimen ID	Colour of tuft on hind tibia	Location
<i>Heterosphecia pahangensis</i> ♂ paratype #3	KM453983.1	GWOTK683-13	yellow	Specimen utilized for wing and genitalia preparations
<i>Heterosphecia pahangensis</i> ♂ paratype #4	KM453982.1	GWOTL1123-13	dark	Kuala Lumpur, Malaysia, private collection of Marta Skowron
<i>Heterosphecia pahangensis</i> ♂ paratype #5	KM453984.1	GWOTL1124-13	dark	Kuala Lumpur, Malaysia, private collection of Marta Skowron
<i>Heterosphecia bantanakai</i> ♂	-	CCDB-04687 E05	-	Research Collection of Arthur Lingenhoele



**FIGURE 4.** Male genitalia of *Heterosphecia pahangensis*.



**FIGURE 5.** Colour variation of hind tibia tufts. Note the difference in the amount of yellow scales.

<p>Nucleotides (654 base pairs):</p> <pre>A ACATTATACTTTATTTGGTGTGACTGGAATATTAGGAACCTCCCTTAAGATTA TTAATTGCGAGCAGAAATTAGGAACCTCCCGATCTTAAATTGGAGATGATCAAATTAT AATACTATTGTAACAGCTCATGCTTTATTATAATTTCCTTATAGTTATGCCATT ATAATTGGAGGATTGGAATTGATTAGTACCTCTAATATTAGGACCTCTGATATA GCTTCCCCGAATAAATAATATAAGATTGACTCTCACCTCAATCTCTT TTAATTCAAGAAGAATTATTGAAAATGGAACCTGGAACAGGATGAACAGTATA<b>TCCC</b> CCACTTCATC<b>TAATATCGCTCATAG</b>GGTAGTTCACTGAGATCTTCATTTTCT CTTCATCTAGCTGGAAATTCTCAATTAGGAGC<b>ATAA</b>TTTATTACTACTATT ATTAATATACGACCTAAAAATATCCTTGATCAAAACCTTATTGTTGAGCC GTTGGAATTACAGCTTTACTACTTTATCTTACCTGTTCTAGCAGGAGCTATT ACCATATTAACTGATCGAAATTAAACTTCCTTTTGATCCTGTTGGTGG GGAGACCCATTCTTACACACTTA</pre> <p>Amino acids:</p> <pre>TLYFIFGVWAGMLGTSLSLLIRAELETPGSЛИGDDQIYNTIVTAHAFIMIFFMVMPI MIGGFGNWLVPMLGAPDMAPRMMNNMSFWLPPSISL<b>LISSII</b>ENG<b>T</b>GWTVYP PLSSNIAH<b>GGSSV</b>DLSISFLHLAGISSILGAINFITTINMRPKNMSFDQMPLFVWA VGITALLLLSLPVLAGAITMLLDRNLNTSFFDPVGGGD<b>PILYQHL</b></pre>	<p>Nucleotides (654 base pairs):</p> <pre>B ACATTATACTTTATTTGGTGTGACTGGAATATTAGGAACCTCCCTTAAGATTA TTAATTGCGAGCAGAAATTAGGAACCTCCCGATCTTAAATTGGAGATGATCAAATTAT AATACTATTGTAACAGCTCATGCTTTATTATAATTTCCTTATAGTTAT<b>ACCC</b>ATT ATAATTGGAGGATTGGAATTGATTAGTACCTCTAATATTAGGAGCTCTGATATA GCTTCCCCGAATAAATAATATAAGATTGACTCTCACCTCAATCTCTT TTAATTCAAGAAGAATTATTGAAAATGGAACCTGGAACAGGATGAACAGTATA<b>CCCC</b> CCACTTCATC<b>CAATATG</b>CCAT<b>GG</b>TGGTAGTTCACTGAGATCTTCATTTTCT CTTCATCTAGCTGGAAATTCTCAATTAGGAGC<b>TATA</b>ATTTTATTACTACTATT ATTAATATACGACCTAAAAATATCCTTGATCAAAACCTTATTGTTGAGCC GTTGGAATTACAGCTTTACTACTTTATCTTACCTGTTCTAGCAGGAGCTATT ACCATATTAACTGATCGAAATTAAACTTCCTTTTGATCCTGTTGGTGG GGAGACCCATTCTTACACACTTA</pre> <p>Amino acids:</p> <pre>TLYFIFGVWAGMLGTSLSLLIRAELETPGSЛИGDDQIYNTIVTAHAFIMIFFMVMPI MIGGFGNWLVPMLGAPDMAPRMMNNMSFWLPPSISL<b>LISSII</b>ENG<b>T</b>GWTVYP PLSSNIAH<b>GGSSV</b>DLSISFLHLAGISSILGAINFITTINMRPKNMSFDQMPLFVWA VGITALLLLSLPVLAGAITMLLDRNLNTSFFDPVGGGD<b>PILYQHL</b></pre>
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**FIGURE 6.** DNA sequencing of the mitochondrial cytochrome c oxidase subunit I gene of two *Heterosphecia pahangensis* individuals and the corresponding amino acid sequences. Differences are emboldened. Note the serine – glycine substitution. GenBank submission numbers: (A) KM453982 (B) KM453983.



