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Moss-inhabiting flea beetles of the West Indies I: New species of *Borinken* Konstantinov and Konstantinova and *Kiskeya* Konstantinov and Chamorro-Lacayo (Coleoptera: Chrysomelidae: Galerucinae: Alticini) from Puerto Rico

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Abstract. Three **new species** of flea beetles (Coleoptera: Chrysomelidae: Galerucinae: Alticini) from moss cushions from Puerto Rico are described: *Borinken toronegro* Konstantinov and Linzmeier and *Kiskeya segarrai* Konstantinov and Linzmeier from the Toro Negro mountain region and *Kiskeya micheliorum* Konstantinov and Linzmeier from the Maricao mountains. New species are compared morphologically with already known species from the same genera. In addition, to determine the similarities between moss inhabiting flea beetles, we sequenced the Cytochrome oxidase I barcode region of larval and adult specimens. In all cases, the distances between species are well outside the 2% species-limit cutoff typically used as an indicator of different species.

Key words. Moss cushions, bryobionts, COI barcodes, species boundaries.

Introduction

Our preliminary list of Puerto Rican flea beetles (Coleoptera: Chrysomelidae: Galerucinae: Alticini) contains 35 genera and 90 species. Recent collecting, particularly studies of moss cushions in three main mountain regions of the island (Fig. 19), uncovered three more species from the West Indian endemic genera *Borinken* Konstantinov and Konstantinova, 2011 and *Kiskeya* Konstantinov and Chamorro-Lacayo, 2006. These species are described below. It brings the total of known flea beetle species inhabiting moss cushions in the West Indies to eight. A morphological key to distinguish newly described *Kiskeya* species from previously known congeners is provided.

As commonly occurs in flea beetles living in highly isolated habitats such as mountain tops or moss cushions, different species often are very similar morphologically. Cytochrome oxidase I (COI) barcode sequence data were obtained and compared between congeners. These molecular barcodes indicate that within both *Borinken* and *Kiskeya,* the congeners discussed below are substantially different, well beyond the level typically considered reflective of distinct species.

Materials and Methods

Dissecting techniques and morphological terminology follow Konstantinov (1998). Although Chrysomelidae have five tarsomeres, the fourth one is very small, hardly visible and is located at the base of the tarsomere five. Because of that, we follow format traditional for flea beetle literature (e.g., Konstantinov 1998), measure four visible tarsomeres, skip the fourth and refer to tarsomere five as the fourth visible. Descriptive format follows previously published papers (e.g., Konstantinov and Konstantinova 2011). Specimen observations were made with a Zeiss Stemi SV11 Apo microscope. Digital photographs of morphological structures were taken with a Macropod Pro photomacrography system (Macroscopic Solutions, LLC) and with an AxioCam HRC Zeiss camera attached to a Leitz Diaplan compound microscope. The specimens are deposited in the USNM, National Museum of Natural History, Smithsonian Institution, Washington DC, USA.

Molecular Methods. To determine the similarities between moss inhabiting flea beetles, we sequenced the mitochondrial Cytochrome oxidase I (COI) barcode region of larval and adult specimens. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). PCR amplification of the DNA barcode region of COI was performed using primers LCO and HCO (Folmer et al. 1994). PCRs were performed on a Tetrad 2 thermocycler (Bio-Rad, Hercules, CA, USA) with the following "touchdown" program: initial denaturation for two minutes at 92° C, 12 touchdown cycles from 58° C to 46° C (10 seconds at 92° C, 10 seconds at 58–46° C, one minute at 72° C), 27 cycles at 10 seconds at 92° C, 10 seconds at 45° C, one minute at 72° C, and a final extension for seven minutes at 72° C. PCR products were enzymatically purified for sequencing by using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). Sequences were generated with the amplifying primers by using the BigDye Terminator v3.1 Sequencing kit (Applied Biosystems, Foster City, CA) and fractionated on an ABI 3730XL Genetic Analyzer. Sequences were edited in Geneious R10 (Biomatters, New Zealand). Uncorrected pairwise distances between COI barcode sequences were calculated in Geneious. Sequences were deposited in the Barcode of Life Data System (BOLD) under sample IDs ASK0019, ASK0023, ASK0027, ASK0034, and ASK0035.

