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Validation of *Chrysina valentini* Zubov and Ivshin, 2019 (Coleoptera: Scarabaeidae: Rutelinae) by morphometric and cuticular reflectance analyses

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Validation of *Chrysina valentini* Zubov and Ivshin, 2019 (Coleoptera: Scarabaeidae: Rutelinae) by morphometric and cuticular reflectance analyses

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Abstract. Micromorphometric analyses of genital capsules and comparison of adult cuticular reflectance of two species of *Chrysina* Kirby (Coleoptera: Scarabaeidae: Rutelinae) support the status of *C. valentini* Zubov and Ivshin, 2019 as a valid species. Compared with its closest relative *C. optima* (Bates, 1888), capsules of *C. valentini* are proportionately wider at the base of the parameres than those of *C. optima*, and taper toward the apex more abruptly. Reflectance of *C. valentini* under natural light appears slightly greenish while *C. optima* is uniformly reddish. The number of teeth on the protibia and the shape of the mesosternal process, characters cited by Zubov et al. (2019) to distinguish the two species, did not prove reliable.

Key words. Rutelini, micromorphometric analysis of genitalia, statistical analyses, diagnosing cryptic species.

Resumen. Los análisis micromorfométricos de cápsulas genitales y la comparación de la reflectancia cuticular adulta de dos especies de *Chrysina* Kirby (Coleoptera: Scarabaeidae: Rutelinae) apoyan a *C. valentini* Zubov e Ivshin, 2019 como especies válidas. En comparación con su pariente más cercano *C. optima* (Bates, 1888), las cápsulas de *C. valentini* son proporcionalmente más anchas en la base de los parameres que las de *C. optima*, y se estrechan hacia el ápice más abruptamente. La reflectancia de *C. valentini* bajo luz natural aparece ligeramente verdosa mientras que *C. optima* es uniformemente rojiza. Número de dientes en la tibia de la pata delantera y forma de la apófisis mesoesternal, caracteres citados por Zubov et al. (2019) para distinguir las dos especies, no resultó confiable.

Palabras clave. Rutelini, análisis micromorfométrico de genitales, análisis estadísticos, diagnóstico de especies crípticas.

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Introduction

Zubov et al. (2019) described *Chrysina valentini* as a new species in the optima group (sensu Hawks 2001) along with *C. optima* (Bates, 1888) and *C. tricolor* (Ohaus, 1922), and compared the three species morphologically and genetically. They cited the number of teeth on the protibia (referred to erroneously as the femur), degree of elytral striation, and size and thickness of the mesosternal process as characters for separating *C. valentini* from *C. optima*. They supported the validity of the new species with genetic distance based on mtDNA COX I sequence data. They reported negligible differences in male genitalia but did state that the apex of the parameres of *C. valentini* appears slightly more curved than that of *C. optima*. We investigate additional morphological characters in our work that may be of value to separate the two species. We also address the reliability of the morphological characters promoted by Zubov et al. (2019) for distinguishing *C. valentini* and *C. optima*.

Materials and Methods

Material examined. Specimens are from the private collections of David C. Robacker, William C. Warfield, Donald B. Thomas, and Charluz Arocho. All specimens of *C. valentini* were collected in Ngäbe-Buglé Comarca in Panama near the type locality of the species (20 ♂, 5 ♀). Specimens of *C. optima* were collected in Costa Rica (7 ♂, 2 ♀) and Panama (13 ♂, 3 ♀). Costa Rican specimens were collected at Parque Nacional Tapantí in Cartago Province. Panamanian specimens were collected near Boquete in Chiriquí Province.

Measurement procedure. Measurements of genital capsules were conducted using a Leica EZ 4W stereo dissecting microscope (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland) set to 8× power. The microscope was equipped with a built-in Leica HD camera system. The method was to photograph capsules in ventral aspect, print the images on paper and measure the structures on the photographs with a plastic ruler calibrated to mm. Capsules were centered for photography to minimize effects of distortion at the margins due to curvature of the lens. Ruler measurements were estimated to the nearest 0.5 mm resulting in measurement error of ± 0.25 mm. Observed magnifications were higher than the nominal values due to printing the images displayed on a computer monitor. Observed magnification was calculated as 22.2 by dividing ruler measurement on a photograph of a capsule by the actual size of the capsule as determined with a CEN-TECH™ Electronic Digital Caliper (Harbor Freight Tools, Camarillo, CA). Repeated measurements of the same capsule indicated that caliper-measurement error was ± 0.2 mm.

