PENSOFT

CAUCASIANA
Journal on the biodiversity of the Caucasus and the adjacent regions

Leptopus hispanus and *Erianotus lanosus*: the first DNA-assisted records of Leptopodidae (Hemiptera, Heteroptera) for the fauna of Georgia

Beka Chitadze[1], Eka Arsenashvili[1], Natalia Bulbulashvili[2]

1 *Institute of Ecology, Ilia State University, Cholokashvili av. 3/5 Tbilisi, 0162*

2 *Rustaveli st. 8, 1400, Gori, Georgia*

http://zoobank.org/7DE73062-76C0-433F-8792-86D672F7D08E

Corresponding author: Beka Chitadze (beka.chitadze.2@iliauni.edu.ge)

Academic editor: Levan Mumladze ♦ **Received:** 13 February 2023 ♦ **Accepted:** 15 March 2023 ♦ **Published:** 21 March 2023

Abstract

The shore-bug (Leptopodidae Brullé, 1836) family is reported from Georgia for the first time upon several finds of *Leptopus hispanus* Rambur, 1840, and *Erianotus lanosus* (Dufour, 1834) (Hemiptera, Heteroptera). These species are widespread from the Mediterranean to Central Asia, including the Caucasus. Barcodes of the species, collecting information, and pictures of specimens are given.

Key words

New species record, shore-bugs, South Caucasus

Introduction

The infraorder Leptopodomorpha consists of four extant families of predaceous bugs that are typically littoral in habits, with the most diverse family being the Saldidae Amyot & Serville, 1843 (ca. 335 species) (Schuh and Polhemus 2009), while the Aepophilidae Puton, 1879, and Omaniidae Cobben, 1970 are very small families of highly specialized species inhabiting intertidal zones in the Old World (Grimaldi et al. 2013). The Leptopodidae contains at least 55 extant species in 38 genera (Henry 2017; Schuh and Weirauch 2020).

Leptopodomorpha are very small to medium-sized predatory bugs (2.5–8 mm), varying in shape from nearly globose, slightly flattened, and ovoid, to elongate and parallel-sided, and often darkly colored. The head is usually relatively short and broad without dorsal trichobothria. The eyes are usually very large, occupying almost the entire side of the head. The antennae are 4-segmented, thin, and distinct in size. The rostrum is 4-segmented; in Leptopodidae, segments II and III are spinose laterally. The pronotum is trapezoidal without a transverse furrow. The forewings are in the form of hemelytra, with a conspicuously coriaceous anterior portion and a membranous posterior region; the membrane usually has 3–5 cells in macropterous forms. The legs are thin, and the front ones in Palaearctic Leptopodidae are armed with 1 row of spines (Péricart 1990; Schuh and Slater 1995; Vinokurov and Kment 2015).

The family Leptopodidae Brullé, 1836, is very close to that of Saldidae. Despite being combined under the name "shore-bugs", Leptopodidae are mainly associated with dry environments (sometimes with no traces of water nearby) and even caves (Khazaei et al. 2020). A single species of the family, *Patapius spinosus* (Rossi, 1790), was known to occur in Georgia (Lindskog 1995; Aukema et al. 2013); however, we failed to find any exact record in the published sources (e.g., Cobben 1968, Hoberlandt 1983). Either the specimen distribution is based on a private collection or has never been published.

Our findings of *Leptopus hispanus* Rambur, 1840 and *Erianotus lanosus* (Dufour, 1834) are thus the first confirmed records of the family Leptopodidae in Georgia.

Materials and methods

Material was collected within the framework of the BMBF-funded project Caucasus Barcode of Life (CaBOL) (https://ggbc.eu/). The specimens were collected by hand and sweep-net, fixed in 96% ethanol and later stored in a freezer under -22°C for further DNA barcoding at Ilia State University, Institute of Ecology. The specimens were identified using the key by Péricart (Péricart 1990; Khazaei et al. 2020). Photos of the specimens with CaBOL-IDs 1011728 (Fig. 1A) and 1012366 (Fig. 1B) were taken using a Canon EOS 90D camera with a Canon EF-S 60 mm f/2.8 Macro USM lens. The digital images were prepared using Zerene Stacker image stacking software and Adobe Photoshop CS6.

Genomic DNA was extracted from tissue samples using the Quick-DNATM Miniprep PlusKit (Zymo Research) (for 25 mg tissue). Partial sequences of cytochrome oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) using the primer pair LCOI490-JJ and HCO2198- JJ (Astrin and Stüben 2008). Thermal conditions included denaturation at 95 °C for 1 min, followed by 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 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). 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 systems.

