

**Comment (Case 3709) – Additional comments on the proposed conservation of names for western North American *Hesperia comma*-group subspecies through designation of neotypes**  
(see BZN 75: 88–121 [Case]; BZN 76: 55–56, 57–59, 135–138; 77: 35 [Comments])

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Our previous comments in opposition to Case 3709 (Calhoun et al., 2019) cited some of our ongoing genomic studies, which were made public on 4 September 2019 (Cong et al., 2019). As promised, we now provide additional details about this work. We are pleased that Scott et al. (2019) agreed with the principal conclusions of our research (based on preliminary information shared by N. V. Grishin), yet several of their statements are problematic and require a response. Also, it should be noted that two coauthors of Case 3709 were conspicuously absent from the subsequent authors' comments (Scott et al., 2019), and at least two other coauthors now oppose the case and have submitted comments that express that opinion (Opler & Davenport, 2020).

To summarize our DNA research, we first sequenced and assembled a reference

genome of *Hesperia colorado* from a specimen collected and preserved for that purpose. Second, we sequenced the c.150-year-old lectotype of *Pamphila colorado* Scudder, 1874, resulting in about 25 percent complete nuclear and entire mitochondrial genomes of that specimen. Third, we obtained a whole genome shotgun of 85 specimens across Colorado, USA. The ages of these specimens varied from 150 years to recently collected. These sequences were mapped onto the reference genome and analyzed by a combination of population genetics tools. The result was unambiguous: the lectotype of *colorado* was geographically placed within an area about 15 km in diameter in Lake County, Colorado.

Using PCA, t-SNE, STRUCTURE and TREEMIX analyses, we determined that *H. colorado* is represented by four major populations in Colorado, which correspond to subspecies. These subspecies intergrade at the boundaries of their ranges, forming hybrids. The lectotype of *colorado* is surrounded exclusively by other specimens from Lake County, implying where it was collected. Moreover, the mitogenome of the lectotype was a 100% match to a single specimen more recently collected near Twin Lakes in Lake County. Previously, historical evidence indicated that the specimen was collected at Twin Lakes (Calhoun 2015a, 2016). The paralectotype of *colorado* also maps to the same area. As t-SNE shows, the lectotype of *colorado* clusters within the Arkansas River Basin population, a portion of which was described as the subspecies *H. c. oroplata* Scott, 1981 (Cong et al., 2019). These new results therefore force the name *oroplata* into synonymy.

Scott et al. (2019) argued that they “already have DNA sequences and phenogram of approximately 500 specimens of the *Hesperia comma*-group, which show that DNA is very similar throughout central Colorado in the ranges of all the Colorado names,” adding that they “have ‘barcode’ mtDNA (COI gene) for over 500 *Hesperia* specimens from throughout the world,” yet they did not provide supporting evidence. Nevertheless, whole genome sequencing has demonstrated that COI barcodes do not correlate well with *Hesperia* taxa. This is because the barcode sequence is too short (654 base pairs), and even entire mitogenomes (approx. 5,000 base pairs) are discordant with the nuclear genome in their evolution, due to hybridization and gene flow between *Hesperia colorado* subspecies. As expected, *Hesperia* phylogenies obtained from nuclear genomes (both from autosomes and the Z chromosome, which gave nearly the same result) are in agreement with phenotypic similarities. Any analysis of COI barcodes will therefore mislead researchers of *Hesperia*.

Scott et al. (2019) stated that “DNA study is no substitute for neotypes that are consistent with the phenotypes of name-bearing types and original descriptions...” This statement contradicts Case 3709, which seeks to replace the lectotype of *colorado* with a neotype that morphologically disagrees with the phenotype of its existing name-bearing type, as well as its original description and the figures that accompanied that description.

Scott et al. (2019) also stated that “if we consider only wing phenotype (ignoring most of the DNA as many people will do) the name *sublima* is a junior synonym of *colorado* and the name *oroplata* is a valid subspecies.” This is simply wrong, as the lectotype of *colorado* is a more brightly-colored individual, similar to the *oroplata* holotype, while the holotype of the higher elevation subspecies, *H. c. sublima*, is dark. Therefore, even by wing phenotype, the name *oroplata* is a junior synonym of *colorado*, and *sublima* is a valid subspecies. As we have demonstrated, DNA analysis supports this conclusion.

Referring to the lectotype of *colorado*, Scott et al. (2019) claimed that it is “from the mostly somewhat-darker near-Twin Lakes populations near the middle of the wing

color cline, which will result in taxonomic instability because most people use wing pattern to define subspecies and identify specimens (they can't use expensive DNA analysis)." In reality, there is no taxonomic instability, as the lectotype of *colorado* is a more brightly-colored phenotype that defines the taxon even without DNA analysis, which was conducted in an effort to corroborate this conclusion. Various individuals from the Twin Lakes area are darker phenotypes, but none of those are the lectotype. The nominal species *colorado* is defined by the lectotype, not by a population at a given locality.

