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SHORT COMMUNICATION

New Records of Gynandromorphism in Heriades and Dianthidium and Images of the First Reported Dianthidium Gynandromorph (Hymenoptera: Megachilidae)

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ABSTRACT: We report two new records of gynandromorph bees: *Heriades carinata* Cresson, 1864 the first reported record of a gynandromorph in the genus and the first of the species *Dianthidium pudicum pudicum* (Cresson, 1879) (Megachilidae) and include descriptions, images, and 3D scans of both specimens. We also provide images and a 3D scan of the first *Dianthidium* gynandromorph originally reported by Hicks (1926), *D. curvatum sayi* Cockerell, 1907. We CT scanned each specimen, though these scans did not reveal any internal morphological structures.

KEYWORDS: Gynandromorph, bees, Hymenoptera, Megachilidae, CT scan, 3D scan

Gynandromorphism is an uncommon condition reported in both vertebrates and invertebrates where a single individual expresses both male and female characters (Michez et al., 2009; Peer and Motz, 2014; Wcislo et al., 2004), a more noticeable condition in sexually dimorphic organisms such as bees (Hymenoptera: Anthophila). Gynandromorphy presents itself as bilateral, mosaic, or transverse (Michez et al., 2009). Bilaterals have equal and symmetric male and female parts, mosaics have sex characters randomly distributed, and transverse individuals have characters distributed asymmetrically throughout the body. Traits expressed in gynandromorphs are consistent with those of brood parasitic bees (Wcislo et al., 2004).

We report the first gynandromorph in the genus *Heriades* Spinola, 1808 and the first for *D. pudicum pudicum*, the third report for the genus (Hicks, 1926; Schwarz, 1926). Gynandromorphy is infrequently observed in wild populations but has been recorded all around the globe in all bee families except for Stenotritidae (Hinojosa-Díaz *et al.*, 2012, Michez *et al.*, 2009, Wcislo *et al.*,

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2004). Nearly half of the gynandromorph records are from bees in the Megachilidae family, especially in the genus *Megachile* Latreille, 1802 (Hinojosa-Díaz *et al.*, 2012). The new records reported here add to the lesser-represented tribes of Megachilidae (Osmiini and Anthidiini) and complete the first reported *Dianthidium* record from Hicks (1926) by including high-quality images.

MATERIALS AND METHODS

We identified these specimens following (Michener, 1938) for *Heriades* and (Schwarz, 1926; Timberlake, 1943) for *Dianthidium*. We compared both gynandromorph specimens to representative conspecific males and females vouchered at the University of Colorado Museum of Natural History Entomology Collection (UCMC) collected from nearby localities of the intermountain western USA. We studied the specimens under a Leica S9i microscope to describe morphological features. For the specimen descriptions, we followed morphological terminology as described in Michener (2007). All three specimens are vouchered at UCMC.

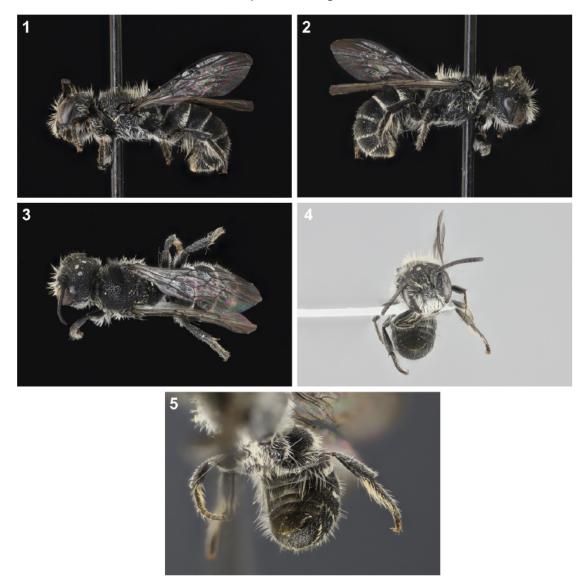
Diagnostic 2D focused-stacked images were taken using a Canon EOS 6D Mark II DSLR camera for the *Heriades carinata* specimen and a Canon 5D Mark III for the *Dianthidium* specimens. All images were stacked using Zerene Stacker (Zerene Systems LLC). In addition, 3D image suites were taken for all three specimens using a Macropod Pro 3D imaging station (Macroscopic Solutions, East Hartford, CT, USA). In brief, 2D focus-stacked images were taken every 2.5 degrees around the specimens using a rotational stage on the macropod. These 144 focus-stacked images were then used to generate a 3D photogrammetric model using Agisoft Metashape version 2.0.2 (Agisoft, LLC). Measurements were taken using a Leica Stereozoom S9i and the Leica LAS X imaging software annotation tool. Measurements were recorded in millimeters and rounded to the nearest hundredth.

