

## RESEARCH ARTICLE

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## Description of a new species of *Chrysina* Kirby, 1828 (Coleoptera: Scarabaeidae: Rutelinae) from *resplendens* group, based on morphological characters and mtDNA COX I molecular marker

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A new species of the genus *Chrysina* Kirby, 1828 is described from Panama. *Chrysina kalinini* sp.n. is related to *Chrysina resplendens* (Boucard, 1875) in habitus, male genitalia morphology and mtDNA COX I molecular marker). The two closely related species differ in the shape of pronotum, mesosternal process and male genitalia. mtDNA COI molecular marker analysis gives a reliable distance for the compared species.

**Key words:** Scarabaeidae; Rutelini; *Chrysina*; *Plusiotis*; new species; Panama

### Introduction

The genus *Chrysina* Kirby, 1828 (Coleoptera: Scarabaeidae: Rutelinae) contains about 120 species (Hawks, 2017; Moore et al., 2017). The genus is widespread in the North of C. America (starting from Texas, Usa) to the North-West of South America (Columbia, Ecuador). Some contemporary *Chrysina* were previously known as *Plusiotis* and *Pelidnotopsis*. Currently these genera are placed into synonymy under the older name, *Chrysina* (Hawks, 2001). According to D.C. Hawks (2006), there is a group of species morphologically closely related to *Chrysina resplendens* (Boucard, 1875): *Chrysina chaltothea* (H. W. Bates, 1888), *Chrysina cupreomarginata* (F. Bates, 1904), *Chrysina resplendens* (Boucard, 1875) and *Chrysina tapantina* (Morón, 1992). We assumed that new species belongs to this group and is similar to *C. resplendens* (Fig. 1–3). *C. resplendens* was described from Costa Rica, San Jose. The species is also known from other localities of Cordillera de Talamanca from Costa Rica and Panama. New species and *C. resplendens* occur in Panama sympatrically. In this article we compare the new species with *C. resplendens* and *C. cupreomarginata* because they appear to be the closest morphologically.

### Material and methods

About 70 specimens from the *resplendens* group were available for the study in research collections of the first and the third authors, the “Museum für Naturkunde - Leibniz Institute for Evolution and Biodiversity Science” (Berlin, Germany) and private collection of V.A. Kalinin (Russia, Moscow). For morphological examination the authors used stereo microscope Zeiss Stemi 2000-C. Tissue samples (abdomen tergites) were taken from 14 specimens of *Chrysina* for DNA analysis, and for comparison and estimation of the pairwise distances. The results were deposited in BOLD public project data records – see the Published Projects section of BOLD (Ratnasingham & Hebert, 2007; [www.barcodinglife.org](http://www.barcodinglife.org)).

DNA was extracted using the standard phenol-chloroform method (Sambrook, Fritsch, & Maniatis, 1989). Amplification reactions were carried out according to BOLD protocol with minor improvements in PCR thermal regime algorithm in the final volume of 25 µL with 12.5 pmol (or 0.5 pmol/µL) of the primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and 12.5 pmol (or 0.5 pmol/µL) of the primer HCO2198 (5'-TAAACTCAGGGTGACCAAAAATCA-3') (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994), 0.1g of the isolated DNA and the

universal Encyclo Plus PCR kit (Evrogen, Moscow) following the manufacturer's protocol. PCR was performed in a T100 thermal cycler (Bio-Rad, USA). The PCR thermal regime consisted of an initial denaturation of 4 min 30 sec at 94°C; five cycles of 30 sec at 94°C, 20 sec at 45°C and 1 min at 72°C; 35 cycles of 30 sec at 94°C, 20 sec at 55°C and 1 min at 72°C, and a final extension of 5 min at 72°C. Amplification success was checked by electrophoresis in 1.5% agarose gel with subsequent identification of PCR fragments under UV light after staining with ethidium bromide. The section of mtDNA is standard "barcode region" of the Cytochrome Oxidase subunit I gene was sequenced using the above primers LCO1490 and HCO2198 at the Evrogen laboratory (Moscow). DNA sequences were verified, aligned and analysed using MEGA version 6.0 (Tamura, Stecher, Peterson, Filipowski, & Kumar, 2013). The maximum likelihood method (Tamura-Nei model) was applied for estimating of divergence. For estimating branch support bootstrapping was carried out. Bootstrap values above 70 (expressed as percentages of 500 replications) were shown at branch points of the likelihood tree.