Molecular Results

Pairwise distances between two *B. toronegro* specimens and one *B. elyunque* specimen ranged from 4.7–4.9 % (similarity: 95.1–95.3%). The pairwise distance between the *K. micheliorum* and the *K. elyunque* specimens was 13.5% (similarity: 86.5%). In both cases, the distances between species are well outside the 2% species-limit cutoff typically used as an indicator of different species (Scheffer and Wiegmann 2000; Hebert et al. 2003).

Morphological Results

Borinken toronegro Konstantinov and Linzmeier, new species

(Fig. 4–9, 19)

ZooBank. lsid:zoobank.org:act:1AF380FF-8AB4-40B0-9AE7-026989B61B8A

Description. Body length 1.08–1.18 mm, width 0.70–0.81 mm, oval, relatively flat in lateral view (2.08 times as long as thick). Color dark yellow without metallic luster, legs slightly darker and antennae dark brown, almost black. Vertex covered with large punctures, shiny, without wrinkles. Oblique fold situated

between orbit and antennal callus. Proportions of antennomere lengths in female: 13:8:7:5:4:4:5:5:6:8:11. Antennomeres widened abruptly beginning from antennomere 7 (it is as long as wide). Pronotum evenly covered with large punctures, their diameter much larger than distance between them. Anterolateral callosity extending slightly beyond lateral margin. Ventral side of body without many setae. Elytron with nine complete rows of punctures, additional scutellar row with six punctures. Punctures large, about as large as space between rows. Interspaces convex, shiny, without wrinkles or punctures. Proportions of tarsomere lengths of female as follows: protarsomeres 7:6:6:14; mesotarsomeres 7:6:6:14; metatarsomeres 10:6:6:13. Spermathecal receptacle separated from pump and as long as pump. Receptacle ovoid, sides convex. Duct without coils, making a loop away from receptacle, its longitudinal part straight. Tignum long, its narrow part slightly curved, posterior part widely sclerotized. Sclerotization fades posteriorly. Vaginal palpi widely separated posteriorly. Free (posterior) part slightly longer than anterior.

Remarks. *Borinken toronegro* differs from *B. elyunque* by the dark yellow color (*B. elyunque* is dark brown, Fig. 1–3). In *B. toronegro* the antennae are much darker than the body (in *B. elyunque* the antennae are about the same color as the beetle body). Elytral interspaces are a bit more convex in *B. toronegro*. In addition, *B. toronegro* may be separated from *B. elyunque* by the following features: antennal calli positioned more or less horizontally (more or less vertically in *B. elyunque*); pronotum slightly wider (Fig. 1, 4); anterolateral corners of pronotum extend farther beyond lateral margin (anterolateral corners of pronotum extend not as far beyond lateral margin (Fig. 1, 4).

Habitat. *Borinken toronegro* was collected at the highest point of Puerto Rico, near the phone towers on Toro Negro. Moss bearing trees are located on the side of the road that leads to the towers (Fig. 20, 21).

Distribution. Puerto Rico.

Etymology. This species is named after its type locality. The epithet is a noun in apposition.

Type material. Holotype, female, with labels as follows: 1) Puerto Rico: Toro Negro, 3.IX.2014 N18.10.335 W66.35.504, h-1350m, moss, WP-478, leg. A. Konstantinov; 2) 2014.09.03 0670; 3) Holotype *Borinken toronegro* new species des. A. Konstantinov & A.M. Linzmeier 2020 (USNM). Paratype, female, with the same labels as holotype (USNM).

Material examined. *Borinken elyunque*: Puerto Rico: El Yunque, 8.IX.2014, N18°16.54 W65°50.23 h-952 m, moss, WP-488 leg. A. Konstantinov (male, USNM). Puerto Rico: El Yunque, 8.IX.2014 N18.16.56 W65.50.28, h-933m, moss, WP-489, leg. A. Konstantinov (2 males, USNM).