To further assess the internal anatomy of the specimens, we CT scanned them with a Zeiss Xradia 520 Versa Xray Microscope and reviewed the resulting .tiff stacked images with both ORS Dragonfly (Version 2022.2) and Fiji ImageJ (Version Java 1.8.0_322) software (Schindelin *et al.*, 2012). Scans of dried specimens did not reveal any internal scleroitized morphological features that were not visible externally (Herhold *et al.*, 2019; Hu *et al.*, 2011). We expect scans of hydrated specimens would have provided further morphological information as in Almada *et al.*, 2022.

Additional media for the specimens reported here, including all 360-degree focused-stacked images used to develop 3D photogrammetric models, 3D meshes, final rendered models, and a CT scan of one specimen, can be found at: https://www.morphosource.org/projects/000549701

RESULTS

Heriades (Neotrypetes) carinata Cresson, 1864 Gynandromorph



Figures 1-5. Gynandromorph of *Heriades carinata* Cresson. 1. Lateral view, left side. 2. Lateral view, right side. 3. Dorsal view. 4. Head showing both antennae with 10 flagellomeres as in females and the face with many pale hairs as in males. 5. Ventral view of metasoma, S6 with scopa.

The Heriades carinata specimen was collected in the Pike-San Isabel National Forest along the Barr Trail (38.8563, -104.9573) on 2 July 2020 on the flowers of Geranium caespitosum James as part of an ecological study on plant-pollinator interactions (NSF-DEB 2102974). Sex-specific behaviors were not recorded at the time of capture, though the lack of pollen in the scopa suggests the bee was not provisioning a nest. Heriades carinata is widespread throughout North America, found in southern Canada and northern Mexico from coast to coast (Ascher and Pickering, 2020; Michener, 1938). We compared our specimen to others of the same species in the UCMC collection including 1 male from Boulder County, Colorado captured on 27 June 1963, and 1 male from Eagle County Colorado on 27 June 1963; and 2 females captured in Boulder County, Colorado on 11 June 1976 and 23 June 1925.

DESCRIPTION: Male and female features appear patchily throughout the body (Figs. 1-5). The specimen is long and slender with black integument, large punctations, and pale hair throughout Figures 1-5. Body length: 5.12 mm.

Head: width: 1.53 mm, length: 1.46 mm, distance between eyes: 0.82 mm. The general appearance of the head is male-like with pale hair all throughout and each mandible has two evenly-spaced teeth. There are 10 flagellomeres on each antenna as is found in females. The two sides do not have distinctly different morphological features (Fig. 4).

Mesosoma: Intertegular distance: 1.15 mm. The mesosoma is male as indicated by the legs. The integument is consistently punctate and the wings are a dusky color (Figs. 1-3).

Metasoma: The metasoma is female-like with six exposed tergites with black, deeply punctate integument. There are apical hair bands with white setae on T1-T5. There are six exposed sternites that are only sparsely hairy and lack a scopa on S1-S5. S1 is medially raised to a bump, a diagnostic feature in males of this species. S6 is flattened and bears a scopa (Fig. 5). All six legs are male-like, and each tarsal claw is bifid. There is a sting, but we did not dissect the genitalia to preserve the integrity of the specimen. A CT scan of the specimen did not reveal male or female specific features in the terminal metasomal segments.

This specimen is vouchered at UCMC with the identification number: UCMC 0134249.

Dianthidium (Dianthidium) pudicum pudicum (Cresson, 1879) Gynandromorph



Figures 6-9. Gynandromorph of *Dianthidium pudicum pudicum* Cresson. **6.** Dorsal view of metasoma with maculations on the right side of T1-6 interrupted. **7.** Head with 11 flagellomeres on the right and 10 flagellomeres on the left. **8.** Ventral view of sternites showing scopa and a rounded T6 on the left and curled hairs medially. **9.** T5 and T6 showing gonostyli, penis valves, and a sting.

The *Dianthidium pudicum pudicum* specimen was collected in Colorado in Weld County on 18. June 2020 along an urban trail as part of a post-flood resurvey (Mullins, 2021). The bee was captured while hovering above the bare ground and the collector captured it too quickly to observe sex-specific behaviors as reported in other examples (Alvarez *et al.*, 2019; Barrett, 2021). This species is widespread throughout western North America from Manitoba, Canada to Sonora, Mexico (Ascher and Pickering, 2023; Semmler *et al.*, 2018). This is the third reported gynandromorph in this genus and the first of the species (Hicks, 1926; Michez *et al.*, 2009; Schwarz, 1926). We compared our specimen to confirmed representatives of the species, including two males from Boulder County, Colorado collected on 20 August 1946 and 17 August 1936; two females from Gilpin County captured on 4 August 1946 and one in Boulder County captured on August 1918. The gynandromorph specimen is a similar size to these representatives.