The sequences deposited in the BOLD database are shown in Table 1.

**Table 1.** Specimen data, sequences and images deposited in the BOLD project database (<http://www.boldsystems.org>).

Taxon name	BOLD sample Id
<i>Chrysina cupreomarginata</i>	BC-lvsh-5476-M14, BC-lvsh-5477-M11
<i>Chrysina kalinini</i>	BC-lvsh-5478-PK11, BC-lvsh-5479-PK12, BC-lvsh-5480-PK13, BC-lvsh-5481-PK21, BC-lvsh-5482-X23, BC-lvsh-5483-X24, BC-lvsh-5484-PP11, BC-lvsh-5485-PP12
<i>Chrysina resplendens</i>	BC-lvsh-5472-X12, BC-lvsh-5473-X11, BC-lvsh-5474-CK3, BC-lvsh-5475-R14

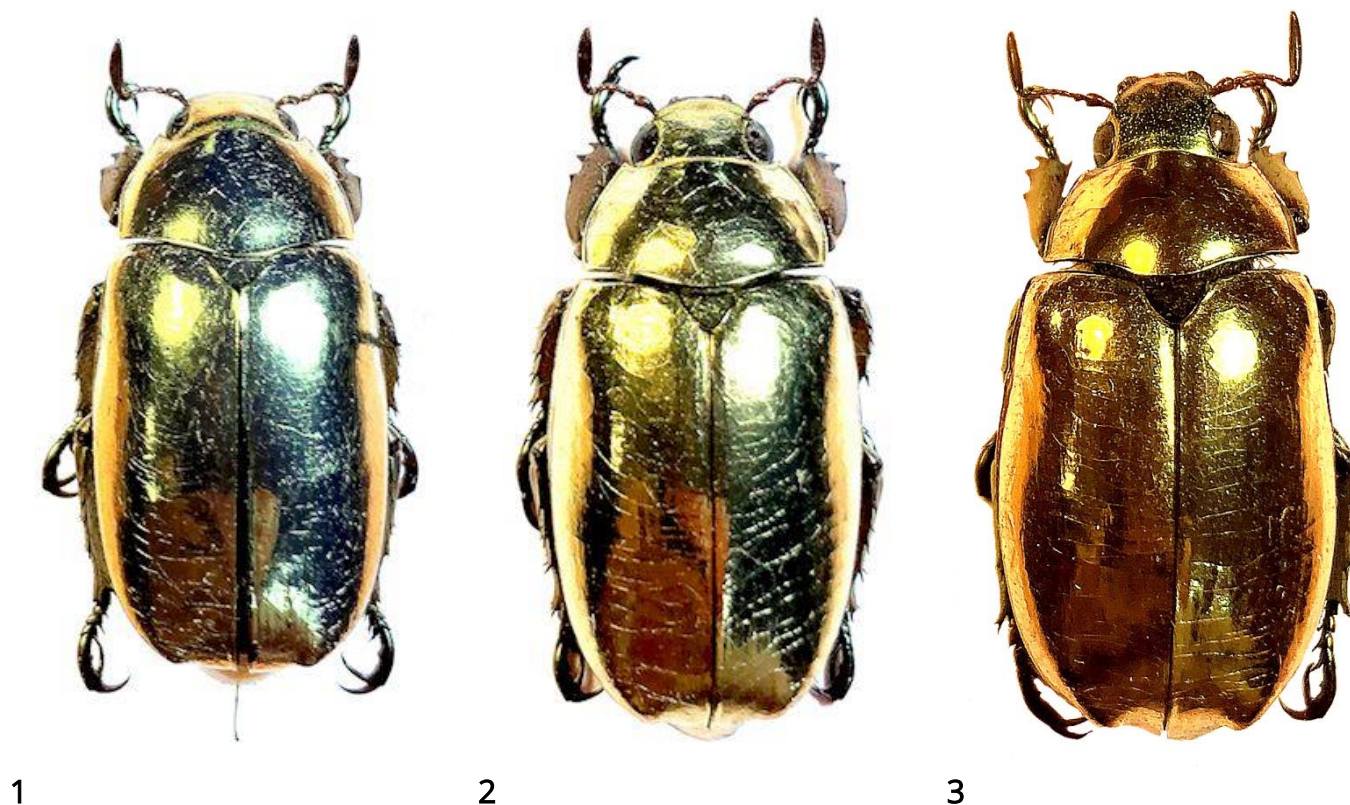
**Abbreviations.** The following abbreviations are used to indicate the location of the paratype specimens:

**MNKB** – Museum für Naturkunde, Leibniz Institut für Evolution und Biodiversitätsforschung (Berlin, Germany)

**VK** – the private collection of V. Kalinin (Moscow, Russia)

**AT** – the private collection of Andrey Yu. Titarenko (Moscow, Russia)

**AZ** – the private collection of A.S. Zubov (Moscow, Russia)



**Figs. 1-3.** 1. *C. kalinini* sp.n., male, Holotype: dorsal view. Panama, Chiriqui prov., Nueva California [08.88324, -82.68500], 1920 m., 17-19.06.2018 (BOLD sample ID BC-lvsh-5482-X23); 2. *C. resplendens*, male: dorsal view. Costa Rica. Limon prov., Lomas de Guacimo, Cerro Bellavista, Atlantic steppe, h-1200.V-VI.2015; 3. *C. cupreomarginata*, male: dorsal view. Costa Rica. Limon prov., Lomas de Guacimo, Cerro Bellavista, Atlantic steppe, h-1200.V-VI.2015. (BOLD sample ID BC-lvsh-5477-M11).

*Chrysina kalinini* Zubov & Ivshin sp. n.

**Holotype.** ♂, Panama, Chiriqui prov., Nueva California [08.88324, -82.68500], 1920 m., 17-19.06.2018, Leg. Zaritskiy V. [BM], BOLD sample ID BC-lvsh-5482-X23 (Fig. 1).

**Paratypes.** 30 sp. (VK), Panama, Chiriqui prov., Nueva California [08.88324, -82.68500], 1920 m., 17-19.06.2018, Leg. Zaritskiy V.; 2♂, 1♀ (AT), Panama Chiriqui, env. Boquete, Rio Palo Alto, 1300 m, 06.05.1980, Porion, Bertrand leg.; 1♀ (AT), Panama, Chiriqui, 06.06.1988, G. Brossard leg; 9♂, 6♀ (AT), Panama, Chiriqui prov., env. Volcan town, h-1700-1900m, 20.05-20.06.2018, A. Kozlov leg; 8♂, 14♀ (AT), Panama, Chiriqui, Bugaba distr., [8.843417N, 82.692W], h-1580m, 22.06, 26.06.2017; 5♂, 1♀ (AT), Panama, Chiriqui prov., Renacimiento distr., [8.8963N, 82.74142W], h-1675m, 25.06.2017; 1♀ (AT), Panama, Chiriqui, Rte Gualaca Fortuna, Hornito – Valle de la Sierpe, h-1000m, 08.05.1980, Porion, Bertrand leg.; Panama, Chiriqui, env. El hato del volcan, Quebrada Tisingal, h-1400m, 02.05.1980, Porion, Bertrand leg.

