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# The first DNA-assisted record of *Hemelytroblatta livida* (Blattodea: Corydiidae) from Georgia with notes on Corydiinae species composition in the Caucasus

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## Abstract

The fossorial sand cockroach *Hemelytroblatta livida* (Brunner von Wattenwyl, 1865) is reported from Georgia for the first time, with a commentary on the species composition of the Corydiinae Saussure, 1864 subfamily in the Caucasus. Collection data, pictures of the male and female, and DNA barcodes are also provided. Furthermore, information on *Polyphaga aegyptiaca* (Linnaeus, 1758) is provided, along with images of the male, female, and juvenile.

## Key words

Dictyoptera, CaBOL, faunistics, sand cockroach, South-Caucasus

## Introduction

The subfamily Corydiinae Saussure, 1864 is the most diverse and most studied subfamily in the family Corydiidae Saussure, 1864. These cockroaches known under the collective name “sand cockroaches” are mainly distributed in arid regions and expose a strong sexual dimorphism, with males being macropterous and females generally apterous (such females are fossorial and possess special adaptations for subterranean life). Although females of several genera, including *Ergaula* Walker, 1868 (Grandcolas 1997), *Eucorydia* Hebard, 1929 (Qiu et al. 2017b; Yanagisawa et al. 2021a, 2021b, 2021c), *Homoeogamia* Burmeister 1838; (Estrada-Alvarez and Guadarrama 2013), *Hypercompsa* Saussure, 1864 (Crespo et al. 2015), *Polyphagoides* Mackerras, 1968 (Mackerras 1968) and *Therea* Billberg, 1820 (Grandcolas 1993) are known to have wings. The reclusive lifestyle of Corydiinae, along with the unfortunate reputation of these sadly neglected insects has always been an obstacle for the study and collection for a long time, with the most recent and major revisions performed on the most species-rich North American genus *Arenivaga* Rehn, 1903 (Hopkins 2014) and Oriental genera *Eupolyphaga* Chopard, 1929 (Qiu et al. 2018b) and *Eucorydia* (Qiu et al. 2017b; Yanagisawa et al. 2021a, 2021b, 2021c).

The most significant work on the taxonomy of Corydiinae has been performed by Chopard (1929), who recognized 54 species within 10 genera in the Palaearctic Region, of which 7 genera and 23 species were new. Bey-Bienko (1950) did the more recent comprehensive work dedicated to Corydiinae and Blattodea overall of the former USSR countries, recognizing 5 genera and 10 species, namely *Anisogamia tamerlana* Saussure, 1893, *Eupolyphaga sinensis* (Walker, 1868), *Hemelytroblatta incerta* (Chopard, 1929), *H. livida*

(Brunner von Wattenwyl, 1865), *H. minuta* (Bey-Bienko, 1935), *H. roseni* (Brancsik, 1898), *Mononychoblatta semenovi* Chopard, 1929, *Polyphaga aegyptiaca* (Linnaeus, 1758), *P. plancyi* Bolívar, 1883 and *P. saussurei* (Dohrn, 1888). While most of these species are of Central Asian origin, only two species of the subfamily occur in the Caucasus – *H. livida* and the most widely distributed *P. aegyptiaca*, also known from Georgia. Since then, no studies on this group of ancient insects have been performed within the country, and here we report *H. livida* from Georgia for the first time.

# Materials and methods

## Sample collection and processing

Specimens of *Hemelytroblatta livida* were collected within the framework of the Caucasus Barcode of Life (CaBOL) project by the CaBOL team of the Institute of Ecology, Ilia State University (https://ggbc.eu/). Male specimens were collected at night by hand with flashlights in xerothermic places on vegetation or via pitfall traps filled with vinegar. Female specimens were collected during the day by hand under the rocks, then preserved in 96% ethanol and deposited in the collection of Ilia State University.

