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The Lepidoptera of White Sands National Monument, Otero County, New Mexico, USA 10. A remarkable new white species of *Chionodes* Hübner (Gelechiidae)

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Abstract

The U.S. National Park Service initiated a 10-year study, in late 2006, of the Lepidoptera at White Sands National Monument, Otero County, New Mexico. *Chionodes bustosorum* **sp. n.**, described here, was discovered in 2010, during the third year of the study. The male imago and male genitalia are illustrated, and its DNA barcode is compared to that of seven other species of *Chionodes* from western North America.

Key words: Endemism, evolution, U.S. National Park Service, U.S. Army, White Sands Missile Range, Tularosa Basin, biological diversity, white gypsum dunes, DNA barcodes

Introduction

The White Sands National Monument preserves 284.9 $\rm km^2$ (110 square miles), about 40%, of the world's largest snow-white gypsum dune field. The remainder of the 275 square miles dune field is under the jurisdiction of the U.S. Army's White Sands Missile Range. The dune field is located in the northern Chihuahuan Desert in southern New Mexico's Tularosa Basin (Schneider-Hector 1993).

In 2006 White Sands National Monument invited Metzler to conduct a 10-year study of moths at the Monument. The primary purposes of the 10-year study were to compile an inventory of moths and describe new species in habitats within and immediately adjacent to the white gypsum dunes in the Monument. In 1950 Stroud reported 20 species of Lepidoptera from White Sands National Monument, none of which is unusual for the region. In the period 9 February 2007 through 30 January 2016, Metzler recorded more than 650 (unpublished data) species of described Lepidoptera from White Sands and approximately 40 undescribed species of moths. This is the twelfth description of a new species of moth emanating from the study (see Metzler 2014b, Metzler *et al.* 2009, Metzler & Forbes 2011b, 2011c, Metzler & Lightfoot 2014, Wright 2012, 2014, Wright & Gilligan 2015).

Hodges (1999) revised the species of *Chionodes* Hübner, [1825], found in North American north of Mexico. As evidence of the poorly known status of the genus, Hodges described 115 (62%) of the 187 known species as new to science. The distributions of many species appear to be highly disjunct. Additional collecting of small moths should fill in some gaps as well as disclose undescribed species. When Hodges (1999) specifically mentioned the need for more collecting in southern New Mexico, he did not know about the high rate of endemism of moths in White Sands National Monument (Metzler 2014a).

Methods and materials

Moths and other night flying insects were collected in U.S.D.A. type black-light traps, as described in Smith *et al.* (1974), in diverse habitats within the white gypsum dunes. A detailed description of the study methods is given by Metzler *et al.* (2009).

All but the most easily identified species of moths (*e.g.*, *Hyles lineata*) were retained for further study. The specimens were sorted, and selected specimens were spread and labeled. All nonlepidopterous insects from the traps were placed in 95% ethanol and deposited in the Museum of Southwestern Biology at the University of New Mexico, Albuquerque, New Mexico.

Genitalia were examined following procedures generally outlined in Clarke (1941), Hardwick (1950), Lafontaine (2004), and Pogue (2002). Abdomens were removed from the moths, dipped in 95% ethanol, and soaked in 10% KOH for 30 minutes at 50°C. Genitalia were dissected in 5% ethanol, dehydrated in 99.9% ethanol, stained with Chlorazol Black in water and Safranin O in 99.9% ethanol. The genitalia were cleared in oil of clove, rinsed in ethyl acetate, and slide mounted in Canada Balsam.

Terms for elements of wing pattern, color, morphology, and genital structures follow Hodges (1999). Terms for regions of the wing follow Mikkola *et al.* (2009). Forewing length was measured to the nearest 0.1 mm, from the base to the apex excluding fringe, using a Leica MZ 12 stereo-microscope with a Wild 15x ocular micrometer.

