Taxonomy

Integrative Taxonomy Reveals a New *Melitaea* (Lepidoptera: Nymphalidae) Species Widely Distributed in the Iberian Peninsula

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Abstract

The *Melitaea phoebe* group is constituted by six species distributed throughout the Palearctic. One of the most widespread species is *Melitaea ornata* Christoph, 1893, present from France (Provence) to Central Asia. Recently, populations of *M. ornata* were discovered in a mountainous region of south-eastern Iberia, although doubts about their taxonomy existed. To clarify the taxonomic status of these populations and to revise the distribution of this taxon in Iberia, we have sequenced mitochondrial (*COI* barcode region) and nuclear (*wg*, *RPS5*, *MDH*, and *EF-1α*) markers, and analyzed the male genitalia for 72 Iberian individuals and for all the species of the *M. phoebe* group. This information was complemented with phenological and ecological data. Our results unveiled that the Iberian *M. ornata*-like taxon is in fact distributed through most of the Iberian Peninsula, except for the south-west and north-east. In contrast to the univoltine *M. ornata*, the Iberian taxon can be bivoltine in the wild. The Iberian taxon was retrieved to be related to *M. ornata*, but the differences in the genetic markers and genitalia were comparable to those found between species in the group. Based on the evidence here presented and according to species delimitation results, we propose to consider the Iberian taxon as a novel species, tentatively named *Melitaea pseudornata* Muñoz Sariot & Sánchez Mesa, 2019, *stat. nov.*

Key words: phylogeography, integrative taxonomy, hybridization, phylogenetics, butterfly
The information available about the taxonomy (Wiemers et al., 2018), evolution (Dappo et al., 2019, Wiemers et al., 2020, Dincă et al., 2021), distribution (Kudrna et al., 2015) and ecology (Settele et al., 2009) of the European butterflies is massive even at local and regional scale, which highlights them as probably the best-known insect group in Europe. Nevertheless, the emergence of genetic techniques revealed that this knowledge was not as complete as believed due to the existence of cryptic diversity: taxa that have remained unnoticed due to the morphological similarity with other species. Indeed, novel butterfly species are still being documented in Europe (e.g., Vodolazhsky and Stradomsky, 2008, Dincă et al., 2011, Hernández-Roldán et al., 2016, Hinojosa et al., 2021). This evidences the existence of potential gaps in the taxonomy of some groups, which brings uncertainty in actions linked to conservation and may hamper a proper understanding of these organisms and their evolution (e.g., Gill et al., 2016, Sales et al., 2018). Thus, further studies addressing the presence of hidden diversity are required, especially in the groups with most debated taxonomy.

The butterfly genus Melitaea Fabricius, 1807 comprises about a hundred species distributed in the Palearctic (van Oorschot and Coutois, 2014). Its taxonomy and systematics have been traditionally problematic since it comprises species characterized by being polymorphic and by having similar adult and larval morphology. In consequence, this genus is particularly affected by descriptions of redundant species and subspecies, as well as by cases of long-overlooked taxa. This scenario is especially true for the Melitaea phoebe species group, which was recently reviewed using DNA and morphological data (Tóth et al., 2014, 2017) and divided in six species: M. abbas Gross & Ebert, 1975, M. ornata Christoph, 1893, M. phoebe ([Denis & Schiffermüller], 1775), M. punica Oberthür, 1876, M. scotiosa Butler, 1878, and M. telona Frustorfer, 1908.

One of the most confusing species of the M. phoebe species group is M. ornata. It was originally described as Melitaea phoebe var. ornata by Christoph (1893) from an individual collected in Guberlya (Orenburg Oblast, Russia). Its similarity with M. phoebe led to the description of a large list of M. phoebe subspecies that corresponded, in fact, to the taxon ornata. It was elevated to the species rank in parallel and with two distinct names, Melitaea empinata (Russell et al., 2005) and Melitaea oggyia (Varga et al., 2005), which were finally synonymized (Tóth and Varga 2011, Russell and Tennent 2016) to M. ornata. The most constant feature in M. ornata is the head color of the 4th and later larval instars, which is typically brick red (Russell et al., 2007, Russell and Tennent 2016) instead of black, as in M. phoebe. The first molecular analysis comparing both taxa (Tóth et al., 2014) confirmed that M. phoebe and M. ornata are distinct species: they were monophyletic and showed considerable divergence in nuclear DNA (nuDNA), albeit some populations share mitochondrial DNA (mtDNA) haplotypes with M. phoebe (Tóth et al., 2017).