Scott et al. (2019) insisted that "If types have DNA from an intergrade area, those names are worthless," thus "the type locality of *colorado* must be moved to the alpine tundra end of the clines, by designating the neotype." Our genomic analysis reveals that the lectotype of *colorado* is relatively 'pure,' much more so than the holotype of *oroplata*, which contains about 20% genes from the subspecies *H. c. idaho* and about 5% genes of the subspecies *H. c. ochracea* (Cong et al., 2019). Nonetheless, the gene pool of the *oroplata* holotype still confidently identifies that nominal subspecies as a junior subjective synonym of nominotypical *colorado* in all analyses.

It is significant that Scott et al. (2019: 137) conceded that "The DNA-based conclusion that the *colorado* lectotype came from near Twin Lakes seems to be correct . . .," which contradicts Case 3709 and previous publications by its senior author, which stated that the name *colorado* threatens the names *idaho* and *ochracea*. After unsuccessfully arguing that the collection date and coloration of the lectotype are incompatible with the Twin Lakes population, Scott et al. (2019) now contend that accepting a Twin Lakes type locality for *colorado* "produces a bad nomenclatural result," as it does not conform to their preconceived concept of *colorado*. This concept was promoted by the senior author of Case 3709 following his failure to examine the extant lectotype in a prominent American museum. The name *colorado*, as defined by its lectotype, poses no nomenclatural threat to the names *idaho* or *ochracea*, thereby eliminating the need to designate a neotype of *colorado* for the reasons given in Case 3709.

As observed by Calhoun et al. (2019), nominotypical *colorado* occurs almost entirely within the state of Colorado. Acknowledging its true type locality affects only the status of the name *oroplata*, which has not been widely used since its description in 1981; its use has mostly been confined to publications by its describer, the senior author of Case 3709. Although Scott et al. (2019) insisted that the *colorado* lectotype "must not be from the middle of a cline," our genomic analyses reveal that this specimen was collected at the upper reaches of an elevational cline, not the middle. Phenotypes at lower reaches of this cline were identified as the subspecies *H. c. oroplata*. Because this cline is geographically limited in size and produces a wide range of phenotypes, it is best to recognize the entire population as representing nominotypical *Hesperia colorado*. Moreover, all our DNA analyses clearly placed the holotype of *oroplata* within the same population as the lectotype of *colorado*. The correct treatment of nominotypical *colorado*, its synonym *oroplata*, and the new subspecies *sublima*, have been recognized since 2015 in the online catalogue of North American butterflies (Pelham, 2020).

The lectotype of *manitoba* was also ignored by *Hesperia* researchers until it was figured by Calhoun (2015b), despite it being deposited in the same museum as the lectotype of *colorado*. Although Scott et al. (2019) agreed with our DNA results that the lectotype of *manitoba* is from the area of Lac la Hache, British Columbia, they argued that this "location throws the taxonomic stability of the names *idaho* and *assiniboia*

into chaos over most of western North America...” This is unfounded, as the lectotype of *manitoba* defines that taxon very well. The lectotypes of *idaho* and *assiniboia* define their taxa equally well. We have determined that the lectotype of *assiniboia* is clearly genetically distinct from that of *manitoba*. We not only fail to recognize an exceptional need for designating a neotype of *manitoba*, we see no need whatsoever.

Case 3709 argued that the locality label on the holotype of *ochracea* is vague, thus the specimen may have originated from higher elevations that are close to the type locality of *sublima*, thereby forcing the name *sublima* into synonymy. Although its label simply reads “Platte Canon [=Canyon] Col.,” this taxon occupies a broad area within the South Platte River drainage of Colorado (Warren & Calhoun, 2015); thus, though imprecise, this locality is not inaccurate. More importantly, the holotype of *ochracea* is morphologically consistent with the popular concept of this name, and our DNA analyses confirm its genetic kinship with such populations. The holotype of *ochracea* contains a very small quantity of *sublima* genes, but not enough to warrant a neotype as requested by Case 3709. In fact, this specimen is more ‘pure’ than the holotype of *oroplata*, which Case 3709 does not dispute. We are confident that continuing genomic studies involving the name-bearing types of the names *cabelus*, *harpalus*, and *oregonia* will likewise clarify their geographical origins, removing any perceived need for their replacement by neotypes.

In our opinion, Case 3709 seeks to justify taxonomy through nomenclature, and protecting the name *oroplata* from synonymy appears to be a primary motivation. Scott et al. (2019) provided a long list of “problems” that influence the acceptability of *Hesperia* type specimens, but such issues affect a large percentage of existing zoological types. Replacing all validly designated name-bearing types that are deemed problematic is not a reasonable solution. Recent advances in genomics have enabled us to overcome uncertainties regarding collection localities, allowing a clearer understanding of species concepts as originally described. Ruling in favor of Case 3709 would not only reward bad science, it would set a dangerous precedent that undermines the very principal of the type concept. We feel that long-term nomenclatural stability in *Hesperia* is best served through ongoing studies of existing name-bearing types, not by rewriting history with the designation of unnecessary neotypes.

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