The photograph of the adult was taken with a Nikon D7100 equipped with an AF-S Micro Nikkor 105mm 1.28 GED lens and a small homemade lightbox of 4" diameter x 4" long white PCV pipe, illuminated with a 60 LED ring light. The photographs of the genitalia and slide-mounted structures were taken with a Nikon D7100 digital camera mounted on a Leitz Aristophot, a Summar 42 mm objective, and a 24 mm condensor. The images were processed with Zerene Systems software and Photoshop CS6 software.

The coordinates for latitude and longitude on the labels of the specimens from the study are in degrees and decimal minutes. The coordinates were obtained with the aid of a Garmin II Plus and confirmed by reference to Google[™] Earth Pro 7.1.4.1529. The specimens cited in this paper are deposited in the United States National Museum of Natural History (USNM), Smithsonian Institution, Washington, DC.

Systematics

Chionodes bustosorum Metzler, sp. nov.

BOLD:ACS7412 Figs 1–3

Holotype \mathcal{S} , pinned, double-mounted, with labels as follows: "New Mexico: Otero Co., White Sands Nat[ional] Mon[ument]; Interdune vegetation; 106°11.38'W; 32°46.60'N 4,000'; 11 June 2010 WSNM8; Eric H. Metzler uv tr[a]p; Accss # White Sands National Monument 00131." [blue label] "Barcode of Life Project Leg(s), DNA extracted"; "USNMENT01142737"; [green label] "Genitalia slide by EHMetzler, \mathcal{S} USNM 146317" [red label] "HOLOTYPE USNM; *Chionodes bustosorum* Metzler 2016". Deposited in USNM.

Paratype ♂, pinned, double-mounted, same locality/date as holotype, [blue label] "Barcode of Life Project Leg(s) removed, DNA extracted"; "USNMENT01142738" [green label] Genitalia slide by EHMetzler, ♂ USNM 146318." Deposited in USNM.

Description. Adult male (Fig. 1). Head: Front and vertex scales broadly spatulate, erect, cream-white; front smooth, scales spatulate, directed forward and ventrally, cream-white; palpi upturned, basal segment scales appressed, mid-segment = $\frac{1}{2}$ length of palpi, slightly shaggy, scales spatulate, apical segment = $\frac{1}{3}$ length of palpi, divergent apically, scales appressed, cream-white; haustellum base densely scaled, cream-white. Antenna, each segment basally ringed with semi-erect cream-white scales. Thorax: dorsal and ventral surfaces with appressed, cream-white scales. Legs with appressed, cream-white scales. Forewing: Length 5.4 mm, mean 5.4 mm, n = 2; Uniformly cream-white, including fringe, apex rounded; underside pale yellow with concolorous fringe. Hindwing mirror-like reflective-white with concolorous, long fringe, apex slightly produced; underside white, male with patch of pale yellow sex scales arising from wing base and extending along inner margin. Abdomen: Scales appressed, pale yellow. T8 (Fig. 2d) with lateral sides parallel, posteriorly broadly convex, anteriorly broadly concave. Male genitalia (Fig. 2) (2 preparations examined) with uncus broad, spoon shaped, setose laterally; culcitula absent; gnathos with base sclerotized, lobed laterally, lobes extending 1/3 length of tegumen, each lobe with a posteriorly directed finger-like projection enclosing a diamond-shaped lateral process; gnathos sharply curved at 0.2x length, gently curved most of length, apex sharply recurved; tegumen broadly A-shaped, excavated to 1/3, robust suture separating pedunculi, each pedunculus narrowing to junction with vinculum, base of each pedunculus twisted 180° at junction with vinculum; vinculum = 0.8x length of tegumen, abruptly narrowed

immediately anterior of juncture with tegumen, distal 2/3 trough-shaped, sides parallel extended to blunt rounded apex; posterolateral lobe from vinculum an extension of twisted pedunculus, sclerotized, trough-shaped, apex bluntly rounded, length = width of base of pedunculus of tegumen; saccus not differentiated from vinculum; valvae asymmetrical, unequal in length and dissimilarly shaped: right valva, maximally extended to 1/2 length of tegumen, then strongly recurved mesially at 2/3 length, robust where recurved, apex weakly bifurcate, posterior projection thorn-like, robust anterior projection pointed, beak-like; left valva narrow, curved, bow-like mesially or laterally, extended to middle of uncus, terminal 1/10 bent approximately 90°, apex doubly bifurcate, posterior projection robust pointed, beak-like, anterior projection bifurcate, not robust. Phallus with distal part sculpting complex, caecum approximately 5x length of distal part, longitudinal sclerotized bar at anterior end.