M. ornata is known to be present from Kazakhstan, the Middle East, and eastern Europe to the Italian Peninsula, Sicily (Russel et al., 2007, Tóth et al. 2013, Tóth et al. 2017) and the Provence (Lafranchis et al., 2015). Based on larval morphology, this species was recently reported for the first time in the Iberian Peninsula, restricted to mountain areas of the south-east (Sánchez Mesa and Muñoz Sariot, 2017, Muñoz Sariot and Sánchez Mesa, 2019a). The same authors named these populations as the subspecies M. ornata baetica (a name that was preoccupied) and, later, M. ornata pseudornata, but they also suggested that it could be related to the north African M. punica due to similarities in the caterpillars or that it could even be a distinct species. Hence, in order to clarify the placement of the Iberian M. ornata-like taxon in the frame of the M. phoebe species group and to document its distribution and ecology, we have sequenced nuDNA and mtDNA markers, performed a geometric morphometry analysis of the male genitalia, and gathered phenological, hostplant and parasitoid data. The results allowed to launch a taxonomic hypothesis grounded on multiple evidence: the Iberian taxon is considered as a distinct species, which we tentatively name Melitaea pseudornata Muñoz Sariot & Sánchez Mesa, 2019 stat. nov.

Material and Methods

DNA Extraction and Sequencing

The bodies of the specimens used in the DNA analyses were stored in 99% ethanol at –20°C and wings were kept separately as vouchers. The DNA analyses were conducted employing sequences retrieved from 191 individuals, including all the species of the M. phoebe group. Sampling sites in the Iberian Peninsula are plotted in Fig. 1; here, the specimens were identified using the wg phylogeny (Supp Fig. S1 [online only]). In total, we used 160 COI and 180 wg, 111 RPS5, 87 MDH, and 90 EF-1α (Supp Table S1 [online only]).

Total genomic DNA was extracted using Chelex 100 resin, 100–200 mesh, sodium form (Biorad), under the following protocol: one leg was removed and introduced into 100 μl of Chelex 10% and 5 μl of Proteinase K (20 mg/ml) were added. The samples were incubated overnight at 55°C in a shaker and were subsequently incubated at 100°C for 15 minutes.

Primers and PCR protocols used for the amplification of COI (barcode region), wg, EF-1α (three fragments), RPS5, and MDH are written in Supp Tables S2 and S3 (online only). Universal tails were included in all primers. PCR products were purified and Sanger sequenced by Macrogen Inc. Europe (Amsterdam, the Netherlands). All sequences have been deposited in GenBank (Supp Table S1 [online only]).

Phylogenetic Reconstruction

Sequences were visualized, edited, and aligned in Geneious Prime 2019.0.3 (https://www.geneious.com). A haplotype network of the COI barcode region was created in POPART v1.7 (Leigh and Bryant 2015) under the TCS method. The best-fitting substitution model was estimated in jModelTest (Darriba et al. 2012) under the Akaike information criterion.

The alignments of the nuclear genes were concatenated and a phylogeny was reconstructed using BEAST v2.5.0 (Bouckaert et al., 2014). Distinct partitions were selected for each gene using the best model indicated by jModelTest and four rate categories if included gamma and base frequencies were estimated. Parameters were estimated using two independent runs of 30 million generations each and convergence was checked with TRACER 1.7.1 (Rambaut 2018). A burn-in of 10% was applied.

A maximum likelihood (ML) inference was obtained with the concatenated (partitioned) alignment of nuclear loci using RAxML v8.2.11 (Stamatakis 2014). RAxML was also used to retrieve a phylogeny for every individual nuclear gene, which was employed in ASTRAL. A thorough bootstrapping was employed and we selected a GTRGAMMA model and 1,000 bootstrap replicates.

Species Delimitation

Joint Bayesian species delimitation was conducted using the program BPP (Yang 2015). The method uses the multispecies coalescent model to compare different models of species delimitation (Yang and Rannala 2010, Rannala and Yang 2013) in a Bayesian framework,
accounting for incomplete lineage sorting due to ancestral polymorphism and gene tree-species tree discordance. Considering that, in butterflies, the median of the theta is 0.0160 (ranging from 0.004 to 0.043) mutations/site (Mackintosh et al. 2019), a sensible diffuse theta prior would be IG(3, 0.045).