Measurement accuracy. Actual sizes of capsule structures were calculated by dividing the ruler measurements on photographs by 22.2, the observed magnification. Accuracy of the actual sizes of capsule structures is dependent on measurement error of the caliper, magnification error, and ruler measurement error. Using rules of propagation of error resulting from arithmetic operations (Anonymous 2007), error of capsule sizes was estimated as ± 0.20 mm. Thus, the actual length of a capsule calculated as 8.0 mm would be within the error range 7.8–8.2 mm.

Measurement precision and size differences. Although error of actual sizes is 0.2 mm, the calculated size differences depend only on the precision of ruler measurements on photographs. For example, a capsule that is 8.0 mm long would be measured as 177.6 mm (8 mm × 22.2) while a capsule that is 8.2 mm long would be measured as 182.0 mm. With ruler measurement error of 0.25 mm, the ranges would be 177.35–177.85 and 181.75–182.25 mm, respectively. Dividing these ranges by 22.2, now used as a constant, results in ranges of 7.99–8.01 and 8.19–8.21 mm. Although actual sizes of the two capsules would be 7.8–8.2 and 8.0–8.4 mm, respectively, the difference in size, (8.19–8.21) versus (7.99–8.0), is reliably about 2 mm. Thus, differences in sizes are more reliable than suggested by error of actual sizes.

Parameres curvature. Curvature of the parameres was measured from photographs of capsules in lateral aspect. An assumption was made that the curvature could be represented as the angle created by two straight lines that intersect somewhere between the base and the apex. This assumption was reasonable because the basal half of the parameres appears to extend in a straight trajectory, the middle section bends ventrally, then the distal half appears to extend in a straight trajectory to the apex. The first line originated at the center of the base of the parameres and extended distally through the center of the parameres, exiting from the parameres where they begin to bend ventrally. The second line originated at the apex of the parameres and extended proximally through the center of the parameres, exiting where they begin to bend toward the base. The angle was measured with a protractor.

Assessment of protibiae and mesosternal processes. We examined protibiae of all 24 *C. valentini* and all 25 *C. optima* to determine if the protibia appeared bidentate or tridentate as indicated by Zubov et al. (2019). We also examined the mesosternal processes of the same specimens to match them to the figures in Zubov et al. (2019).

Color assessment. Silver-form specimens of *C. valentini* and *C. optima* were observed in natural light and elytral reflectance was scored as either greenish or reddish. Because five of the *C. optima* were red or gold color forms, we used 24 specimens of *C. valentini* and 20 specimens of *C. optima*.

One photograph of one specimen of each species was taken with a Canon EOS 30D (1:2.8) camera with a Canon Macro EF 100 mm lens (Canon USA Inc., Melville, New York). The photograph was done in direct sunlight with camera settings: F stop ⅛, ISO 200, exposure ⅟₁₀₀ sec.

Statistical analyses. *t*-tests of means of independent samples with equal variance were used to test differences in capsule measurements and various ratios of measurements. Analyses were conducted with Microsoft Excel

2010 (Microsoft Corporation, Redmond, WA, USA). Data used in these analyses were the highly precise ruler measurements on photographs.

Chi-square tests for independence of categories in 2×2 contingency tables, with Yates continuity correction, were used to compare proportions of adults with protibia bidentate vs tridentate, with mesosternal process thin and pointed vs thick and rounded, and with cuticular reflectance reddish vs greenish. Analyses were conducted using GraphPad QuickCalcs (Motulsky 2018).

Results

Capsule measurements. Table 1 shows lengths of capsules and parameres, maximum widths of parameres measured at the base of the parameres, ratios of capsule length to maximum width of parameres, ratios of parameres length to maximum width, and curvature of parameres. No significant differences were found for lengths of capsules or parameres. Parameres of *C. valentini* were significantly wider at the base than those of *C. optima*. Widths ranged from 2.13–2.36 mm for *C. valentini* and 1.95–2.13 mm for *C. optima*. This difference is evident in Fig. 1–2, photographs of capsules representing specimens with average parameres widths. The wider parameres of *C. valentini* also resulted in significantly lower ratios of either capsule or parameres lengths to parameres widths. Thus, capsules of *C. optima* are proportionally narrower. The angles created by the ventral curvature of the parameres did not differ significantly.

Figures 1–2. *Chrysina* spp. male genital capsule ventral habitus. **1)** *C. valentini*. **2)** *C. optima*.

Table 1. Male capsule measurements. Data entries are means tested for significant differences using *t*-tests. n = 10 for each mean. Significance levels of *t* values: ns, no significant difference at the 5% level; * $P < 0.001$.

