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The first barcode-assisted record of *Tropidopola turanica* Uvarov, 1926 (Orthoptera, Acrididae) from Georgia

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Abstract

The genus *Tropidopola* (Insecta, Orthoptera) is recorded in Georgia for the first time. Detailed information on the barcode of the species, pictures of the live and preserved specimen, collecting information, and remarks on the ecology of *T. turanica* Uvarov, 1926, are given.

Key words

CaBOL, barcoding, faunistic, new record, South Caucasus, Tropidopolinae

The knowledge of the Orthoptera fauna of Georgia has been constantly growing in recent years (Mulder and Gorochov 2019; Stalling et al. 2019; Stalling and Seropian 2022; Arsenashvili et al. 2022). In this communication, we provide evidence on the occurrence of one more grasshopper species of the family Acrididae in Georgia. Acrididae is one of the most diverse lineages within Orthoptera, comprising about 80 species in Georgia (Tarkhnishvili et al. 2013). The genus *Tropidopola* Stål, 1873 (Acrididae, Orthoptera) has a wide distribution, extending to the Mediterranean region, the Middle East, the Republics of Central Asia and the Caucasus, India, the French Sudan, Egypt, and tropical Africa, which includes a total of 7 species: *T. cylindrica* (Marschall, 1836); *T. daurica* Uvarov, 1926; *T. graeca* Uvarov, 1926; *T. longicornis* (Fieber, 1853); *T. nigerica* Uvarov, 1937; *T. syriaca* (Walker, 1871); *T. turanica* Uvarov, 1926 (Childebaev and Storozhenko 2001; Cigliano et al. 2023). A single species, *T. turanica* is found in the South Caucasus, more precisely in Azerbaijan and Armenia (Tarbinsky 1940; Savenko 1941; Bey-Bienko and Mishchenko 1951; Avakyan 1968; Snegovaya and Kerimova 2022). *Tropidopola* occurs in damp meadows, inhabiting the beds of reeds, *Cyperus*, *Juncus*, and similar plants growing in desert or semi-desert areas near water bodies (Brunner 1882; Bey-Bienko and Mishchenko 1951). The external morphology of *T. turanica* responds well to the peculiar needs of the habitat and its behavior. *Tropidopola* species are characterized by a very elongated, cylindrical body; their slender hind tibia muscles are weakly developed; this is due to the lifestyle of these locusts: during the day, they sit motionless in an ambush on the grass and avoid potential danger by moving to the other side of the grass, not by jumping (Buxton and Uvarov 1924; Uvarov 1926) (Fig. 1).

Recently, *Tropidopola turanica* was collected in Georgia for the first time. Georgia is marked as the species distribution range at the Orthoptera Species File Online database (Cigliano et al. 2023), but there is no literary data available on the previous finds of the species in Georgia.

Despite the numerous expeditions and collecting efforts within the CaBOL project (Caucasus Barcode of Life, https://ggbc.eu/) in urban and protected areas in different parts of Georgia, the species was caught in Dighomi Park by accident (Tbilisi, Georgia: 41.769476°N, 44.773614°E, 426 m a.s.l.), and a single female was collected in the thickets of *Phragmites australis* (20.VI.2022; leg. Seropian A.). The specimen was preserved in 96% ethanol and stored in a freezer at -30 °C. The preserved specimen was photographed using a Canon EOS 60D camera with a Canon EF 60 mm

Figure 1. General habitus of *Tropidopola turanica* Uvarov, 1926; live female.