Photos of the pre-cleaned from dust and grease adult male and female of *Hemelytroblatta livida* (Plate 1: A, B, C, D) were taken with a Canon EOS 60D camera and Canon EF-S 60mm f/2.8 Macro USM lens, while an adult male, female, and juvenile of *Polyphaga aegyptiaca* (Plate 1. E, F, G, H) were taken using a Canon EOS 550D camera and Canon EF 100 mm f/2.8 Macro USM lens. Digital images were prepared using Zerene Stacker image stacking software and Adobe Photoshop CS6. For specimen identification, we followed Brunner (1882), Chopard (1929), and Bey-Bienko (1950). Specimens of *P. aegyptiaca* examined during this research are deposited in the collections of Ilia State University.

**Institutional abbreviations:** ISUIE – Ilia State University, Institute of Ecology; ISUIZ – Ilia State University, Institute of Zoology.

# DNA processing

DNA was extracted from tissue samples using the Quick-DNA Magbead Plus Kit (Zymo Research). Partial sequence of cytochrome oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR) using the primer pairs LCOI490-JJ and HCO2198-JJ (Astrin and Stüben 2008). Thermal conditions included denaturation at 95°C for 1 min, followed by the first cycle set (15 cycles): 94°C for 30 sec., annealing at 55°C for 1 min (–1°C per cycle), and extension at 72°C for 1:30 min. Second cycle set (25 cycles): 94°C for 35 sec., 45°C for 1 min., 72°C for 1:30 min., and a final extension step at 72°C for 5 min. PCR amplicons were visualized on 1% agarose gels using 1.7 µl of PCR product. Sequencing of the unpurified PCR products in both directions was conducted at the Beijing Genomics Institute (Hong Kong, CN) by using the amplification primers. Sequence analysis was performed using Geneious Prime 2022.1.1 (http://www.geneious.com). Extracted DNA was deposited in the scientific collections of Ilia State University, Tbilisi, Georgia, and aliquots will be deposited at LIB Biobank at Museum Koenig, Bonn, Germany, while the sequences have been submitted to Barcode of Life Data System (BOLD) databases. The newly obtained DNA barcodes of COI sequences were checked out against the BOLD Systems database (http://www.boldsystems.org/index.php). The Barcode Index Number (BIN) (Ratnasingham and Hebert 2013) for the sequenced taxa and for their nearest neighbor in BOLD systems (if they had a BIN) are also given. For the calculation of sequence differentiation, we used the k2p distance as performed in the BOLD system

# Results

From the collected material, 6 specimens were submitted for the barcoding pipeline, and 4 quality, BOLD compliant barcodes (658 bp length, with no stop codons, indels, or deletions) representing 2 species have been generated so far. Barcode information is given under each barcoded species listed below.

## Taxonomic account

## Family Corydiidae Saussure, 1864

## Genus *Hemelytroblatta* Chopard, 1929

### *Hemelytroblatta livida* (Brunner von Wattenwyl, 1865)

**Materials exemined. GEORGIA** • 1♂; Dighomi village (8 km E of Tbilisi); N41.7807°, E44.7021°; 734 m a.s.l.; heathland, xerothermic slope, on *Rhamnus pallasii* at night; leg: A Seropian and L-G Japaridze; 10 Sep 2020; CaBOL-ID 1009797 (Fig. 1A,B). • 1♂; same locality; on *Pyrus salicifolia* at night; leg. A Seropian and L-G Japaridze; 11 Sep 2020. • 1♂; Didgori (10 km E of Tbilisi); N41.7855°, E44.6764°; 801 m a.s.l.; deciduous forest, slope with loose soil; leg: A Seropian and L-G Japaridze; 20 Sep 2020. • 1♂; Tbilisi; N41.7300°, E44.7048°; 710 m a.s.l.; heathland, xerothermic slope, on vegetation at night; leg: L-G Japaridze; 02 Nov 2020. • 1♀; Kumisi; N41.6259°, E44.8111°; 657 m a.s.l.; steppe, under rock; leg: S Japarashvili; 25 Sep 2021; CaBOL-ID 1013031. • 1♀; Kvernaki ridge (Gori); N41.9848°, E44.1389°; 696 m a.s.l.; heathland, under rock; leg: N Bulbulashvili; 24 Oct 2021; CaBOL-ID 1020260 (Fig. 1C,D).

**Genetics.** We obtained a single barcode from the specimen with CaBOL-ID 1020260 (BOLD: AEP4515). Neither the species nor its congeners are present in BOLD as we provide the first COI sequence.