Results

Order Hemiptera Linnaeus, 1758
Family Leptopodidae Brullé, 1836

Erianotus lanosus (Dufour, 1834)

Materials examined. • 3 specimens; Gori; N41.9771°, E44.0984°; 589 m a.s.l.; under rocks in steppe near Mtkvari River; leg. N Bulbulashvili; 10/11-Sep-2021; CaBOL-IDs 1012366, 1012367, 1012379.

Genetics. We obtained three barcodes from the specimens with CaBOL-IDs 1012366, 1012367 and 1012379 (BOLD:AEY2001, mean p-distance 1%), with the nearest neighbor in BOLD systems being *Valleriola* sp. from China (mean p-distance 14.6%). There are no other barcodes of *E. lanosus* available in BOLD systems at the time of publishing

Remarks. From the neighbouring countries *E. lanosus* has been previously reported from Armenia, Azerbaijan and Turkey (Lindskog 1995, Fent et al. 2011). The species is widespread in the Palaearctic region while its general distribution is classified as Centralasiatic-Mediterranean.

Leptopus hispanus Rambur, 1840

Materials examined. • 2 specimens; Telovani; N41.8044°, E44.6880°; 903 m a.s.l.; under rocks at the roadside; leg. A Seropian; 25-Jul-2021; CaBOL-IDs 1011728, 1011740 • 3 specimens; Gori; N41.9788°, E44.0960°; 584 m a.s.l.; under rocks in steppe near Mtkvari River; leg. N Bulbulashvili; 05-Sep-2021; CaBOL-IDs 1012380, 1012392, 1012404 • 1 specimen; Kodistskaro; N42.0116°, E44.3446°; 736 m a.s.l.; under rocks in steppe; leg. N Bulbulashvili; 03-Jun-2021; CaBOL-ID 1025745 • 1 specimen; Vardzia; N41.3529°, E43.2518°; 1315 m a.s.l.; under rocks at Mtkvari River bank; leg. N Bulbulashvili; 13-Oct-2022; CaBOL-ID 1032753 • 1 specimen; Mashavera River; N41.4516°, E44.4941°; 528 m a.s.l.; swept from vegetation; leg. E Arsenashvili; 25-Oct-2022; CaBOL-ID.

Genetics. We obtained five barcodes from the specimens with CaBOL-IDs 1011728, 1011740, 1012380, 1012392 and 1012404 (BOLD:AEX9285, maximum p-distance 0.3%), with the nearest neighbor in BOLD systems being *L. marmoratus* (Goeze, 1778) from France with a Private status (mean p-distance 13.5%). There are no barcodes of *L. hispanus* in BOLD systems available at the time of pubishing.

Remarks. From the neighboring countries *L. hispanus* has been previously reported from Armenia, Azerbaijan and Turkey (Lindskog 1995; Fent et al. 2011; Dioli et al. 2019). The species is widespread in the Palaearctic region while its general distribution is classified as Centralasiatic-Mediterranean.

Discussion

Despite the wide distribution of *Erianotus lanosus* and *Leptopus hispanus* and their presence in neighboring Azerbaijan, Armenia, and Turkey (Lindskog 1995), these and other Leptopodidae family representatives remained undiscovered in Georgia for a long time. This was not only due to their small size or secretive lifestyle, but mainly because of a lack of sufficient and targeted research on local Heteroptera. Moreover, our research has revealed that the previous anecdotal record of *Patapius spinosus* in Georgia (Lindskog 1995; Aukema et al. 2013) is most likely an extrapolation of data on the species occurrence in neighboring Azerbaijan and Armenia (Cobben 1968; Hoberlandt 1983), which, along with Georgia, form the South Caucasus ecoregion. However, given its wide distribution range and presence in neighboring Turkey, along with another spiny-legged bug, *Leptopus marmoratus* (Goeze, 1778) (Lindskog 1995; Yazici 2020; Yazici & Bal 2022), the occurrence of both species on the territory of Georgia is highly likely. Further studies should be carried out in order to establish the actual distri-

