POINTS OF VIEW

The Detection and Naming of Geographic Variation Within Species

Subspecies is the only taxonomic rank below species recognized by the International Code of Zoological Nomenclature (1999). The use of subspecies to denote morphologically distinct races that occupy different parts of a species range became standard practice in zoology in the late 1800s (Mallet 2013). Subspecies differ from species in that there are no reproductive or genetic barriers among subspecies, so they typically exhibit a genetically continuous zone of intergradation from one subspecies to the next across geographical space (Mayr 1969, 1982). As noted by Mayr (1969), this means that subspecies are not units of evolution (independent evolutionary lineages, in modern terms). Instead, Mayr (1969) argued that subspecies represent geographic areas of recognizable morphology within a species, such that the appearance of the animals in one area of a species range is distinctly different from that in another area of the species range.

Mayr (1969:41) defined subspecies formally as:

> "A SUBSPECIES IS AN AGGREGATE OF PHENOTYPICALLY SIMILAR POPULATIONS OF A SPECIES, INHABITING A GEOGRAPHIC SUBDIVISION OF THE RANGE OF A SPECIES, AND DIFFERING TAXONOMICALLY FROM OTHER POPULATIONS OF THE SPECIES."

He further explained that "differing taxonomically" means that they differ by diagnostic morphological differences. These differences may result from different selective conditions in different parts of the species range (for example, related to differences in habitat or climate, or the presence of mimicry models or predators). They also can result from formerly isolated historical lineages that subsequently regain reproductive contact, such that they are no longer on independent evolutionary trajectories.

The use of subspecies in herpetology became widespread throughout the early 1900s, as many species of reptiles and amphibians were shown to exhibit considerable geographic variation in morphology in different parts of their ranges (e.g., Ruthven 1908). However, many species exhibit continuous clines in morphological variation, rather than sudden geographical

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transitions, and some herpetologists began to abuse the concept of subspecies to designate arbitrary slices of continuous clines as subspecies. This practice led to objections (e.g., Wilson and Brown 1953) about the overuse of subspecies, as authors noted that different morphological features varied across a species range in different ways, so that the application of subspecies was often arbitrary. More recently, other authors have argued that some subspecies designations are misleading about non-morphological geographic variation or historical sublineages within species (Frost et al. 1992; Zink 2004).

Despite the objections to the often-arbitrary nature of subspecies, they continue to be useful in some contexts, such as field guides and studies of color-pattern selection, for designating strikingly different-looking geographic races within species. For example, in his second edition *A Field Guide to Reptiles and Amphibians of the Eastern and Central North America,* Conant (1975:8) defended the need for subspecies in his guide thusly:

> "A UNIFORMLY BLACK SNAKE, A YELLOW ONE WITH DARK STRIPES, AND OTHERS WITH BOLD SPOTTED PATTERNS LOOK QUITE DIFFERENT, BUT ALL THE SERPENTS ON PLATE 28 FROM THE BLACK RAT SNAKE TO THE BOTTOM OF THE PAGE, INCLUSIVE, ARE MEMBERS OF THE SAME SPECIES. TO ILLUSTRATE JUST ONE AND BURY THE OTHERS IN THE TEXT WOULD BE UNFAIR—BOTH TO THE AMATEUR NATURALIST AND TO THOSE DISTINCTIVELY MARKED ANIMALS."

Conant (1975:9) further noted that:

> "THE SUBSPECIES… PROVIDES A NAME FOR DISTINCTIVE POPULATIONS, AND HELPS POINT OUT EVOLUTIONARY TRENDS AND THE RESPONSES OF SPECIES TO HABITATS THAT MAY DIFFER DISTINCTIVELY IN CONCORDANCE WITH THE VARIOUS PHYSIOGRAPHIC REGIONS INHABITED BY THE SPECIES AS A WHOLE. … I STRONGLY DISAGREE WITH THE HANDFUL OF HERPETOLOGISTS WHO WOULD IGNORE SUBSPECIES ENTIRELY AND WHO REFER TO ANNECTANT POPULATIONS AS THAT PORTION OF THE SPECIES WHICH IS BLACK IN COLORATION, THE ONE THAT HAS A DISTINCTIVE SPOTTED PATTERN…, ETC. HOW MUCH SIMPLER IT IS TO REFER TO EACH FRACTION OF THE FULL SPECIES BY A SUBSPECIFIC NAME THAT IS APPLICABLE TO THE ANIMALS IN QUESTION."