**Description.** Body length 20-24 mm. All body parts except antennae have shiny golden coloration, abdomen mostly brownish to golden and almost mat. Antennae short, brown, antennal club small. Body shape almost oval, twice long as wide. Pronotum slightly trapezoid, approximately 1,5 times wider than long, sides slightly triangular (Fig. 4–5). Elytra almost 1,5 times longer than wide. Legs short, hind tibia slightly longer than middle tibia and approximately 1,3 times longer than foreleg tibia. Head, pronotum and elytra glabrous in small rare punctuation. Clypeus brownish. Claws long and curvy. Mesosternal process relatively short, thinned to the apex, greenish. Prosternal plate has rounded triangular shape, flat, shiny golden (Fig. 6–9). Hind femur of males and females slightly thickened.

**Comparative analysis and remarks.** The new species is very close to *C. resplendens* and has only few morphological differences from it. Clypeus of *C. kalinini* sp.n. is slightly longer than in *C. resplendens*.

Pronotum in *C. kalinini* sp.n. is slightly longer in relation to its width than in *C. resplendens*, its sides have smaller angles, whereas in *C. resplendens* the sides of pronotum are rounded (Fig. 4–5).

Mesosternal process shiny, shorter and wider than in *C. resplendens*, where the process is long and narrow and its apical half is greenish-golden (Fig. 6-8).

Prosternal plate of *C. kalinini* sp.n. is rounded triangular and flat, in *C. resplendens* it is square and has a clear dent (Fig. 6–9).

Phallus of *C. kalinini* sp.n. is more narrow and more narrowed to the apex than in *C. resplendens* (Fig. 10–12).



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Fig. 4-5. 4. *C. kalinini* sp.n., male Holotype: pronotum (BOLD sample ID BC-lvsh-5482-X23); 5. *C. resplendens*, male: pronotum

In comparison by molecular markers (mtDNA COX I “barcoding region”), *C. resplendens*, *C. cupreomarginata* and *C. kalinini* demonstrated strong divergence. A maximum likelihood tree based on 14 DNA barcode sequences of the new taxa and those of their putative closest relatives in the genus is shown in Fig. 13. Sequences ranged from 500 to 700 bp (sequences shorter than 500 bp were excluded). In the Table 2 the number of base substitutions per site from averaging over all sequence pairs between groups are shown. Standard error estimates are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model (Tamura, Nei, & Kumar, 2004). The analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 612 positions in the final dataset.

According to the average values of the interspecies pairwise comparisons and the tree topology (Fig. 13, Table 2), *Chrysina kalinini* sp.n. reliably differs from its closest relatives, and molecular divergence of these three species is in concordance with their morphological divergence.

**Table 2.** Interspecies pairwise distances: the average values for number of base substitutions per site between sequences estimated as the divergence over sequence pairs between groups. Standard error estimate(s) are shown above the diagonal.

Taxon name	<i>Chrysina cupreomarginata</i>	<i>Chrysina kalinini</i>	<i>Chrysina resplendens</i>
<i>Chrysina cupreomarginata</i>	-	0.036	0.026
<i>Chrysina kalinini</i>	0.076	-	0.021
<i>Chrysina resplendens</i>	0.054	0.042	-

### Etymology

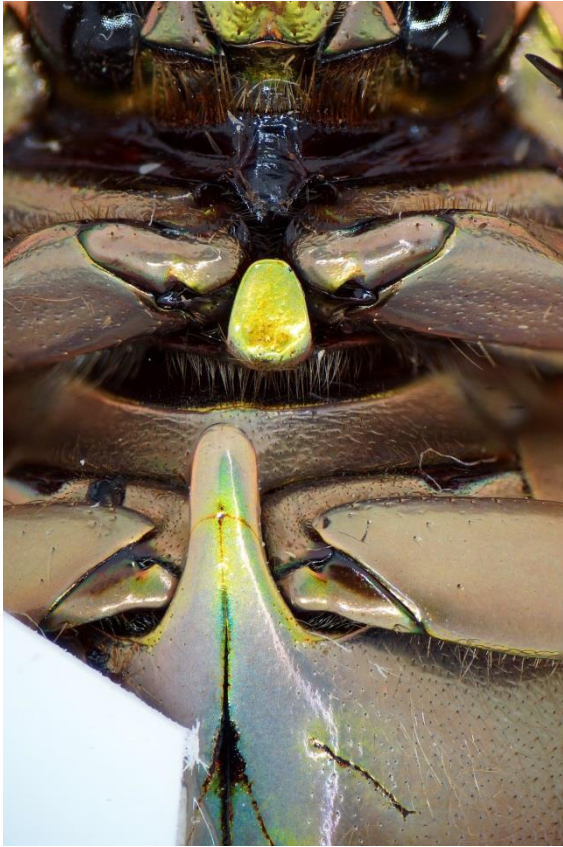
The species is named after the Russian collector Valentin Alexandrovich Kalinin (Russia, Moscow), who organized the research on this group and who supported a number of research expeditions which were crucial for this study.