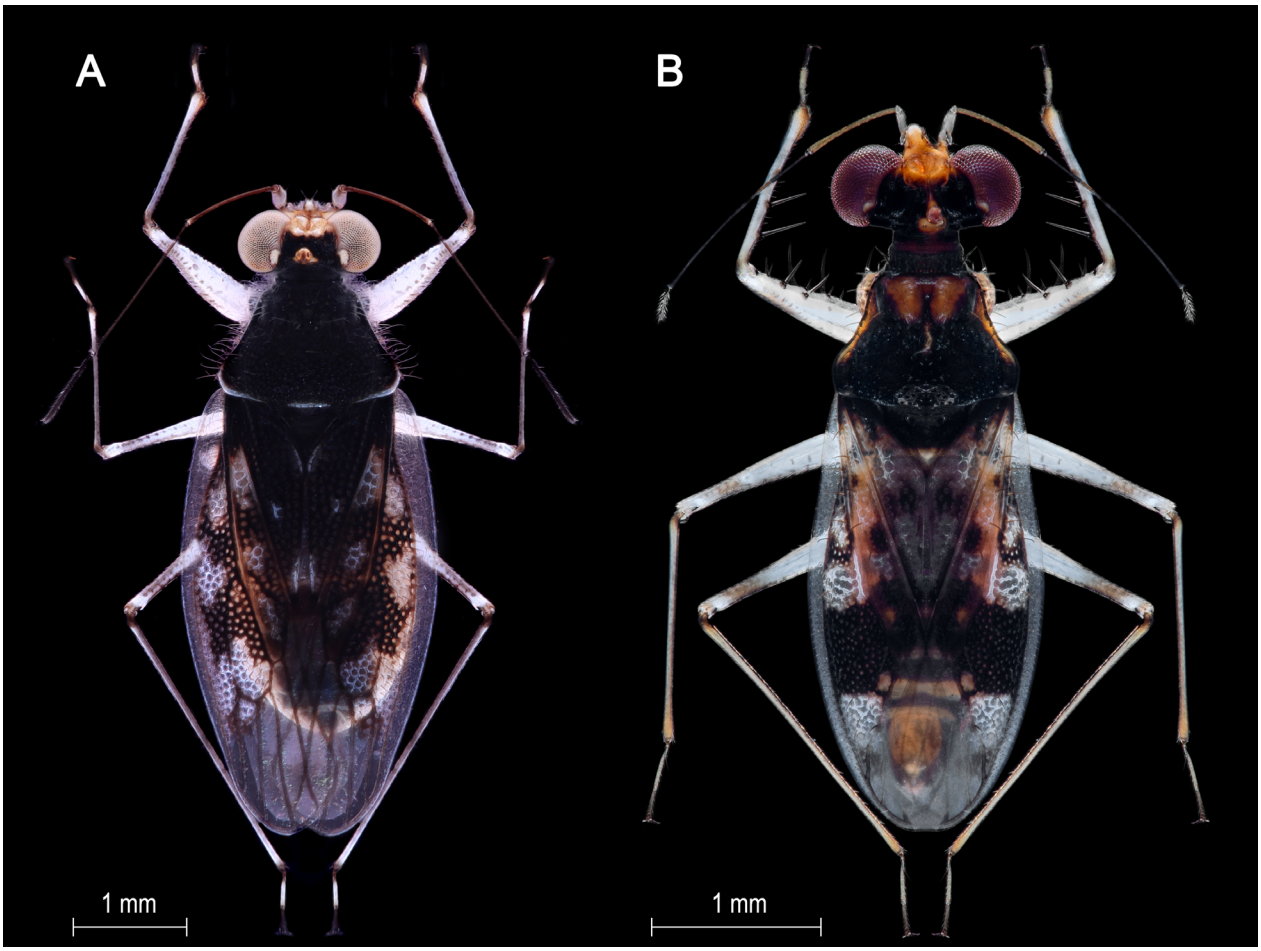

Figure 1. *Erianotus lanosus* (Dufour, 1834) (A) and *Leptopus hispanus* Rambur, 1840 (B). (Photo credit: Armen Seropian)

bution and population status of *E. lanosus* and *L. hispanus*, which will allow for a more accurate assessment of their protection status, as in some countries they are regarded as rare species (Gueorguiev et al. 1998; Dorow et al. 2003; Küssner 2011; Protic 2018).

Acknowledgements

We would like to express our gratitude to Armen Seropian for his valuable comments and images of voucher specimens. Prof. Levan Mumladze provided valuable suggestions during the preparation of the manuscript. We would also like to thank the CaBOL (Caucasus Barcode of Life) Entomology team and Genetic Laboratory at Ilia State University. This study was funded by the Federal Ministry of Education and Research under the grant number 01DK20014A. The responsibility for the content of this publication lies with the author.