Kiskeya micheliorum Konstantinov and Linzmeier, new species

(Fig. 13–15, 19)

ZooBank. lsid:zoobank.org:act:BB43DE7B-DF54-487F-952F-261E263D2373

Description. Body length 0.76–0.86 mm, width 0.60–0.69 mm, ovoid, relatively convex in lateral view (1.5 times as long as thick). Color shiny black, with silvery luster. Legs and antennae brown, antennomeres 2 and 3 paler, 2 nearly whitish. Vertex smooth, with small, sparse, sharply impressed punctures, bearing short setae. Supraantennal sulcus present. Antennal club with 3 antennomeres. Pronotum with punctures as small and sparse as on vertex. Posterolateral callosity of pronotum nearly as large as anterolateral. Elytron (Fig. 13) convex in lateral view [length (from apex to connection with pronotum) nearly equal to height], with punctures smaller and sparser than those on pronotum. Proportions of protarsomeres in male and female (starting with first) 4:2:3:8; mesotarsomeres 4:2:3:8; metatarsomeres 11:2:3:9. Median lobe of aedeagus simple. In ventral view, apex subtriangular, without acute denticle, surface convex. In lateral view, evenly curved throughout, abruptly curved near base; basally narrower than medially; apex nearly straight ventrally. In dorsal view, opening ovoid and relatively wide.

Remarks. *Kiskeya micheliorum* can be easily separated from the Dominican Republic species *K. baorucae* Konstantinov and Chamorro-Lacayo, 2006 and *K. neibae* Konstantinov and Chamorro-Lacayo, 2006 based on the smaller body, and the aedeagus without apical denticle (the aedeagus of both Dominican Republic species has a well-developed denticle). *Kiskeya micheliorum* differs from *K. elyunque* by having

antennomeres 2 and 3 paler than antennomeres 4 and 5 (in *K. elyunque* antennomeres 2 and 3 are about as dark as the remaining antennomeres). *Kiskeya micheliorum* may be separated from other Puerto Rican species with the help of the key at the end of this paper.

Habitat. *Kiskeya micheliorum* was collected along an unpaved road off the main road in Maricao at the 9.2-mile mark. Two moss bearing trees are located along this small road.

Distribution. Puerto Rico.

Etymology. This species is named after Mona and Julio Micheli and the rest of Micheli family (Ponce, Puerto Rico) in recognition of their contribution to studies of Puerto Rican nature. The epithet is a noun in the genitive case.

Type material. Holotype male is labeled as follows: 1) Puerto Rico: Maricao, 4.IX.2014 N18.08.041 W66.57.290 h-702 m, moss, WP-483 leg. A. Konstantinov; 2) 2014.09.04 0672; 3) Holotype *Kiskeya micheliorum* new species des. A. Konstantinov & A.M. Linzmeier 2020 (USNM). Paratype, female, with the same labels as holotype except 2014.09.04 0507 (USNM).

***Kiskeya segarrai* Konstantinov and Linzmeier, new species**
(Fig. 16–18, 19, 20, 21)

ZooBank. lsid:zoobank.org:act:9AF0A2F2-BBD6-42BD-B730-8A8B88EE6371

Description. Body length 0.73–0.84 mm, width 0.58–0.69 mm, ovoid, relatively convex in lateral view (1.5 times as long as thick). Color shiny black, with pink luster. Legs and antennae light brown, antennomeres 2 and 4 paler, 3 nearly white. Vertex dull, with tiny, sparse, weakly impressed punctures, bearing short setae. Supraantennal sulcus present. Antennal club with 3 antennomeres (Fig. 17). Pronotum with punctures as small and sparse as those on vertex. Posterolateral callosity of pronotum much smaller than anterolateral. Elytron (Fig. 16) convex in lateral view [length (from apex to connection with pronotum) nearly equal to height], with punctures as small and sparse as those on pronotum. Proportions of protarsomeres in both male and (starting with first) 4:2:4:8; mesotarsomeres 3:2:3:8; metatarsomeres 8:2:4:8. Median lobe of aedeagus simple. In ventral view, apex subtriangular, without acute denticle, surface slightly convex. In lateral view, evenly curved throughout, including base; basally slightly narrower than medially; apex nearly straight ventrally. In dorsal view, opening elongate, relatively narrower basally than at middle.

Remarks. *Kiskeya segarrai* can be easily separated from the Dominican Republic species *K. baorucae* and *K. neibae* based on the smaller body and the aedeagus without apical denticle (the aedeagus of both Dominican Republic species has a well-developed denticle). It may be separated from Puerto Rican species with the help of the key at the end of the paper.

Habitat. *Kiskeya segarrai* was collected in the same moss cushions as *Borinken toronegro*, at Toro Negro, the highest point in Puerto Rico, near the phone towers. Moss bearing trees are located on the side of the road that leads to the towers.