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**Figs. 6-9.** 6. *C. kalinini*, male, Holotype: mesosternal process (BOLD sample ID BC-lvsh-5482-X23); 7. *C. kalinini*, female: mesosternal process; 8. *C. resplendens*, male: mesosternal process; 9. *C. cupreomarginata*, male: mesosternal process (BOLD sample ID BC-lvsh-5477-M11).

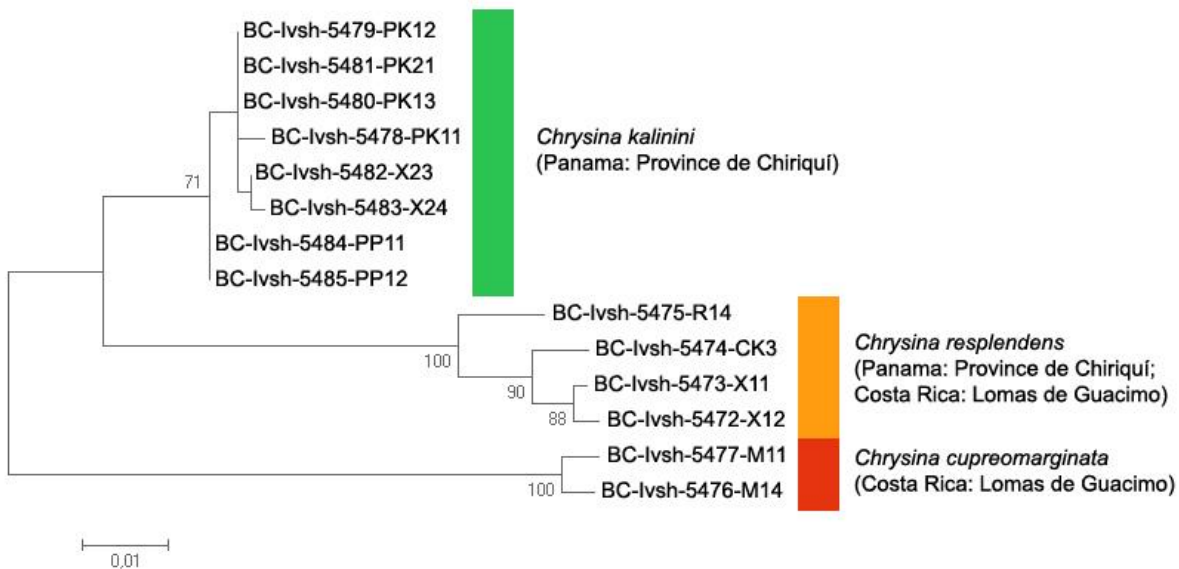


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**Figs.10-12.** 10. *C. kalinini*, Holotype: phallus (BOLD sample ID BC-lvsh-5482-X23); 11. *C. resplendens*: phallus; 12. *C. cupreomarginata*: phallus (BOLD sample ID BC-lvsh-5477-M11).



**Fig. 13.** Maximum likelihood tree of the studied *Chrysina*. Bootstrap support values of > 70% are indicated near the branches (500 bootstrap replicates).

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