Supplementary material, Doc S1

# DNA extraction and sequencing protocols

## Eggs

The eggs were punctured using a sterile medical needle (0.2 x 20 mm), and genomic DNA was extracted using the Dneasy® Blood & Tissue Kit (Qiagen Inc., Valencia, California, United States of America) following the manufacturer’s instructions. The barcoding region was amplified using the primer pair LepF1/LepR1 (Hebert *et al.* 2004). Each polymerase chain reaction was carried out in a volume of 20 μl [1 μl of DNA, 11.8 μl of H2O, 2 μl of High Yield Reaction Buffer A (1 × 1.5 mM MgCl2), 1.8 μl of MgCl2 (2.25 mM), 1.2 μl of dNTP (0.6 mM), 1 μl of each primer of the pair LepF1/LepR1 (0.5 μM), and 0.2 μl of FastGene Taq DNA polymerase (NIPPON Genetics Europe). The polymerase chain reaction protocol consisted of an initial denaturation at 95 °C for 5 minutes, 35 cycles, each consisting of three steps, i.e., 1 minute at 94 °C, 1 minute at 48 °C and 1.5 minutes at 72 °C, with a final extension step at 72 °C for 7 minutes at the end of amplification protocol. After polymerase chain reaction amplification, the product was run on 1% agarose gel, stained with ethidium bromide, and visualised under a ultraviolet transilluminator. The amplified product was sequenced by Macrogen Inc. (Seoul, Korea). To obtain 658 bp of the barcoding region, sequencing was performed with both forward and reverse primers.

## Adults

Dry legs from adults were sent to the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Guelph, Ontario, Canada and were analysed following standard protocols (deWaard et al. 2008).

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**References**

Please see the References section in the main paper (“Citizen science reveals establishment of *Chamaesphecia empiformis* (Esper) (Lepidoptera: Sesiidae), a long-lost biological control agent for *Euphorbia cyparissias* (Euphorbiaceae), in Ontario, Canada”) for the bibliographic information for references cited here.

Comparative morphology of *Chamaesphecia empiformis*

Some superficial resemblance may be seen in a few dark-colored, similarly sized North American species with forewing that are heavily overlaid with dark scales; for example, *Albuna fraxini* (c.f. form “*vitriosa*” shown in Eichlin and Duckworth 1988, plate 1 fig 47), *Synanthedon exitiosa* (c.f. form “*graefi*” shown in Eichlin and Duckworth 1988, plate 2 fig 43), *Synanthedon bibionipennis* (c.f. Eichlin and Duckworth 1988, plate 3 fig 33), and some *Carmenta* such as *C. prosopis* (c.f. Eichlin and Duckworth 1988, plate 4 fig 11). This resemblance is more noticeable in worn or preserved specimens, particularly in the latter as they tend to become greasy resulting in more subdued coloration. However, all of these other species have a long, well-developed PTA extended to the discal spot.

Size: wingspan 11–22 mm [from Špatenka et al. (1999) and Laštůvka & Laštůvka (2001)]. Preserved Ontario specimens measure between ~ 12–16 mm, the male being the smallest, however, their incompletely spread wings render measurements approximate. Špatenka et al. (1999) noted that the species is variable in size and in the amount of yellow scaling on the forewing and abdomen.

In the male *C. empiformis*, the forewing PTA is narrow, distally tapered; in the female it is thinner and may be reduced to an inconspicuous sliver barely tapering to the middle of ATA, or absent/indistinct (as on some of the preserved specimens in the CNC). In male the forewing is black with bronze-violet sheen, with scattered yellow scales, particularly in the apical area, discal spot, and around the external transparent area (ETA). The ETA has five elongate cells, the anteriormost of which has yellow scales. The anal area has six elongate, yellow spots. The female forewing is similarly colored but less shiny. The posterior margin of the head has a pair of dense tufts of orange scales which are extended ventrally behind the eyes. The thorax has yellow tegulae forming lateral stripes and a median longitudinal yellow line, and each side of the metascutellum has a prominent yellow tuft of hairlike scales. The abdomen is shiny black with a significant amount of yellow on the posterior half of tergites 2, 4 and 6 and a narrow, white or pale yellow band on the posterior margin of the same tergites. The male anal tuft flat or slightly convex, yellow medially, black laterally. The female anal tuft is dorsally V-shaped, yellow at base, laterally and distally black.

There are other characters that are useful when attempting to run through identification keys found in Eichlin and Duckworth (1988) or when examining preserved specimens. In Eichlin and Duckworth *C. empiformis* keys out to the combined key to species of *Palmia*, *Carmenta*, and *Synanthedon*, but no further. The first and second labial palpomeres have expanded ventral vestiture of hairlike scales. This vestiture is more developed in the male so that the palpus has a hairy appearance in lateral or latero-ventral view. In the female the hairlike scales are restricted to the first palpomere. The 2nd labial palpomere is yellow with a black stripe on the outer side of the second in the male; it is mostly yellow in the female. The third palpomere has a mixture of yellow and black.The antenna is clavate with a scale tuft at tip, is ciliate in the male (as opposed to bipectinate), and has some yellow externally in the middle of the flagellum. The haustellum well developed, coiled, longer than labial palpus, and dark piceous brown (for example, it is pale rufous in some co-occurring species such as *Carmenta ithacae*). The forewing R4 and R5 are stalked. The hindwing Cu1 and M3 veins are stalked, the common stalk arising from the crossvein. The hindwing cell is shorter than two-thirds of the wing length. In the hindleg the tibia is about two times as long as the femur; and the first tarsomere is about 1.5x the length of the femur and is not thickened with tufts of scales.

References

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Laštůvka, Z. and Laštůvka, A. 2001. The Sesiidae of Europe. Apollo Books, Stenstrup, Denmark.

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