Distribution. Puerto Rico.

Etymology. This species is named after Alex Segarra (University of Puerto Rico, Mayaguez) for his contribution to study of insect diversity in Puerto Rico. The epithet is a noun in the genitive case.

Type material. Holotype, male, is labeled as follows: 1) Puerto Rico: Toro Negro, 3.IX.2014 N18.10.335 W66.35.504 h-1350 m, moss, WP-478 leg. A. Konstantinov; 2) 2014.09.03 0671; 3) Holotype *Kiskeya segarrai* new species des. A. Konstantinov & A.M. Linzmeier 2020 (USNM). Paratypes, with the same labels as holotype (9, USNM).

Key to *Kiskeya* species of Puerto Rico

1. Median lobe of aedeagus wider and relatively short, in lateral view curved in middle. Vertex with a few larger punctures in between small ones . . . . . ***Kiskeya elyunque* Konstantinov and Konstantinova**

— Median lobe of aedeagus narrower and relatively long, in lateral view curved near base. Vertex with relatively uniform small punctures **2**

2(1). Upper side of body black, with silvery luster. Posterolateral callosity of pronotum nearly as large as anterolateral. Median lobe of aedeagus in dorsal view with opening ovoid and relatively wide (Fig. 15) . . . . ***Kiskeya micheliorum* Konstantinov and Linzmeier, new species**

— Upper side of body black, with pink luster. Posterolateral callosity of pronotum much smaller than anterolateral. Median lobe of aedeagus in dorsal view with opening elongate, relatively narrower basally than at middle (Fig. 18) . . . . . ***Kiskeya segarrai* Konstantinov and Linzmeier, new species**