**Remarks.** The records of *H. livida* in the Caucasus region are known from Julfa and Ordubad (Azerbaijan) (Bey-Bienko 1950), and our records from Georgia extend its known distribution range by almost 680 km to the north-east. Originally described from Cyprus (Brunner von Wattenwyl 1865), the species is also known to occur in Greece (Chopard 1929), Iran, Iraq, Syria, and Turkey (Bey-Bienko 1950; Caesar et al. 2015). Due to the hidden lifestyle of *H. livida*,

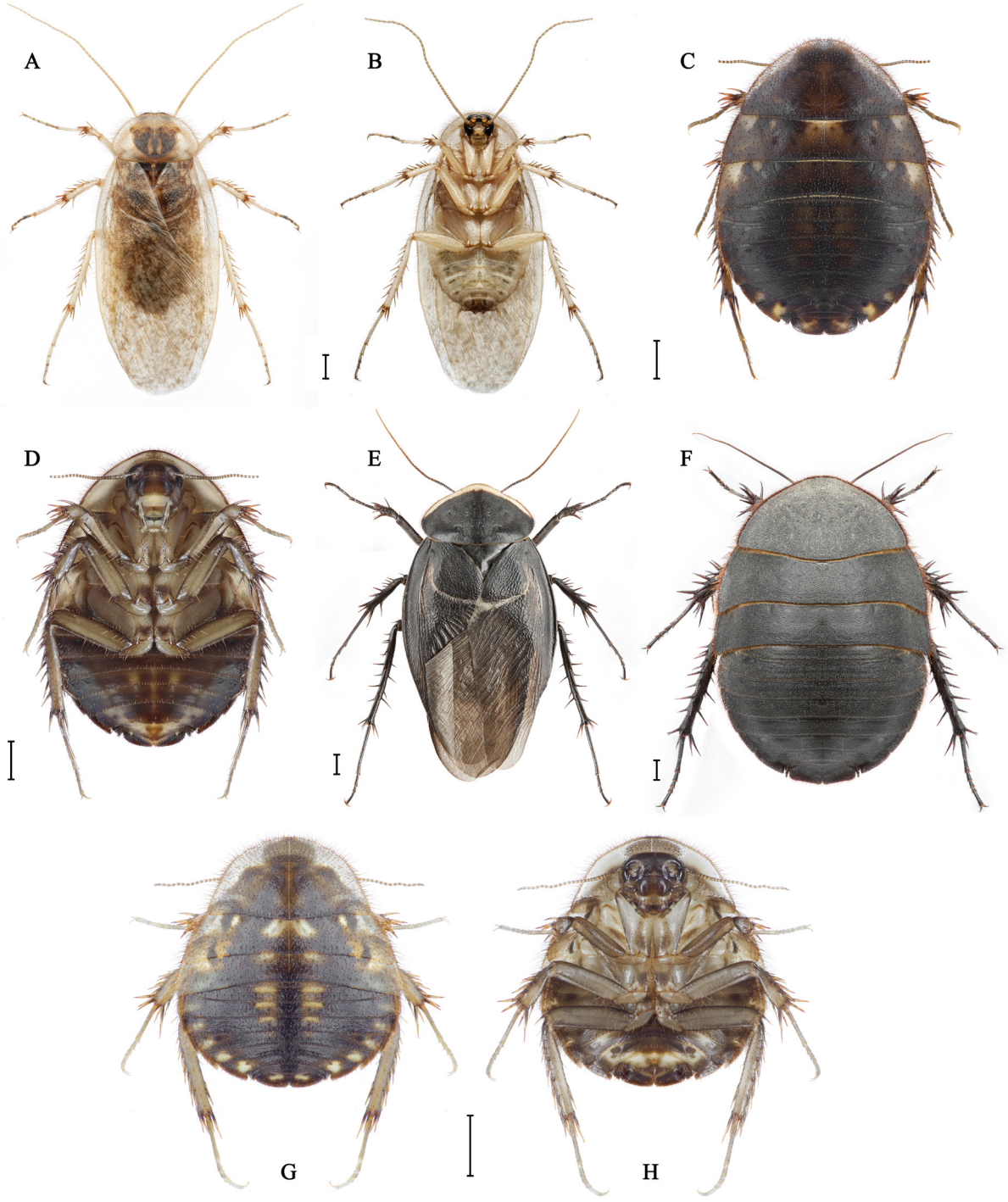


**Figure 1.** A-D: *Hemelytroblatta livida*. A-B: Male, dorsal and ventral view. C-D: Female, dorsal and ventral view. E-H: *Polyphaga aegyptiaca*. E-F: Male and female, dorsal view. G-H: Nymph, dorsal and ventral view. Scale bars = 2 mm.