**FIGURE 7.** Habitat of *Heterosphecia pahangensis*: rocky river bank surrounded by a lowland dipterocarp forest.

**Distribution and habitat.** Pahang State, Peninsular Malaysia. Observed in six different locations very close to the Taman Negara National Park border, on rocky or sandy banks of the Tembeling and Tahan rivers running through a lowland dipterocarp forest, at elevations below 130 m (Fig. 7). Several individuals were seen in Kelantan on the Sungai Lebir river bank.

This diurnal species flies from about 9h30 until 17–19h on sunny days, in full sunlight or half-shaded sites, at temperatures ranging from 28 to 32 degrees Celsius in the shade and humidity from 59% to 92%. At dusk, a few individuals were seen flying off into the forest. The likelihood of observations was dependent on water level — during heavy rains that caused flooding of the rocky beaches, *H. pahangensis* was absent from its type locality. It is

worth noting that during dry periods with very low water level, *H. pahangensis* was also absent. This might mean the sesiids appear in this location in search for certain minerals, which are present only on surfaces moistened by river water.

**Behaviour and bionomics.** The new species was found in August (but was also observed in June and July in the following year), mud-puddling on a rocky river bank (Fig. 8, Supplementary video: TC 00:06–01:53), often in large numbers. Mud-puddling, or simply puddling, serves the purpose of gaining essential nutrients, such as sodium or proteins (Beck *et al.* 1999). Previous studies (Adler 1982; Boggs & Jackson 1991) have shown that puddling behaviour is typical for male Lepidoptera and occurs very rarely among females. Unsurprisingly, all of the captured specimens were males. It has also been previously noted that Lepidoptera eject an excess of water during puddling. *Heterosphecia pahangensis* also displayed such behaviour (Supplementary video: TC 01:36, 01:38). Individuals most preferably fed on yellow quartz cobbles and pebbles (from river sediments) with traces of feldspars, but also on sand, mud and in one case on organic matter, most likely feces. A salt (NaCl) solution was experimentally poured on several cobbles, these rocks seemed to be preferred by *H. pahangensis*. Baits containing honey solutions, as well as commercially available pheromone lures (lures alone, not encased in a trap) were also placed at the type locality, however, *H. pahangensis* showed no attraction to them.

*Heterosphecia pahangensis* displays distinct behavioural mimicry. Its jerky flight closely resembles that of bees (Supplementary video: TC 02:01–03:27). In addition, the tufted hind legs described above are not used for motion. Some individuals have been observed to use them to drive away conspecifics during mud-puddling (Supplementary video: TC 01:09) but they seem to serve mainly for mimicry. The yellow bands present in some individuals on the hind tibia are most probably an imitation of the corbicula (pollen basket) of bees. During flight hind legs are left hanging downwards and the abdomen is pointed upwards (Supplementary video: TC 03:18–03:24). Due to their mimic behaviour and morphology, it is very difficult at first glance to distinguish these sesiids from bees, which occur in the same habitat in large numbers. This species rests with its wings folded back. The larval hostplant is unknown.



**FIGURE 8.** Puddling behaviour on quartz. Males of different species of Lepidoptera are often observed feeding on mud, sand or organic matter in order to obtain sodium and proteins, which probably increase reproductive success. The discovered species was found licking quartz cobbles on a river bank.

**Etymology.** The name derives from the state Pahang in Malaysia, where the species was discovered.

**Authors' contributions.** M.S. discovered and described the new species from Peninsular Malaysia, collected the specimens, took measurements, made photographic and video documentation, prepared dissections and slide mounts, analysed DNA sequencing results, wrote the manuscript and named the species. S.A.H. assisted in expedition logistics. G.W. provided research consulting. B.M. assisted in sampling.

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