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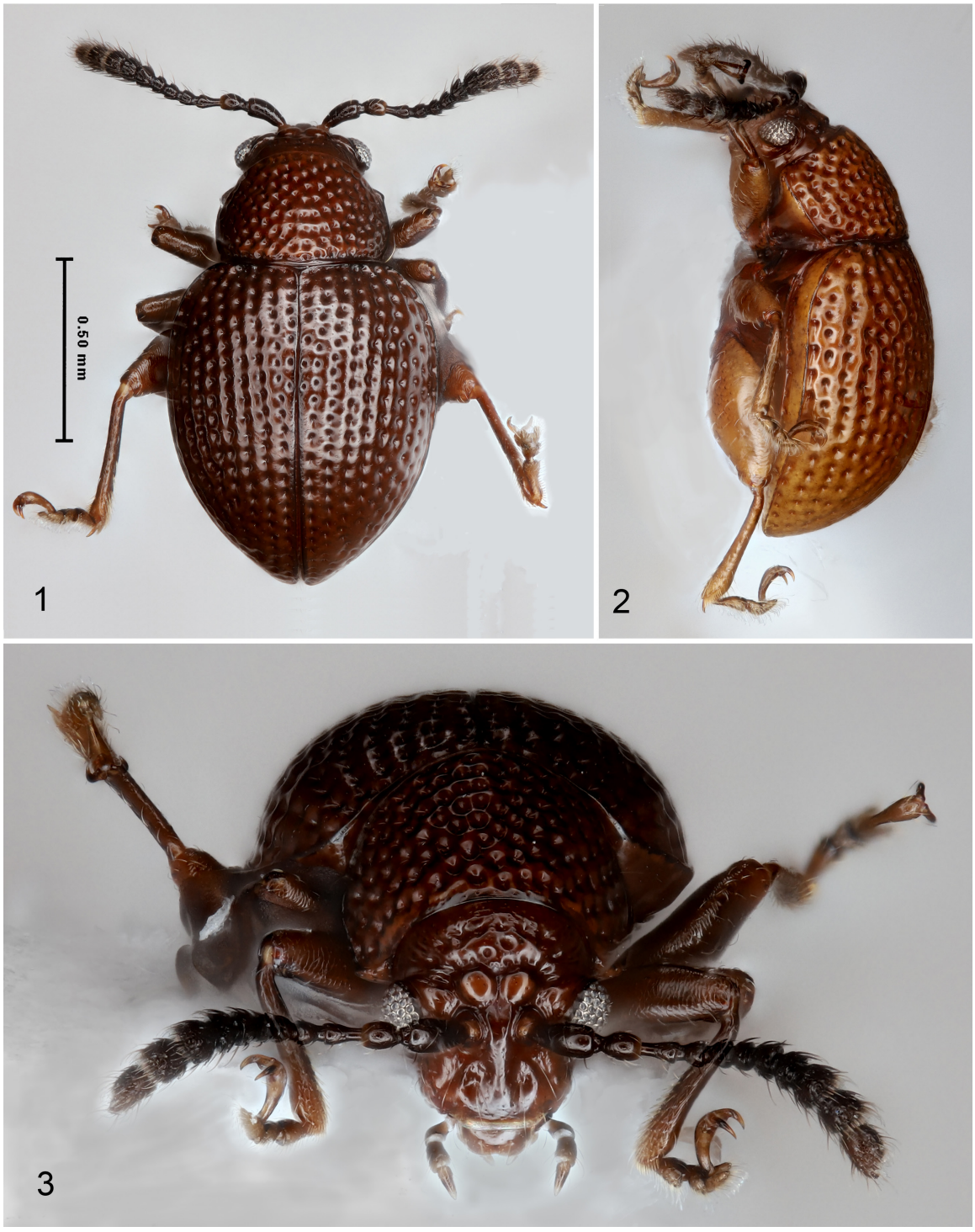
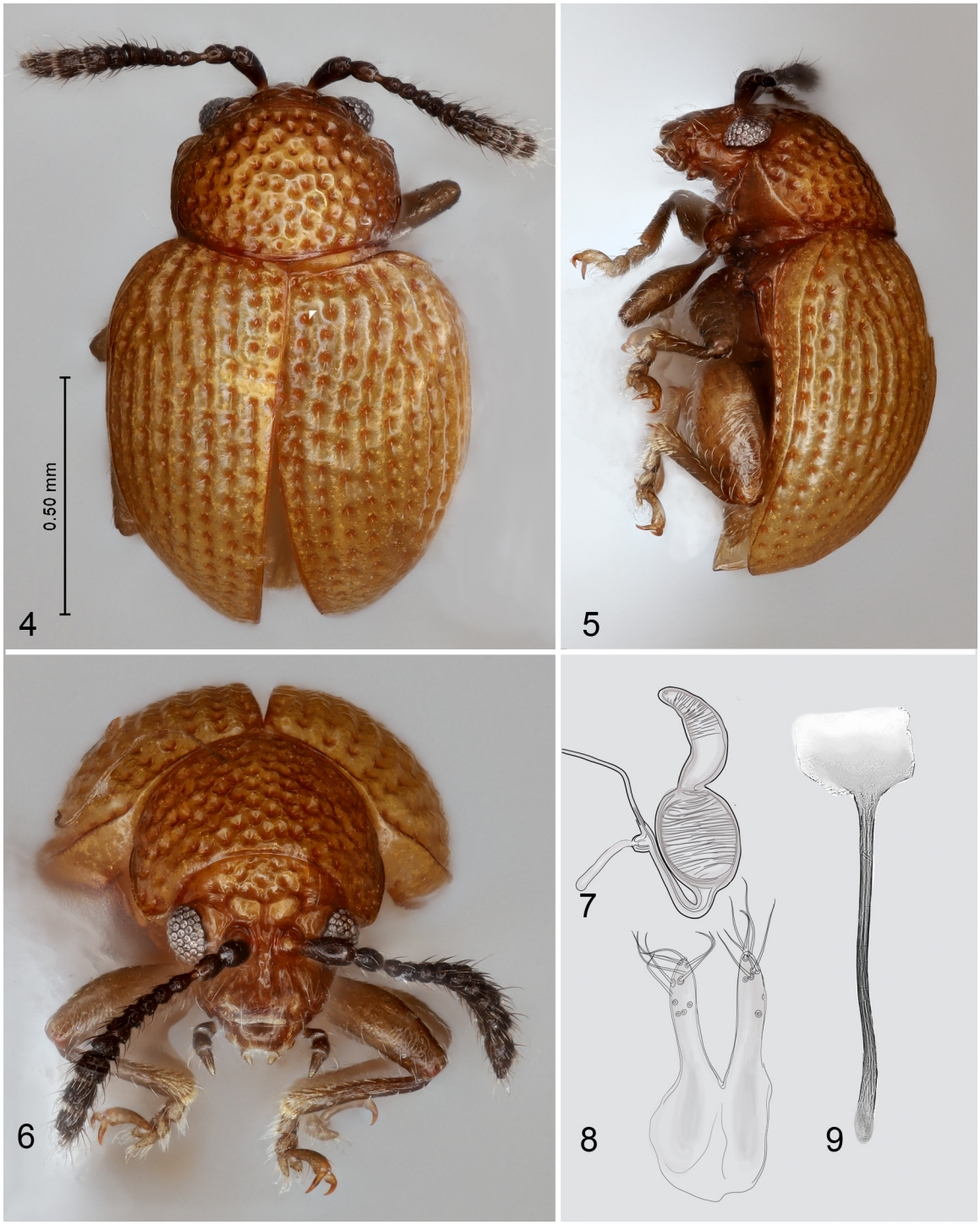