I accept Conant's defense of subspecies in this context, and understand the practicality of subspecies in applications where it is useful or important to refer to morphologically distinctive geographical races. Nonetheless, in 1990, Darrel Frost and I published a paper that many people mistook to be an attack

on the concept of subspecies in herpetology. Our paper was on species concepts in herpetology (Frost and Hillis 1990). In that paper (p. 92), we argued for the recognition of species as evolutionary lineages "…whose components, if distinguishable, are not incontrovertibly on different phylogenetic trajectories (i.e., sublineages, if distinguishable, are reproductively compatible)." This was a somewhat tortured way of saying that species are the independently evolving branches on the Tree of Life. We were arguing that species are the lineages that are maintained as separately evolving units over time, and that are held together as lineages (at least in sexual species) largely by the exchange of genes in reproduction. Barriers to such genetic exchanges among species allow them to evolve independently of one another. We noted that this was a mainstream idea in biology, and was consistent with the evolutionary lineage view of species articulated by many authors (e.g., Hennig 1950 1966; Simpson 1961; Ghiselin 1974; Wiley 1978). Furthermore, this concept combined the ideas of reproductive compatibility (as a mechanism that held lineages together; Dobzhansky 1937; Mayr 1942, 1957, 1969) with the long-term evolutionary lineage views of Simpson (1961) and Wiley (1978). Since our paper (Frost and Hillis 1990), other authors have expanded and further developed this general evolutionary lineage view of species (e.g., Mayden 1997; de Queiroz 1998).

In Frost and Hillis (1990), we made a few passing references to the use of subspecies. In one passage, we noted (p. 92) that

"THE SUBSPECIES CATEGORY COULD BE USED THEORETICALLY FOR SUBLINEAGES NOT INCONTROVERTIBLY REMOVED FROM THE POSSIBILITY OF INTERACTION WITH OTHER SUBLINEAGES, BUT THE USE OF THIS CATEGORY WOULD NECESSARILY FOLLOW RECOVERY OF THE HISTORICAL RELATIONSHIPS OF THE SUBPOPULATIONS."

In other words, we acknowledged that species could contain historically distinct sublineages that had since merged through reproductive interaction, and that such historical sublineages could be logically recognized as subspecies. We did not, however, specifically address the use of subspecies to designate different selective conditions (which result in distinct morphologies) in different parts of a species' range.

On the other hand, we were critical of two other uses of the subspecies category. We (Frost and Hillis 1990:93) argued that application of an evolutionary species concept would "…remove all of the 'subspecies' denoting sections of clines …and would, in fact, 'clean up' our taxonomy significantly." We further argued (p. 94) that

"…THERE IS NOTHING IN THE EVOLUTIONARY SPECIES CONCEPT THAT NECESSARILY ABJURES TRINOMIALS (=SUBSPECIES). HOWEVER, THE SUBSPECIES CATEGORY HAS LONG BEEN ABUSED FOR USE IN NAMING ARBITRARILY DEFINED 'SLICES' OF CLINES (E.G., THE ARBITRARY DELIMITATION OF A NUMBER OF 'SUBSPECIES' IN OPHEODRYS AESTIVUS BY GROBMAN, 1984; AND IN LAMPROPELTIS TRIANGULUM BY WILLIAMS, 1978). THESE 'SLICES' ARE CLEARLY NOT EVOLUTIONARY ENTITIES UNDER ANY DEFINITION OF THE TERM (MAYR, 1982; WILSON AND BROWN, 1953)."

As a second objection to the contemporary use of subspecies, we stated that

"IT SEEMS THAT WE HAVE GOOD REASON TO BE SUSPICIOUS OF ALL POLYTYPIC SPECIES AND PARTICULARLY OF UNUSUAL PHENOMENA, LIKE SYMPATRY OF SUBSPECIES IN LAMPROPELTIS TRIANGULUM (WILLIAMS, 1974) OR ENSATINA ESCHSCHOLTZII (STEBBINS, 1949). FOR THIS REASON, WE PREDICT THAT MOST OF THE LARGE POLYTYPIC SPECIES LEFT (SUCH AS AMBYSTOMA TIGRINUM, NECTURUS MACULOSUS, SCELOPORUS JARROVII, S. UNDULATUS, DIADOPHIS PUNCTATUS, PITUOPHIS MELANOLEUCUS, LAMPROPELTIS GETULA, TANTILLA RUBRA, THAMNOPHIS SIRTALIS, AND CROTALUS DURISSUS) WILL BE FOUND TO BE COMPOSED OF SEVERAL EVOLUTIONARY SPECIES."

Indeed, that prediction proved accurate, as almost all of those named taxa (as they were then known) have been subsequently split into multiple species. Some of them (such as *L. getula*) have been split well beyond anything that we intended, to the point that some recognized "species" in that complex intergrade across broad geographic contact zones (see discussion below). So the taxonomic pendulum has now swung fully in the opposite direction of where it was in 1990.

Despite the fact that we argued *for* one particular use of subspecies (for historical sublineages within species that are now reproductively interacting), and *against* two others (the use of subspecies to carve up continuous clines, or as a proxy for species that overlap sympatrically), our paper was wildly viewed as being "anti-subspecies." Some people (e.g., Collins 1991) quickly embraced the opportunity to raise distinct, non-reproductively connected subspecies to the rank of species. But others were furious with our argument that subspecies should not be used for arbitrary slices of geographic clines. In the early 1990s, the late Charles Lowe printed up "buttons" for herpetologists to wear at scientific meetings that read "Save a Subspecies: Kill a Cladist" (and he made it clear to me that the buttons were meant as a response to our paper).