Adult female. Unknown.



FIGURE 1. *Chionodes bustosorum* adult male paratype (scale bar = 1 mm).

Diagnosis. Ron Hodges (personal communication to EHM) considered C. bustosorum to be undescribed based on the diagnosis in combination with the details of the description. Chionodes bustosorum (Fig. 1) is a small (forewing length 5.4 mm) creamy white moth with no discernable markings on the surface. Two nearly identical specimens were captured in black light traps with Scythrididae of similar size and appearance. The apex of the forewing of C. bustosorum is rounded, whereas the forewing of Scythrididae terminate in an acute apex. The apex of the hindwing of C. bustosorum is rounded and produced, a wing shape that is typical for most Gelechiidae. The apex of the hindwing of Scythrididae is acute. The male genitalia of C. bustosorum are typical for the genus Chionodes (Fig. 2) with a broad prominent spoon-shaped uncus. Chionodes bustosorum keys out to couplet 6, obscurusella or formosella groups, in the Hodges (1999: 25) key. The male sex scales of C. bustosorum are on the ventral surface of the hindwing, similar to species in the obscurusella group. Based on characters of the male genitalia as figured by Hodges, C. bustosorum appears to be in the abella complex of the formosella group, however, C. bustosorum fails to match any features in the early couplets in the key to species of the formosella group (see Hodges 1999: 33). The diagnostic features of the male genitalia of C. bustosorum are the markedly asymmetrical valvae. The left valva is long and narrow, whereas the right valva is shorter, more robust, and recurved mesially like the cutting blade of a hand-held scythe. In a comparison of male genitalia, based on the photographs in Hodges (1999), the genital capsule of C. bustosorum is most similar to a lateral mirror image of C. abella as illustrated on p. 261, fig. D-1 of that work. The angle of the recurved right valva of C. bustosorum is broad, like a hand-held scythe, whereas the angle of the recurved left valva of C. abella is acute. The area between the pedunculi of the tegumen of C. bustosorum is not sclerotized, whereas in C. abella it is lightly sclerotized. The lateral sides of the uncus of C. abella appear to be nearly parallel; the uncus of C. bustororum is oval. The genital capsule also resembles a mirror image of that of C. abdominella as illustrated on p. 263, fig. F-20 of Hodges (1999).



FIGURE 2. *Chionodes bustosorum* male genitalia and male eighth abdominal segment. 2a, male genitalia paratype USNM slide # 146318; 2b, male aedeagus paratype USNM slide # 146318; 2c, male eighth abdominal segment (tergum on left) holotype USNM slide # 146317 (scale bars = 1 mm).



FIGURE 3. *Chionodes bustosorum* type locality and distribution map. 3a, type locality; 3b, *Chionodes bustosorum* is known from White Sands National Monument, Otero Co., New Mexico.

Remarks. This new species is placed in the genus *Chionodes* based on the presence of the caecum on the aedeagus as defined by Hodges (1999). The holotype and paratype are identical in habitus and genital structure. The paratype imago is illustrated because it possesses complete antennae. The holotype was selected because it is the specimen that yielded a nearly complete barcode sequence.