little is known about its phenology, and there is no information on its reproductive biology, which is likely somewhat similar to that of other congeners. The general appearance of males and females as well as the somatic characteristics correspond well to the descriptions and drawings by Chopard (1929) and Bey-Bienko (1950). Bey-Bienko also mentions the small differences in coloration between specimens from Parnassus (Greece) and Azerbaijan compared to the conspecifics originating from Asia Minor and Central Asia, with the first ones sometimes being more brightly colored with almost no dark spots on elytra. The juveniles strongly resemble the adult females, but with more prominent ornamentation.

## Genus *Polyphaga* Brullé, 1835

### *Polyphaga aegyptiaca* (Linnaeus, 1758)

**Materials examined. GEORGIA** • 1♂; Dighomi village (8 km E of Tbilisi); N41.7805°, E44.7034°; 775 m a.s.l.; heathland, under rocks at night; leg: A Seropian and L-G Japaridze; 13 Sep 2020; CaBOL- ID 1010347 (Fig. 1E). • 1♀; Dighomi village (8 km E of Tbilisi); N41.7805°, E44.7034°; 775 m a.s.l.; heathland, under rocks at night; leg: A Seropian and L-G Japaridze; 05 Sep 2020; CaBOL- ID 1010359 (Fig. 1F). • 1juv.; Dighomi village (8 km E of Tbilisi); N41.7776°,

E44.7132°; 654 m a.s.l.; heathland, under artificial garbage; leg: A Zukakishvili; 03 May 2021; CaBOL- ID 1021022 (Fig. 1G-H). • 2♀♀; 2juv.; Uplistiskhe; N41.966004°, E44.219267°; 563 m a.s.l.; steppe, under rocks; leg. L Mumladze; 02 Sep 2010; ISUIZ. • 1♂, 3♀♀; Chachuna Managed Reserve; N41.29366°, E45.95407°; 400 m a.s.l.; semidesert, under rocks; 19 Oct 2020; ISUIZ.

**Genetics.** We obtained two identical barcodes from the specimens with CaBOL-IDs 1010347 and 1010359 (BOLD: AEP4515) and provided the first COI sequences of *P. aegyptiaca*.

**Remarks.** The distribution range of the species covers the entire Mediterranean region, the southern parts of Europe, and southwestern Asia, reaching Iran in the east. With such a wide distribution, one might think it's a well-studied species in Georgia. In fact, there is almost no location-specific data on *P. aegyptiaca* finds in Georgia, as we provide one based on the material collected within CaBOL, museum material, and also photographic observations available at the Georgian Biodiversity Database (Tarkhnishvili et al. 2013), iNaturalist, and the Facebook group "Wildlife in Georgia". The distribution in Georgia is as follows: Batumi (Bey-Bienko 1950), Chachuna Managed Reserve, Gori, Kumisi, Tbilisi, Rustavi, Poladauri, Kutaisi, Mtskheta, and Uplistsikhe.

## Discussion

The taxonomy of Corydiidae has significantly progressed in the last decade, indicating greater species diversity within the family (Gutierez 2012; Estrada-Álvarez and Guadarrama 2013; Qiu et al. 2016, 2017a, 2017b, 2018a, 2018b; Lucanas 2018; Yanagisawa et al. 2021a, 2021b, 2021c). However, the DNA-based phylogenetic works are not that optimistic due to the difficulty in obtaining specimens (Djernæs et al. 2012, 2015; Legendre et al. 2015; Wang et al. 2017). Further targeted studies of these invertebrates may shed some light on their actual diversity, distribution within the country and phylogenetic relationships.

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