Assuming neutrality, the mutation rate for butterflies has been estimated to be about $2.9 \times 10^{-9}$ mutations/site/year (Keightley et al. 2015). A divergence time between 3.3–9.6 Mya (95% credibility interval) has been estimated for the *M. phoebe* species group (Tóth et al. 2017). By multiplying these values, we considered a divergence between the root of the species tree and the present time ($\tau$) between 0.0096 and 0.0278 mutations/site and we assigned a diffuse $\tau$ prior of IG(3, 0.03).

The other divergence time parameters are specified by the uniform Dirichlet distribution (Yang and Rannala 2010: equation 2). We selected 500,000 MCMC and 50,000 burn-in and the analysis was run twice to confirm consistency between runs.

**Genitalia Morphometry**

Geometric morphometry on the processus posterior (male genitalia) was applied to determine the morphological relationships between *M. pseudornata* and the other species of the *M. phoebe* species group. In total, 315 specimens have been measured. These individuals are partly identical with the material used in Tóth and Varga (2011), but it has been completed with 12 specimens from Iberia that were identified based on nuclear DNA data (Supp Table S1 [online only]).

A standard genital preparation method was followed. The abdomens were removed and heated in 15% KOH solution in 80°C for 30 min. Next, genitalia were cleaned and dehydrated in ethanol and mounted in euparal. Genitalia slides were digitalized using a stereomicroscope and a digital camera.

TpsDig2 was used to record nine fixed landmarks at the tips and origin of the main processus (Tóth and Varga 2011). The raw coordinates were transformed using Procrustes generalized least squares using geomorph R package (Adams et al. 2021).

All further analysis was performed based on the transformed coordinates. We used linear discriminant analysis (LDA) to determine the morphological relationships between the studied taxa using MASS R package (Venables and Ripley 2002). Leave-one-out cross-validation classification was also used to quantify the classification success. The significance of the visible pattern was analyzed by pairwise permutational MANOVA using Bonferroni-corrected significance levels using RVAideMemoire R package (Hervé, 2021). Average landmark coordinates of the processus posterior were calculated for all the studied species, then a PCA was performed on these mean shapes.

**Flight Time Data (Navarre, Northern Spain)**

Adult records were retrieved from the data of two transects located at Taxoare (Aranguren, Navarre). In this locality, only orange-headed caterpillars were found, and DNA results classified all the sequenced specimens as *M. pseudornata*. Adults were counted following a standardized methodology that consists of sampling every two weeks, from the first week of April to the last week of September, along a walked transect at distances of 2.5 m on both the sides and 5 m ahead of the recorder (Pollard and Yates 1993).

**Nomenclature**

This paper has been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:D1410808-7450-4190-BA1A-C57BA477AD46
Results

Phylogenetic Inference and Species Delimitation

The haplotype network based on the barcode region of the COI (Fig. 2A,B) showed that *M. ornata* and *M. pseudornata* shared barcode haplogroups with *M. phoebe* individuals. However, *M. ornata* from the Balkan Peninsula and eastwards maintained very distinct barcodes. Some individuals of *M. pseudornata* displayed exclusive haplotypes within Haplogroup 2, although separated from those of *M. phoebe* by only one or two substitutions.

Nuclear markers retrieved all the taxa as monophyletic with a posterior probability (PP) of 1 (Figs 2B, Supp Fig. S2 [online only]) and a bootstrap value (BS) > 70, except for *M. phoebe* in the ML inference, which had a lower support (Supp Fig. S3 [online only]). *Melitaea pseudornata* was recovered sister to *M. ornata* in the Bayesian and ML inferences. Interestingly, the Bayesian phylogeny retrieved two clades within *M. phoebe*: a clade formed by all the Iberian specimens and a French individual (PP = 0.82), and a clade with the rest of individuals (PP = 0.77).

Both BPP runs selected the seven-species hypothesis as the most likely (PP = 1). All the taxa of the *M. phoebe* species group and the Iberian taxon were separated as distinct species.