Measurement	*C. valentini*	*C. optima*	*t*
capsule length (L) (mm)	8.5	8.4	0.14 ns
parameres L (mm)	2.41	2.52	1.8 ns
parameres maximum width (W) (mm)	2.23	2.06	5.48*
capsule L / parameres maximum W	3.79	4.11	4.17*
parameres L / maximum W	1.08	1.23	4.34*
parameres curvature angle (°)	108.2	110.6	1.03 ns

3 4

Figures 3–4. Dorsal habitus of male *Chrysina* spp. photographed together in direct sunlight. **3)** *C. valentini*. **4)** *C. optima*.

Protibiae and mesosternal processes. Tibiae of both species were difficult to assign as bi- or tridentate as shown in Zubov et al. (2019) Fig. 5–6. Most had at least a slight enlargement at the position of the third tooth. Using the criterion that the third tooth was present if a black tip could be identified, *C. valentini* males were mostly bidentate (13/19) and females about equally bidentate (2/5) and tridentate (3/5). Fewer *C. optima* males (8/20) and no females (0/5) were bidentate. An apparent tendency toward bidentate in *C. valentini* and tridentate in *C. optima* was not supported by Chi-square tests which were not significant at the 5% level for either males ($\chi^2 = 2.1$, 1 df) or females ($\chi^2 = 0.6$, 1 df).

Mesosternal processes did not match those in Zubov et al. (2019) Fig. 7–8. We observed no processes as thin and pointed as Fig. 7 and very few that approached the thick rounded process shown in Fig. 8. Using the criterion that a process more closely resembled the thin pointed one in Fig. 7 or the thicker more rounded one in Fig. 8, only four processes in each species could be classified as thin and pointed (*C. valentini* 4/24, *C. optima* 4/25).

Adult cuticular reflectance. Under natural light, all specimens of *C. valentini* (24/24), including both males and females, appeared dark silver with a hint of greenish reflectance on both the pronotum and the elytra. All specimens of *C. optima* (20/20), both males and females from both Costa Rica and Panama, appeared dark reddish on these surfaces. The difference is significant by Chi-square analysis ($\chi^2 = 40.1$, 1 df, $P<0.0001$). These reflectance differences are illustrated in Fig. 3–4.

Discussion

Zubov et al. (2019) provided evidence, including DNA sequences and elytral striation differences, for their description of *C. valentini* as a new species distinct from *C. optima*. We agree with their conclusion.

Other characters cited by Zubov et al. (2019) to separate the two species were not reliable. They stated the protibia of *C. valentini* is bidentate whereas that of *C. optima* is tridentate. We did not find this character consistently different between the two species. Zubov et al. (2019) also indicated the shape and size of the mesosternal process were different between the two species although their information was conflicting as to which species had the larger, more rounded process. Regardless, we found no perceptible differences in this character. Finally, they suggested the parameres of *C. valentini* are more curved (Zubov et al. 2019 Fig. 9–10). Our data in Table 1 show that the curvature of the parameres of the two species is not significantly different.

Except for curvature of the parameres, Zubov et al. (2019) did not address morphology of male capsules. Our analyses indicate that *C. valentini* and *C. optima* differ in the shape of the capsules. The *C. valentini* capsules are proportionally wider because the base of the parameres protrudes laterally when viewed in ventral aspect. This character is reliable as indicated by the non-overlapping ranges of our data provided in the Results. Although consistent, use of this character to identify the species is difficult. Although we did not collect data, viewed in ventral aspect, parameres of *C. valentini* usually taper abruptly toward the apex compared with the more even taper of *C. optima* parameres (Fig. 1–2).

Zubov et al. (2019) provided photographs of the male holotype of *C. valentini* and a silver-form male *C. optima*. In their photographs, *C. valentini* appears light green and *C. optima* appears silver (Zubov et al. 2019 Fig. 1–2). Despite the different appearances in their own photographs, they stated the two species have a similar golden-silver coloration. Our observations under most types of indoor lighting are similar in that both species appear a similar golden-silver color. However, under some lighting conditions, these two species appear quite different. Our photograph shows that *C. valentini* appears green-silver whereas *C. optima* appears red-silver in direct sunlight (Fig. 3–4). All specimens we examined exhibited this difference in reflectance. This suggests a strong genetic difference between the two species that probably expresses as structural differences in the layers of chitin of the exocuticle (Thomas et al. 2007). While possibly interesting to optical physics, the consistency of this effect is certainly valuable as a character to easily distinguish these two species in the field.

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