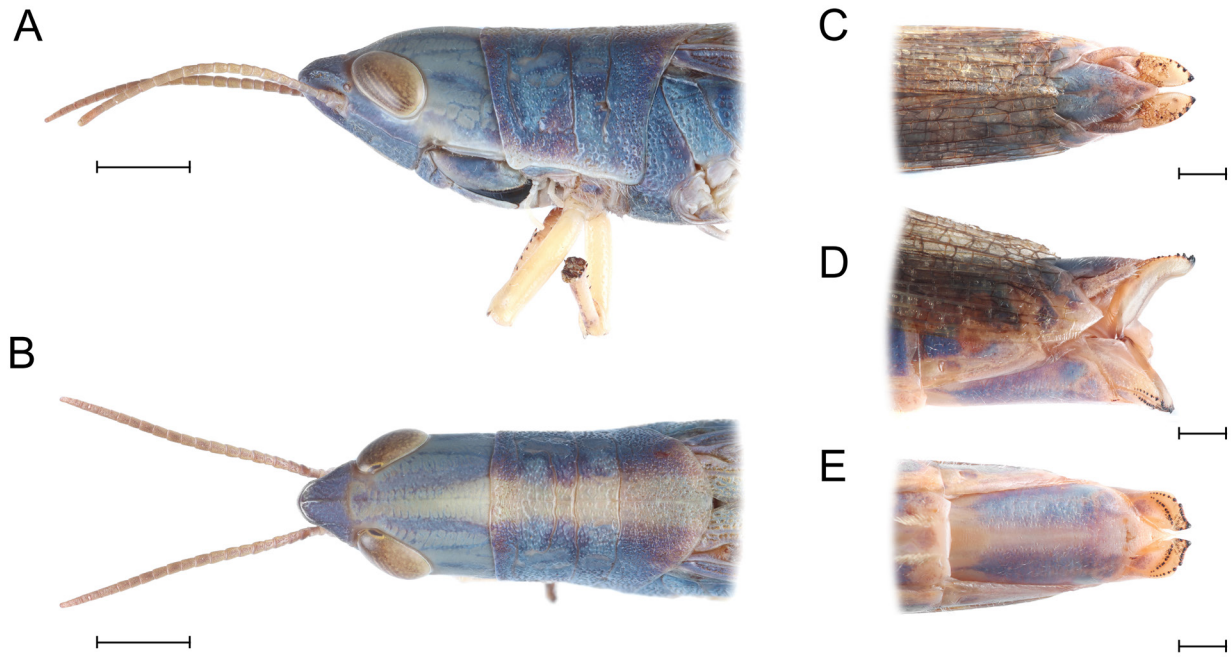

Figure 2. Head of *Tropidopola turanica* Uvarov, 1926, female: **A**: lateral view; **B:** dorsal view. Scale bar = 3 mm. Genital plate: **C**: dorsal view; **D:** lateral view; **E**: ventral view. Scale bar = 1 mm.

f/2.8 Macro USM lens. Digital images were prepared using Zerene Stacker image stacking software and Adobe Photoshop CS6. The specimen was identified as *Tropidopola turanica* Uvarov, 1926 (Uvarov 1926) (Figs. 2C,D). The voucher specimen is kept at Ilia State University under the number CaBOL-ID 1023967.

DNA was extracted from leg tissue using the Quick-DNA Magbead Plus Kit (Zymo Research). The partial sequences of cytochrome oxidase subunit I (COI) were 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 cycles set (25 cycles): 94°C for 35 sec., 45°C for 1 min, 72°C for 1:30 min, followed by 1 cycle at 72°C for 3 min and a final extension step at 72°C for 5 min. The PCR amplicons were visualized on 1% agarose gels using 1.7 µl of PCR product. The sequencing of the unpurified PCR products in both directions was performed at the Beijing Genomics Institute (Hong Kong, China) using the amplification primers. Sequence analysis was performed using Geneious Prime 2022.1.1 (http://www.geneious.com). The DNA was deposited in the scientific collections of Ilia State University, Tbilisi, Georgia, and the aliquots will be deposited in the LIB Biobank at Museum Koenig, Bonn, Germany, while the sequences have been submitted to the Barcode of Life Data System (BOLD)

databases. The newly obtained DNA barcodes of COI sequences were checked against the BOLD Systems database (http://www.boldsystems.org/index.php) (Ratnasingham and Hebert 2007). 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 p-distance as performed in the BOLD System.

The barcode obtained from the sample with CaBOL-ID 1023967 (BOLD:AFB9057) is the first *T. turanica* submitted to the BOLD Systems database. The nearest neighbor to our newly obtained barcode in BOLD systems is the unidentified Acrididae from Pakistan (BOLD:AAP6100, p-distance 6.25%). The second and third best matches are also unidentified Acrididae from Pakistan (BOLD:AAP6100, p-distance 6.27%). The nearest congener in BOLD systems is *T. cylindrica* (Marschall, 1836), with a private status from France, Corsica (p-distance 6.45%). Given a maximum p-distance of 0.2% between the unidentified Acrididae from Pakistan and *T. cylindrica* from France, the former ones most likely belong to the same species.

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