

POINTS OF VIEW

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An Updated Concept of Subspecies Resolves a Dispute about the Taxonomy of Incompletely Separated Lineages

A recent controversy in the herpetological literature concerns the taxonomic treatment of detectable lineages that are exchanging genes, which I will hereafter refer to as *incompletely separated lineages*. Based on results obtained from a Bayesian implementation of the multispecies coalescent model applied to the problem of species delimitation (Yang and Rannala 2010), Burbrink and Guiher (2015) recognized incompletely separated lineages as different species in the case of both cottonmouths (*Agkistrodon piscivorus* and *A. conanti*) and copperheads (*Agkistrodon contortrix* and *A. laticinctus*). These proposals were criticized by Hillis (2019, 2020), who argued that the entities in question may have been geographically isolated in the past but currently are not geographically or reproductively isolated and therefore should not be recognized as species. He also stated that subspecies designations are ideal for the copperhead case. Because some of my publications on the species concept (de Queiroz 1998, 2007) were cited by authors on both sides, it seems useful to examine how my proposals concerning the species category bear on this controversy. As it turns out, the different sides are not as far apart as they might seem, with reconciliation provided by a revised concept of subspecies (proposed below) that has been brought into line with the modern concept of species. Although I use the previously mentioned *Agkistrodon* cases as examples, my proposal has broad applicability within and beyond herpetology.

In some respects, the taxonomic treatment of incompletely separated lineages is an old problem. Indeed, disagreements about whether to recognize certain groups as species versus subspecies have existed for centuries. Darwin (1859:51), for example, noted that “Certainly no clear line of demarcation has yet been drawn between species and sub-species” and that (1859:47)

“CASES OF GREAT DIFFICULTY, WHICH I WILL NOT HERE ENUMERATE, SOMETIMES OCCUR IN DECIDING WHETHER OR NOT TO RANK ONE FORM AS A VARIETY OF ANOTHER, EVEN WHEN THEY ARE CLOSELY CONNECTED BY INTERMEDIATE LINKS; NOR WILL THE COMMONLY-ASSUMED HYBRID NATURE OF THE INTERMEDIATE LINKS ALWAYS REMOVE THE DIFFICULTY.”

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(Note that Darwin commonly used the term “variety” for taxa that later authors would call “subspecies.”) Similarly, Mayr (1966:24) noted:

“EVOLUTION IS A GRADUAL PROCESS AND, IN GENERAL, SO IS THE MULTIPLICATION OF SPECIES (EXCEPT BY POLYPLOIDY). AS A CONSEQUENCE ONE FINDS MANY POPULATIONS IN NATURE THAT HAVE . . . ACQUIRED SOME OF THE ATTRIBUTES OF DISTINCT SPECIES AND LACK OTHERS. ONE OR ANOTHER OF THE THREE MOST CHARACTERISTIC PROPERTIES OF SPECIES—REPRODUCTIVE ISOLATION, ECOLOGICAL DIFFERENCE, AND MORPHOLOGICAL DISTINGUISHABILITY—IS IN SUCH CASES ONLY INCOMPLETELY DEVELOPED. THE APPLICATION OF THE SPECIES CONCEPT TO SUCH INCOMPLETELY SPECIATED POPULATIONS RAISES CONSIDERABLE DIFFICULTIES.”

Despite the long history of this problem, some aspects are relatively new. As new forms of data and new analytical methods have been brought to bear on the problem of species delimitation, there has been an accompanying risk of either overestimating or underestimating the number of species. Thus, authors have raised concerns about species delimited solely on the basis of data from mitochondrial DNA (e.g., Ballard and Whitlock 2004) and relatively few loci (e.g., Dupuis et al. 2012). Recently, Hillis (2019, 2020; see also Chambers and Hillis 2020) discussed the possibility of over-splitting resulting from unrealistic assumptions of current species-delimitation methods based on the multispecies coalescent model.

The cases that I want to focus on in this commentary are not those in which species are inferred incorrectly because of limitations concerning the data or analytical methods used but those in which lineages are correctly inferred based on real signal in the data, yet those lineages also continue to exchange genes—that is, cases of incomplete lineage separation. Incomplete lineage separation can result from earlier geographic separation (allopatry) followed by secondary contact or from *in situ* divergence along an environmental gradient (e.g., Harrison and Larson 2016). The taxa at the center of the current debate—Eastern and Broad-banded Copperheads on the one hand, Northern and Florida Cottonmouths on the other (I use common names to remain neutral as to whether these are species or subspecies, but see below)—appear to represent cases of incomplete lineage separation.