Genitalia

Despite interindividual variability was present in this group of taxa, the processus posterior of *M. pseudornata* showed on average a unique shape, distinct to *M. ornata* and *M. phoebe* (Fig. 3B–D). The average shape of the processus posterior was very similar to *M. ornata* as the inner process have the similar length. On the other hand, it showed some similarities to *M. abbas* and *M. punica* as the outer process pointed upward. On the LDA scatterplots, the centroid of *M. pseudornata* was positioned at a distance from all the other groups but close to *M. ornata* and *M. phoebe* considering the first three axes, which explain 74.81% of the variance between groups (Fig. 3). The first axis explained 38.17%, the second 20.53%, and the third 16.11% of variance between groups. Leave-one-out cross-validation classification assigned 77.78% of the individuals correctly in the case of *M. pseudornata* (Supp Table S4 [online only]). This success was very similar to the overall precision of the classification in the group, which was 78.34%.

The pairwise permutational MANOVA indicated statistically significant differences (*P* < 0.05 in all cases) in the processus posterior shapes among the taxa analyzed (Supp Table S5 [online only]).

![Fig. 2. A. Haplotype network of the Melitaea phoebe species group based on the barcode fragment of the COI gene. The area of the circles is proportional to the number of sequences they represent. B. European distribution of the three haplogroups of Melitaea pseudornata stat. nov., M. phoebe and Melitaea ornata. C. Bayesian inference phylogeny obtained using a partitioned alignment of the nuclear genes EF-1a, MDH, wg and RPS5. Posterior probabilities at species-level and for higher relationships are indicated, and scale units are presented in substitutions per site.](image-url)
M. pseudornata showed a similar morphological differentiation compared to the closely related species [Supp Table S6 [online only]].

Notes About Morphology and Ecology of M. pseudornata

Larval Morphology. Black with white dots and black head until the last stage (L7), when it turns orange; nevertheless, locally some individuals retain the black head in the last stage (Sánchez Mesa and Muñoz Sariot 2017). In south-eastern Iberia (Baetic System), caterpillars have orange scoli (Sánchez Mesa and Muñoz Sariot 2017, Muñoz Sariot and Sánchez Mesa 2019a). In northern Iberia, larvae have black scoli and present an orange lateral stripe, similar to the Iberian specimens of M. phoebe (Fig. 4), although sometimes very diffuse.

Adult Morphology. Wings were very variable and similar to M. phoebe (Supp Fig. S4 [online only]); after a visual inspection of the wing traits partially diagnostic between M. ornata and M. phoebe—mentioned in Russell and Tennent (2016)—no clear differential patterns were found between M. pseudornata and M. phoebe. Regarding the tip of the antennae, stubbier shapes are more frequent in M. pseudornata compared to M. phoebe, although it does not seem a fully diagnostic trait (Sánchez Mesa and Muñoz Sariot 2017). Pictures of the wings of individuals used in this study have been deposited in figshare (DOI: 10.6084/m9.figshare0.16832830)

Flight Time. In the Baetic System, only one generation was recorded (Sánchez Mesa and Muñoz Sariot 2017). Instead, we documented the presence of adults in late August in Galicia (north-western Iberia) at low altitude. In Navarre (north-central Iberia), in a locality where only M. pseudornata has been recorded, biweekly adult counts conducted for three consecutive years showed a consistent bi-modal shape, with peaks at end of May/beginning of June, and at end of July/beginning of August, consistent with the existence of two generations (Supp Fig. S5 [online only]). Thus, this taxon seems to be uni- or bivoltine depending on the locality, which is possibly related to the desiccation of the host plant during summer or, in high altitude areas, to a shorter summer period.
Habitat. Similar to *M. phoebe*, but typically inhabiting mid-mountain biotopes, between 500 m and 1500 m (Fig. 1). Present at sea-level in Galicia. It occupies the Atlantic and Mediterranean biogeographic regions.