Etymology. The specific name of this species, *bustosorum*, a noun in the genitive case, recognizes David Bustos, Chief of Resources at White Sands National Monument since 2007, and his wife, Andrea. David aggressively pursues research; his efforts contributed greatly to the immense accumulation of scientific data during his years at White Sands National Monument. Metzler knows from personal experience that David works long and crazy hours in support of the Monument. He is enthusiastic about the research on moths, to wit, he often sends emails to Metzler from home, long after Metzler is in bed. David is the recipient of the U.S. National Park Service's 2014 Director's Trish Patterson Student Conservation Association Award for Natural Resource Management in a Small Park. His efforts would not be possible without the support and encouragement of his lovely wife Andrea. Metzler takes great pleasure in naming this moth in honor of David and Andrea Bustos.

Distribution and biology. *Chionodes bustosorum* occurs in White Sands National Monument, Otero County, New Mexico (Fig. 3). The immature stages and host plant are unknown.

DNA barcode analysis

Tissue samples (dry legs) were shipped to the Canadian Centre for DNA Barcoding in Guelph for DNA extraction, amplification, and sequence analysis. The protocol followed is as outlined in Landry & Hebert (2013). Barcoding efforts included the holotype and paratype of *C. bustosorum*. Records of other *Chionodes* species already in BOLD were selected for barcode comparison and analysis. The Barcode Identification Numbers (BINs) (Ratnasingham & Hebert 2013) in BOLD are used as registry designations for barcode clusters. Neighbor-joining trees and genetic distances were calculated with the Taxon ID Tree and Distance Analysis tools available in BOLD using the Kimura two-parameter (K2P) model of base substitution with pairwise deletion for ambiguous positions, and Kalign sequence alignment. Details of the barcoded specimens (collecting data, photos, sequence data, GenBank accessions) are available through the online dataset http://dx.doi.org/10.5883/DS-CHIOWHIT.

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	abdominella	abella	bustosorum	fructuaria	landryi	pinguicula	sistrella	sp near fructuaria
abdominella BOLD:ACG5519 (n=4)	0.2 (±0.10)							
abella BOLD:AAB6065 (n=6)	8.0 (±0.08)	0.0 (±0.00)						
bustosorum BOLD:ACS7412 (n=1)	5.9 (±0.14)	7.4 (±0.00)	-					
fructuaria BOLD:AAN5596 (n=4)	6.3 (±0.19)	6.2 (±0.14)	5.0 (±0.22)	0.4 (±0.21)				
<i>landryi</i> [no BIN] (n=1)	4.0 (±0.13)	5.9 (±0.00)	4.8 (±0.00)	4.1 (±0.25)	-			
pinguicula BOLD:AAH5117 (n=2)	6.6 (±0.12)	8.4 (±0.00)	6.9 (±0.25)	6.8 (±0.49)	5.4 (±0.00)	2.0		
sistrella BOLD:ACI6713 (n=7)	8.3 (±0.36)	8.4 (±0.28)	5.5 (±0.53)	6.1 (±0.39)	6.8 (±0.63)	6.8 (±0.31)	0.4 (±0.37)	
sp near fructuaria BOLD:AAN5597 (n=2)	7.2 (±0.13)	7.6 (±0.09)	5.5 (±0.12)	5.8 (±0.20)	4.2 (±0.19)	4.2 (±0.21)	7.0 (±0.33)	0.8

TABLE 1. Percentage of divergence in DNA barcode (cytochrome c oxidase I gene) sequences among eight species of *Chionodes.* "BOLD:ABC1234" = Barcode Index Number. Diagonal cells = intra-specific distances; cells below diagonal = inter-specific distances; means with standard errors in parentheses.

The holotype of *C. bustosorum* yielded a slightly abbreviated sequence of 636 bp which was of sufficient quality to be assigned to a BIN (BOLD:ACS7412). Barcoding of the paratype failed. Twenty-six specimens representing seven other *Chionodes* species were selected from BOLD for comparative barcode analysis. To make that selection, an initial neighbor-joining (NJ) tree was generated by searching BOLD for all available *Chionodes* records from North America: this resulted in a large tree comprising over 1500 sequences representing more than 100 species (not shown). A smaller subset of species was then selected based on their genetic proximity to the type of *C. bustosorum* in the larger tree. These included *C. abdominella* (Busck), *C. fructuaria* (Braun), *C. landryi* Hodges, *C. pinguicula* (Meyrick), *C. sistrella* (Busck), and one unidentified BIN provisionally named *C. sp.* near *fructuaria*. Additionally, *Chionodes abella* (Busck), although appearing genetically quite distant, was also selected because it is the species whose male genitalia are compared to those of *C. bustosorum* in the diagnosis. A tree was then generated and distances calculated for the smaller subset (Fig. 4; Table 1).