Burbrink and Guiher (2015) inferred two species each of cottonmouths and of copperheads based on the results of Bayesian species delimitation analyses of mitochondrial and nuclear gene sequences, explicitly acknowledging gene flow between

the inferred species (a major focus of their paper was delimiting species in the presence of gene flow). Both pairs of lineages were identified as separate populations (clusters) by analyses of population structure prior to the species-delimitation analyses, and in neither case do the proposed species appear to be artifacts of uneven geographic sampling (sampling in contact zones was denser than in other parts of the distributions). Moreover, in both cases, the boundaries between the proposed species correspond closely to those between previously recognized subspecies (e.g., Gloyd and Conant 1990), suggesting concordant morphological divergence between the lineages. Hillis (2019:8) acknowledged that the entities recognized as species by Burbrink and Guier “may well have been geographically isolated at some point in the past,” and he referred to Eastern Copperheads and Broad-banded Copperheads as “historical sublineages” (Hillis 2020:54). According to Strickland et al. (2014), cottonmouths show a signature of two historically separated populations that have come into secondary contact with gene flow between them.

Because my proposals about the species concept provide background for resolution of this controversy about whether such incompletely separated lineages ought to be recognized as species or as subspecies, I briefly review them here. I attempted to resolve a long-standing debate about the definition of the species category by pointing out that all modern species definitions have implicitly or explicitly equated species with separately evolving lineages at the population level of biological organization (e.g., de Queiroz 1998, 1999, 2007). The main differences among those definitions involved additional properties (e.g., reciprocal monophyly, ecological differences, intrinsic reproductive isolation) that were used to decide when two lineages had diverged enough to be considered separate species. I proposed that a single unified species concept could be achieved (and the problem of how to define the species category resolved) by rejecting all of those additional properties as *necessary* properties of species and treating them instead as different lines of evidence concerning the separation of lineages. The unified species concept that results from this proposal is one in which species are equated with separately evolving metapopulation lineages. Such lineages do not have to be reciprocally monophyletic, ecologically differentiated, or intrinsically reproductively isolated to be considered different species; they only have to be evolving separately from one another.

This proposal about the definition of the species category has several benefits, including unifying the diversity of views on species, more clearly distinguishing between the theoretical concept of species and the operational criteria that are used to apply that concept in practice, and making the definition of the species category more consistent with the theoretical importance commonly attributed to species. However, application of the unified species concept to many real-world situations requires clarification, because lineage separation is not always an all-or-nothing phenomenon. In particular, cases of incomplete separation are common among closely related lineages. As noted above, the cases at the center of the current controversy regarding the taxonomy of copperheads and cottonmouths appear to be examples of incomplete lineage separation. Authors on one side of the controversy have recognized those incompletely separated lineages as species, while those on the other side argue that the entities in question should be treated instead as subspecies. I contend that these seemingly conflicting taxonomic proposals are in greater agreement than they may appear. Reconciliation hinges on updating the concept of subspecies to bring it into line with the unified species concept.

Historically, the species and subspecies categories were, first and foremost, taxonomic ranks. The very fact that the species and the subspecies were treated as separate categories implied a discreteness that does not exist in nature, as noted by Darwin (1859). I have argued (de Queiroz 2011) that Darwin’s (1859:119) proposal to equate species with “branches in the lines of descent” (“segments of lineages” in my terminology) was associated with a subtle shift in the concept of subspecies (and variety). In Darwin’s view, not only are subspecies and species impossible to separate clearly in practice, they are also entities of fundamentally the same kind in theory. Thus, Darwin repeatedly referred to subspecies and varieties as “incipient species”—that is, species at an early stage in their existence. I have argued that, by pushing the segment of a lineage that is conceptualized as a species closer to its initial separation from other lineages and by implicitly treating both subspecies and species as representing a level of biological organization, this subtle shift in the conceptualization of subspecies also represents an early stage in the emergence of the unified species concept (de Queiroz 2011). The point I want to make here is that to be consistent with a modern evolutionary concept of species, the concept of subspecies must be modified so that it is no longer considered a distinct category from that of species. Subspecies are entities of the same fundamental kind as species: they are both population-level lineages. In other words, subspecies *are* species. More specifically, the phenomenon of incomplete lineage separation implies the existence of partially separated lineages within a more inclusive lineage (de Queiroz 2005). In this context, it makes sense to conceptualize subspecies as incompletely separated species (lineages) within a more inclusive species (lineage).