Host Plants. Baetic System: eggs and/or L1 larvae on *Carduus platypus* subsp. *granatensis* (Willk.) Nyman (Asteraceae), *Carduncellus hispanicus* Boiss. ex DC. (Asteraceae), *Cirsium arvense* (L.) Scop. (Asteraceae), *Cirsium vulgare* (Savi) Ten. (Asteraceae), *Cirsium pyrenaicum* (Jacq.) All. (Asteraceae), *Cirsium acaulon* subsp. *gregarium* (Boiss. ex DC.) Talavera (Asteraceae); caterpillars in the last instar were found in the previously cited host plants and on *Onopordum acanthium* L. (Asteraceae) and *Onopordum illyricum* L. (Asteraceae). Navarre: L1 caterpillars on *Centaurea jacea* subsp. *angustifolia* (DC.) Gremli (Asteraceae).

Parasitoids. Baetic System: *Cotesia melitaeorum* (Wilkinson, 1937) (Hymenoptera: Braconidae). Navarre: *Dolichogenidea* sp. Viereck, 1911 (Hymenoptera: Braconidae), which is a novel parasitic relationship in the genus *Melitaea*, and *Cynipoidea* (Hymenoptera).

Discussion

The Iberian Taxon, a New Species

The results here presented suggest that the Iberian taxon should be elevated to the species status. First, molecular evidence (nuclear markers) retrieved the Iberian individuals as a monophyletic clade, well-diverged from *M. ornata*, although sister to it (Figs. 2C, Supp Figs. S2–S3 [online only]); species delimitation analyses supported the specific status for this clade. Second, differences in the genitalia between the Iberian taxon and *M. ornata* were comparable to those found interspecifically among other species of the group (Fig. 3B–D; Supp Tables S4–S6 [online only]). Third, their phenology is distinct since *M. ornata* has apparently only one generation each year—second generations have been obtained only in captivity (Russell and Pateman 2013, Russell et al. 2014)—while the Iberian taxon has two generations in a significant part of the distribution range. Worth mentioning, there is no evidence of a close relationship of the Iberian taxon with the north African *M. punica*, a hypothesis that was suggested due to similarities present in the caterpillars (Sánchez Mesa and Muñoz Sariot 2017, Muñoz Sariot and Sánchez Mesa 2019a).

The denomination of this novel species, however, is not straightforward. Russell et al. (2020) attributed several taxa to the Iberian...
M. ornata-like taxon. They based their proposals on the external morphology of the adults, but in these traits the Iberian taxon cannot be reliably differentiated from M. phoebe. Several of our specimens showed typical ornata-like characteristics but, based on nuDNA, they proved to be M. phoebe, and vice versa. The thickness of the tip of the antennae seems not to be a defining trait either because thick tips are found in both taxa (see Sánchez Mesa and Muñoz Sariot 2017, Muñoz Sariot and Sánchez Mesa 2019a), although thicker shapes are more common in M. pseudornata than in the Iberian M. phoebe. Overall, the evidence available points that this taxon can only be reliably distinguished by nuDNA data and by the reddish head of the last (L7) instar caterpillars (Sánchez Mesa and Muñoz Sariot 2017)—distinct to M. phoebe, with invariably black head, and to M. ornata, with reddish head from L4 to the last instar (Russell and Tennent 2016). Given the absence of these data in the taxonomic proposals made by Russell et al. (2020), we think that further analyses of the type specimens are required in order to confirm the identifications. In consequence, here we used the name of the first taxon whose identification was based on the color of the head of the caterpillars, which is pseudornata (Muñoz Sariot and Sánchez Mesa 2019a, b). Thus, we tentatively name the novel species as M. pseudornata Muñoz Sariot & Sánchez Mesa, 2019, stat. nov.

The Distribution Range of M. pseudornata

Identifications based on the w̄g gene (Supp Fig. S1 [online only]) confirmed the presence of M. pseudornata across most of the Iberian Peninsula, apparently restricted to areas of oceanic influence and/or mountain ranges. So far, it has been only found in Spain. This distribution is similar to the prediction made by Tóth et al. (2013, 2017), who showed through ecological niche modelling analyses that virtually all the Iberian Peninsula represents a climatically suitable habitat for the sibling species M. ornata. Although further exploration is required, M. pseudornata was the sole species found in a vast area of north-western Spain. In contrast, only M. phoebe was found in the south-west of the Iberian Peninsula (although sampling in this region is low) and in Catalonia (except in the southern mountains of Els Ports, where it is replaced by M. pseudornata). Both species are locally sympatric in some mountain ranges in the Baetic System (southeastern Iberia), but this seems not to be usual and the pattern documented agrees with a situation of parapatry, in which contact zones may reflect some kind of competition or incompatibility.