Chionodes bustosorum is the sole representative of its BIN, congruent with the distinctiveness in morphology and confirming its status as a uniquely distinct species. It is nested within a cluster with *C. sistrella* as nearest neighbor, and with *C. pinguicula* and *C. sp.* near *fructuaria* forming a neighboring subcluster. It is interesting to note that the similarity in male genitalia with *C. abella* is not reflected in barcode proximity, as the latter is the most distant of all clusters included in the subset. *Chionodes abella* is also the most different in coloration, being a dark-colored species. It is noteworthy that the genetically closest species all have a substantial amount of pale creamy white on the head, thorax, legs, and forewings, suggesting the possibility that *C. bustosorum* may be derived from an ancestor with a predominantly pale coloration.



FIGURE 4. Neighbor-joining tree of K2P distances for the barcode region of the cytochrome *c* oxidase I gene among 27 specimens representing eight species of *Chionodes*. End-branch labels are Specimen IDs. Barcode Index Numbers (BIN) where available are indicated below each species name.

Discussion

Species of the family Gelechiidae are diverse, and a satisfactory way to identify genera from external characters is lacking. Fortunately, Hodges (1999) provided a definition of *Chionodes* in his revision of species in America North

of Mexico. His work greatly aids identification of species of this large genus. The surprisingly rich endemic fauna of moths from White Sands National Monument includes several very pale or nearly white species, including *Chionodes bustosorum* described here. At first the plethora of white and pale species in the snow white dunes seems an obvious outcome of crypsis, yet the explanations are probably more complex. Because most lepidopterans are herbivores, an analysis of the plants growing in the gypsum soils is needed. David Bustos' preliminary findings show that some of the species of plants in the dunes have genetic markers and associated microbes that differ from the same species of plants growing outside, and immediately adjacent to the gypsum soils. These data suggest that larvae eating plants inside the dunes are consuming diets unlike larvae eating the same species of plants outside the dunes, but the relationship between diets of plants in dunes that are genetically different from the same species outside the dunes is unknown relative to the evolution of white colors of individuals. More research must be done before drawing any conclusions, yet the natural, nearly unspoiled by humans, laboratory of the Monument offers an unusual opportunity to explore the evolution of so many species of moths. The lack of lepidopteran specimens from the Monument seen until now can probably be attributed to the dearth of insect collecting in the gypsum dunes ecosystem in New Mexico because the dunes were formerly private property, and are now under the control of the U.S. National Park Service and the U.S. Army.

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The National Park Service provided permits to take samples of moths and provided access to areas normally closed to the public. Representatives from research collections and other institutions provided insect pins, alcohol, identification services, research consultation, and storage space for specimens collected leading to discovery and naming of this species. Metzler thanks the following persons for offering support from their respective institutions: Kelly B. Miller, Sandra L. Brantley, and David C. Lightfoot (University of New Mexico), Frederick W. Stehr, Anthony I. Cognato, and Gary L. Parsons (Michigan State University), J. Donald Lafontaine, Vazrick Nazari, and B. Christian Schmidt (Canadian National Collection of Insects, Arachnids, and Nematodes), Larry Berger (Ohio Department of Agriculture), and David Adamski, John W. Brown, Mark E. Metz, David G. Furth, Patricia Gentili-Poole, and M. Alma Solis (United States National Museum of Natural History). Patricia A. Metzler faithfully assisted the first author on many aspects of this study, and she provided funding. We thank Marie Frias-Sauter, Peter Huemer, and one anonymous reviewer for reading the paper and offering valuable suggestions.

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