A couple of aspects of this proposal deserve clarification. First, when I say that subspecies *are* species, I do not mean to imply that the two categories are identical. Instead, one is a subset of the other: all subspecies are species but not all species are subspecies. The point is that subspecies and species are entities of fundamentally the same kind: population-level lineages. Second, under the definition of subspecies as incompletely separated species (lineages) within a more inclusive species (lineage), the subspecies category is no longer being treated as a taxonomic rank and especially not as one indicating something less than a species, as it has been explicitly or implicitly treated by nearly all previous authors (including Darwin, at least in part, and Mayr, as quoted above). That some species (lineages) are incompletely separated from others is simply a biological fact; it should not be taken to mean that they are anything less than “full” or “good” species (lineages). Related to this point, my proposal also does not depend on the ultimate fate of incompletely separated lineages—that is, whether they subsequently fuse into a single lineage without detectable sublineages, remain partially separated long-term, or eventually become completely separated.

To elaborate, I am *not* proposing that the subspecies category continue to be conceptualized as a rank below that of species that could be used for incompletely separated lineages (e.g., Frost and Hillis 1990; Hillis 2020). Instead, I am proposing that the very concept of subspecies be redefined. Subspecies are not members of a low-level taxonomic rank; *subspecies are incompletely separated lineages within a more inclusive lineage* (note that this definition makes no reference to a taxonomic rank). More precisely, subspecies are not members of a separate and subordinate category relative to species (analogous to the categories sublieutenant, subfossil, and subadult); instead, subspecies are members of the same category as species that are nested within other members of

that category (analogous to the categories subclade, subpopulation, and subsample). In this context, the meaning of trinomials must also be reconsidered. Trinomials are no longer indicative of the rank of subspecies (because the subspecies category is not a taxonomic rank). Instead, trinomials are simply a representational device that can (but need not) be used to indicate the nesting of incompletely separated lineages within a more inclusive lineage. This difference may be subtle, but it is fundamental.

It should also be clear that this reformulated concept of subspecies does not justify the continued recognition or resurrection of all manner of dubious subspecies taxa that have been recognized historically. Conceptualizing subspecies as incompletely separated lineages is strongly at odds with recognizing as subspecies things such as artificial partitions of unitary gene pools based on single characters, arbitrary divisions of gradual clines, and phenotypes that represent individual variation rather than lineage differences—that is, all those questionable uses of the subspecies category that have contributed to its current unpopularity. Conceptualizing subspecies as incompletely separated species requires that any hypothesized subspecies be supported by the same kinds of evidence that would be required to infer that an entity is a species, as well as evidence that its separation from one or more other species is incomplete.

Under the updated concept of subspecies just described, treating Eastern and Broad-banded Copperheads as species is not at odds with treating those same incompletely separated lineages as subspecies. The binomina *Agkistrodon contortrix* and *A. laticinctus* and the trinomina *A. contortrix contortrix* and *A. c. laticinctus* are simply representational devices (see O'Hara 1993; de Queiroz 1999), the purpose of which is to convey our current understanding of lineage diversity within the *Agkistrodon* clade. In either case, the named entities are detectable but incompletely separated lineages, and our taxonomies should represent that situation clearly and unambiguously. The use of trinomina, as advocated by Hillis (2020), would obviously imply incomplete separation under the updated concept of subspecies proposed in this article. In the case of binomina, as adopted by Burbrink and Guiher (2015), indicating incomplete lineage separation would require some additional form of annotation (see, for example, the entry for *Aspidoscelis marmoratus* regarding incomplete separation from *A. tigris* in de Queiroz et al. 2017). This alternative may be preferable in cases toward the higher end of the continuum in degrees of lineage separation or when the incompletely separated lineages are not sister species. In any case, the point is that incomplete lineage separation can be represented using either approach.

In sum, under a modern lineage-based concept of species, the phenomenon of incomplete lineage separation suggests a reformulation of the concept of subspecies, and this updated concept of subspecies largely resolves the current controversy regarding the taxonomic treatment of incompletely separated lineages. I hope that this updated concept of subspecies will make the subspecies category more useful to contemporary biologists and perhaps rescue it from a period of unpopularity. I want to emphasize, however, that this updated concept of subspecies represents a fundamental change in the way that we think about subspecies. Subspecies are no longer entities that are assigned to a lower taxonomic rank to indicate that they are not quite or not yet species; instead, subspecies are simply incompletely separated species within a more inclusive species. Updating the subspecies concept in this way transforms the subspecies from an artificial taxonomic rank into a biologically meaningful category. The traditional concept of subspecies, however, has more than 150 years of inertia

behind it; so this new concept of subspecies will likely face resistance despite its benefits.

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