DESCRIPTION: Male and female features present partially bilateral throughout the body (Figs. 6-9). The specimen appears female at first glance. Body length: 6.89 mm. The integument is black with pale yellow maculations with pale hair.

Head: width 2.40 mm; length 2.21 mm; distance between eyes 1.52 mm. The right half of the head has a male appearance with 11 flagellomeres and a pale yellow mandible and the left half of the head has 10 flagellomeres and a black mandible with a straight cutting edge. The clypeus is almost entirely black with lateral pale yellow maculations, consistent with females. In males, the clypeus is almost entirely pale yellow. There are lateral pale yellow markings along the compound eyes tapered into a narrow line above the antennae level (Figs. 7 and 9). Consistent with other specimens from the Front Range of Colorado, the vertex does not bear any maculations.

Mesosoma: Intertegular distance: 2.05 mm. Both tegulae are black with a pale yellow maculation on the apical half and around the lateral edges. There are two maculations on the left and right side of the apical mesosoma. An impunctate line bisects the mesosoma. Legs: The leg integument is black and there are pale yellow maculations on the middle and hind femora and a basal spot on the tibiae. The integument on legs is more black than yellow, consistent with female specimens. The left coxa is narrowed to a spine as in male specimens.

Metasoma: The general shape of the mesosoma is consistent with female specimens, shorter and more rotund than in representative males of the same species. The specimen has six tergites, typical of female bees, and the sixth tergite is rounded. The mesosoma has black integument with pale yellow maculations that are bilaterally gynandromorphic. The banded maculations on T1 are interrupted on each side to make two yellow spots laterally and one uninterrupted narrow band in the center. T2-T4 are medially interrupted on the right, typical of male D. pudicum pudicum. Maculations on the left half of T2-T5 are deeply emarginate, but not entirely interrupted as on the right half (Fig. 6). T6 is immaculate and rounded, the specimen lacks T7. It is important to note that maculations are highly variable among non-gynandromorph specimens even from the same region. The sternites lack a ventral scopa on the right half of the specimen and there are pale hairs forming the ventral scopa on the ventral left half. The scopa continues on the left half of S6. The margin on the left side of S6 is rounded as found in females and reduced on the right half of the specimen. The right sternites are more similar to those of a male specimen, yet S7 is not present. S6 is lamellate with two emarginations. There is a lateral hairband on S5 with long, wavy hair medially as is typical in male specimens, some of these hairs are also found on the left (female) side of the bee. We did not dissect the genitalia to keep the specimen intact; however, both a sting, gonostyli, and penis valves are present and visible (Fig. 9). A CT scan of the specimen revealed no further sex-specific internal morphological information.

This specimen is vouchered at UCMC with the identification number: UCMC 0328326.

Dianthidium curvatum sayi Cockerell, 1907 Gynandromorph



Figures 10-13. Gynandromorph of *Dianthidium curvatum sayi* Cockerell. **10.** Lateral view of left side, T7 visibly male. Legs ferruginous. **11.** Lateral view of the right side with lighter legs that are only faintly ferruginous. **12.** Dorsal view of mesosoma and metasoma. Scutellum and axilla black on the right side and red on the left. **13.** Head with clypeus and mandible yellow on the right. The left side of the clypeus black medially and yellow on the left half. Left mandible larger and black. Antennae with 10 flagellomeres on the right and 11 flagellomeres on the left.

For the first time, we include photographs of the specimen that marks the first record of a gynandromorphic *Dianthidium* also collected on the University of Colorado campus in Boulder on September 27, 1925 from the flowers of *Sideranthus spinulosus* Pursh, now recognized as *Xanthisma spinulosum* (Pursh) D. R. Morgan and R. L. Hartm. Hicks (1926) reported a bilateral gynandromorph of *Dianthidium curvatum sayi*, originally identified as *D. sayi*. The specimen's head and mesosoma are bilaterally divided with male features on the right and female characters on the left. The metasoma is male, with seven tergites. Body length: 10 mm. See (Hicks, 1926) for a detailed description of the specimen. A CT scan of the specimen revealed no further internal morphological information.

This specimen is vouchered at UCMC with the identification number UCMC 0295698.

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