The ranges of the species pair M. pseudornata and M. ornata match with a distribution pattern typically produced by glacial cycles, even if initial divergence predate them (Ebdon et al. 2021). Glacial periods caused the isolation of populations in the southern peninsulas, which promoted allopatric differentiation and, sometimes, speciation (Hewitt, 2000). In the Iberian Peninsula, there are about twenty butterfly species that have a sibling widespread through Europe (Dincă et al. 2015). Many of them establish contact zones around the Pyrenees—typically in the Ebro River valley, the Pyrenees themselves, or in S. France—such as the pairs Ipictides feisthamelii (Duponchel, 1832) (Papilionidae)–Ipictides podalirius (Linneaus, 1758) (Gauet et al. 2019) or Aricia cramera (Eschscholtz, 1821) (Lycaenidae)–Aricia agestis (Denis & Schiffermüller, 1775) (Voda et al. 2015). In our case, the existence of a contact zone cannot be determined since it is unknown whether M. pseudornata is present in France; meanwhile, the closest area where M. ornata has been reported is Provence. Hence, as far as we know, M. pseudornata is allopatric with respect to M. ornata.

M. pseudornata and M. phoebe, Two Interacting Species

Mitochondrial DNA (Figs. 2A,B) showed that M. pseudornata shared two haplogroups (here, groups of haplotypes linked by two or fewer mutations) with M. phoebe. One of the shared haplogroups, Haplogroup 1, is exclusive to Iberia. The second shared haplogroup, Haplogroup 2, was also found in M. phoebe from all Europe (including Iberia) and in M. ornata. Considering that M. ornata conserves a well-diverged COI lineage in the Balkan Peninsula and eastwards (Haplogroup 3), the fact that in other parts of Europe this species is clustered in the same haplogroup with M. phoebe and M. pseudornata while nuclear markers differentiate them suggests mitochondrial introgression. Thus, M. ornata would have partially lost its original mtDNA in favor of an introgressed mtDNA presumably coming from M. phoebe, a scenario already proposed by Tóth et al. (2017). The same situation may apply to M. pseudornata, whose mtDNA could have been completely erased after the introgression events with M. phoebe—as occurred in other Iberian species such as Ipictides feisthamelii (Gauet et al. 2019). Overall, we cannot determine from these data how common hybridization between M. phoebe and M. pseudornata might be at present but, given that they share two well-differentiated haplogroups, introgressive hybridization seems to have occurred at least twice in the past.

Past hybridization between M. phoebe and M. pseudornata could have had an impact on the morphology of the larvae and the adults. A sign of this can be the presence, in populations of northern Iberia, of the orange lateral stripe in the caterpillars, very similar to those present in the Iberian M. phoebe (Fig. 4); these stripes are absent in M. ornata (Russell and Tennent 2016). Regarding the adults, a combination of traits of the wing underside such as the premarginal markings and color tone of the hindwings and are considered to be relatively useful to distinguish between M. ornata and M. phoebe (Russell and Tennent 2016). However, between the Iberian M. phoebe and M. pseudornata, these traits are regularly shared (Supp Fig. S4 [online only]). Furthermore, no other external traits seem to unambiguously differentiate the adults of M. pseudornata and M. phoebe.

M. pseudornata populations may also be affected by ecological character displacement regarding the larval host plant. In the Baetic System, M. pseudornata females are known to oviposit (or L1 larvae were found) on Carduncellus Adans, Cardaus L., and Cirsium Mill., whereas M. phoebe oviposits on Centaurea L. Contrastingly, in central Navarre, an area where only M. pseudornata has been found, we only observed larvae (including L1) feeding on Centaurea jacea. This behavior could be influenced by a potential competitive pressure caused by the more generalist M. phoebe, a hypothesis already suggested by Tóth et al. (2015) for M. ornata.

Supplementary Data

Supplementary data are available at Insect Systematics and Diversity online.

Specimen Collection Statement

Insect Systematics and Diversity supports compliance with the Nagoya Protocol. The authors attest that all legal and regulatory requirements, including export and import collection permits, have been followed for the collection of specimens from source populations at any international, national, regional, or other geographic level for all relevant field specimens collected as part of this study.

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