References

Astrin JJ, Stüben PE (2008) Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebrate systematics 22(5): 503–522.

Aukema B, Rieger Ch, Rabitsch W (2013) Catalogue of the Heteroptera of the Palaearctic Region. VI. Supplement. The Netherlands Entomological Society, Amsterdam, 629 pp.

Cobben RH (1968) A new species of Leptopodidae from Thailand (Hemiptera-Heteroptera). Pacific Insects 10: 529–533. https://doi.org/10.3897/zookeys.917.46887

Dioli P, Özgen İ, Cianferoni F (2019) First record of *Leptopus hispanus* Rambur, 1840 (Hemiptera: Leptopodidae) for Eastern Anatolia (Turkey). Journal of the Heteroptera of Turkey 1(1–2): 1–3. https://doi.org/10.5281/zenodo.4297478

Fent M, Kment P, Camur-Elipek B, Kirgiz T (2011) Annotated catalogue of Enicocephalomorpha, Dipsocoromorpha, Nepomorpha, Gerromorpha, and Leptopodomorpha (Hemiptera: Heteroptera) of Turkey, with new records. Zootaxa 2856(1): 1–84. https://doi.org/10.11646/zootaxa.2856.1.1

Grimaldi DA, Engel MS, Singh H (2013) Bugs in the biogeography: Leptosaldinae (Heteroptera: Leptopodidae) in amber from the Miocene of Hispaniola and Eocene of India. Journal of the Kansas Entomological Society 86(3): 226–243. https://doi.org/10.2317/JKES130128.1

Henry TJ (2017) Biodiversity of Heteroptera. In: Foottit RG, Adler RH (Eds) Insect Biodiversity: Science and Society. John Wiley & Sons, 279–335. https://doi.org/10.1002/9781118945568.ch10

Hoberlandt L (1983) Results of the Czechoslovak-Iranian entomological expeditions to Iran 1970, 1973 and 1977. Heteroptera, Leptopodidae. Acta Entomologica Musei Nationalis Pragae 41: 99–105.

Khazaei Z, Polhemus DA, Tahami MS (2020) A new species of *Leptopus* (Heteroptera: Leptopodidae) from caves in Iran, with notes on other cavernicolous Iranian Heteroptera. Zootax, 4763(2): 246–258. https://doi.org/10.11646/zootaxa.4763.2.7

Lindskog P (1995) Infraorder Leptopodomorpha. In: Aukema B, Rieger C (Eds) Catalogue of the Heteroptera of the Palaearctic Region. I. The Netherlands Entomological Society, Amsterdam 115–141.

Péricart J (1990) Hemiptères Saldidae et Leptopodidae d'Europe occidentale et du Maghreb. Éditions Faune de France 77: 1–238.

Protić L (2018) Species of the family Leptopodidae (Heteroptera) in Serbia. Acta Entomologica Serbica 23(1): 9–17. https://doi.org/10.5281/zenodo.1421669

Ratnasingham S, Hebert PD (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7(3): 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Schuh RT, Slater JA (1995) True bugs of the world (Hemiptera: Heteroptera): classification and natural history. Cornell University Press, 800 pp.

Schuh RT, Polhemus JT (2009) Revision and analysis of *Pseudosaldula* Cobben (Insecta: Hemiptera: Saldidae): a group with a classic Andean distribution. Bulletin of the American Museum of Natural History 323: 1–102. https://doi.org/10.1206/323.1

Schuh RT, Weirauch C (2020) True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History. Cornell University press, Ithaca, New York.

Vinokurov NN, Kment P (2015) Contribution to the faunistics of shore bugs (Hemiptera: Heteroptera: Leptopodomorpha) in the Palaearctic Region and the Himalayas. Zootaxa 4028(3): 367–387. https://doi.org/10.11646/zootaxa.4028.3.3

Yazici G (2020) Overview of the zoogeographical distribution of aquatic and semi-aquatic Heteroptera (Hemiptera) in Turkey. Journal of Insect Biodiversity and Systematics 6(2): 135–155. https://doi.org/10.52547/jibs.6.2.135

Yazici G, Bal N (2022) Diversity, ecological properties of Dipsocoromorpha, Enicocephalomorpha, Gerromorpha, Leptopodomorpha and Nepomorpha (Heteroptera: Hemiptera) in Turkey. Arch Lif Sci Nutr Res 6(1): 1–13.