Figures 1–3. *Borinken elyunque* Konstantinov and Konstantinova, 2011. **1)** Habitus, dorsal. **2)** Habitus, lateral. **3)** Habitus, frontal.

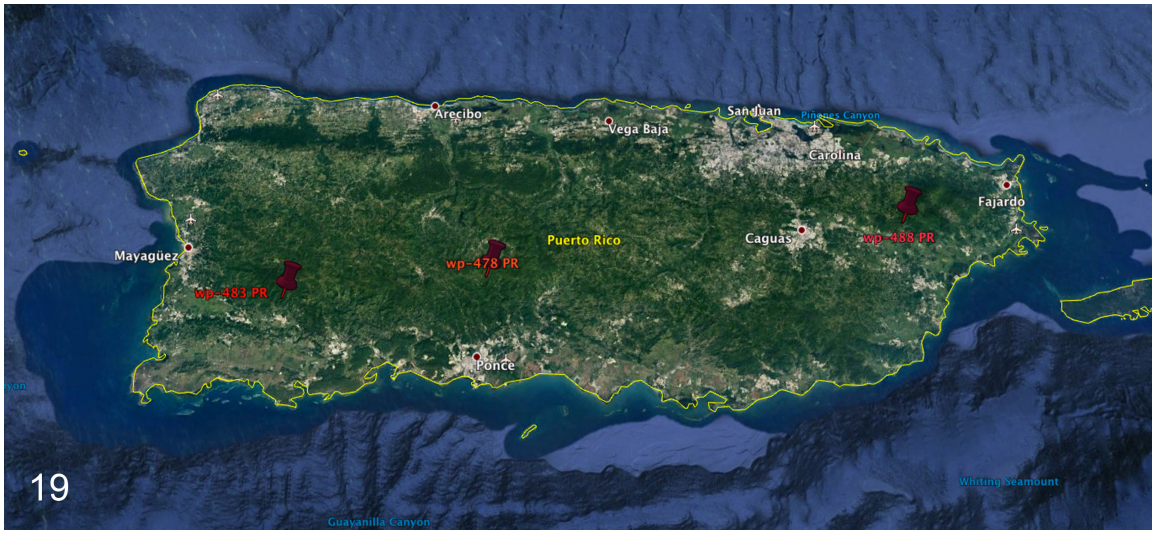


Figures 4–9. *Borinken toronegro* new species. **4)** Habitus, dorsal. **5)** Habitus, lateral. **6)** Habitus, frontal. **7)** Spermatheca. **8)** Vaginal palpi. **9)** Tignum.

Figures 10–12. *Kiskeya elyunque* Konstantinov and Konstantinova, 2011. **10)** Habitus, lateral. **11)** Habitus, frontal. **12)** Median lobe of aedeagus, ventral, dorsal, and lateral views.

Figures 13–15. *Kiskeya micheliorum* new species. **13)** Habitus, lateral. **14)** Habitus, frontal. **15)** Median lobe of aedeagus, ventral, dorsal, and lateral views.

Figures 16–18. *Kiskeya segarrai* new species. **16)** Habitus, lateral. **17)** Habitus, frontal. **18)** Median lobe of aedeagus, ventral, dorsal, and lateral views.



Figures 19. Map of Puerto Rico with marked collecting localities for moss-inhabiting flea beetles.

Figures 20–21. Views of the collecting locality of *Borinken toronegro* and *Kiskeya segarrai*. **20)** Cell towers of Toro Negro, from below. **21)** View from the cell towers on the landscape below.