But have herpetologists confused the three parts of our discussion about subspecies? Note that (1) we argued against any recognition of subspecies in *L. triangulum* that represented arbitrary slices of a reproductively continuous geographic cline. (2) We also noted that sympatric "subspecies" (e.g., the reproductively isolated *L. t. triangulum* and *L. t. elapsoides* of the time) should be recognized as species. And (3), we noted that evidence of historical lineages that were now intergrading where they met geographically *could* be recognized as subspecies.

The last point deserves additional discussion. Wide-ranging species often vary geographically. Some of this geographic variation is a result of historical lineages that were temporarily isolated, but then expanded geographically and began to merge again after coming back into reproductive contact. As a result, individuals occupying different geographic regions of a species range may differ considerably in morphology and genetics, even though there are no clear reproductive or genetic boundaries where these phenotypes or sublineages come into contact. Humans (*Homo sapiens*) are a familiar example. We can identify historical lineages of humans associated with the world's major continents, but we also recognize that these lineages interact reproductively and exchange genes through time, which holds us all together as an evolutionary species.

There are often reasons that biologists (or others) need or want to refer to geographic races within an evolutionary species. How can we do this? There are three obvious ways: Option (1): We could name identified geographical races as "species" (in which case we would recognize many "species" of humans). The obvious problems with this solution are that the common meaning of the word "species" would be essentially equivalent to "geographical race" (or even "tribe" or

"population," if we continue the process of splitting), and there is no end to how small we might make the divisions. Down this road lies taxonomic chaos.

Option (2): A second solution is to simply use common names, rather than formal taxonomic categories, to refer to geographical races within species. This is essentially the solution we use with humans, as we give common names to major geographic races (as well as to tribes, populations, and even family lineages that we wish to discuss). As Conant (1975) noted, some herpetologists prefer to use this option with geographic races (subspecies) of reptiles and amphibians as well, and this option is generally necessary for units that are smaller than subspecies (such as local populations).

Option (3): A third solution is to use the subspecies category to refer to geographic races. Why would we want to do this? Many applications, such as field guides, rely on the appearance of organisms for identification. A Broad-banded Copperhead (*Agkistrodon contortrix laticinctus*) certainly looks different from an Eastern Copperhead (*A. contortrix contortrix*), and there is evidence that the two may represent historical sublineages that have come back into contact and now extensively intergrade (Burbrink and Guiher 2015). They are not reproductively isolated, and the two are connected by a continuous series of interbreeding populations across their combined range (Gloyd and Conant 1990; Guiher and Burbrink 2008). Copperheads across a broad area of eastern Kansas, eastern Oklahoma, and eastern Texas are considered "admixed" between Broad-banded Copperheads and Eastern Copperheads by Burbrink and Guiher (2015). Given the evidence that these two historical sublineages intergrade across many hundreds of kilometers, it is clear that these are not independently evolving lineages. So, they should not be recognized as separate species, but we might still wish to recognize the phenotypic divergence and historical (temporary) separation of the two sublineages within the species *A. contortrix*. Subspecies designations are ideal for this application.

I do not have a strong preference between options (2) and (3) above; I think either is reasonable. A field guide (or other application that relies on morphology) could use the common name Broad-banded Copperhead, and/or the scientific name *Agkistrodon contortrix laticinctus* to refer to the same geographic race. However, I strongly object to option (1). This is, nonetheless, a common trend among many of today's systematists. Indeed, Burbrink and Guiher (2015) recommended that the geographic races of copperheads discussed above be recognized as distinct species, despite overwhelming evidence of the reproductive continuity of adjacent populations across their broad area of intergradation. They based their recommendation on the results of a Bayesian phylogenetics and phylogeography (BPP) analysis (Yang and Rannala 2010; Yang 2015), which is an application of the multispecies coalescent (MSC) model. However, BPP identifies genetic structure within samples, rather than boundaries among species (Sukumaran and Knowles 2017). Application of BPP to geographically structured species can lead to delimitation of artifactual "species" along boundaries that exhibit no barriers to gene flow (Barley et al. 2018; Chambers and Hillis 2020). Geographic structure within species commonly presents problems for species delimitation methods when individuals are sparsely sampled from across a species range (Schwartz and McKelvey 2008; Rittmeyer and Austin 2012; Barley et al. 2018). Indeed, when applied to human populations, BPP divides living humans into multiple "species" (Jackson et al. 2017). So, using BPP to delimit species is equivalent to a version

of option (1) above: delimited "species" may actually represent artifactual splits of genetically structured (but geographically and reproductively contiguous) populations that have little to do with the concept of species as distinct, independent evolutionary lineages. In some cases, these delimited units may be consistent with the traditional concept of subspecies: geographical races that intergrade with one another without any apparent barriers to gene exchange. Indeed, when the starting tree was not assumed, the probability of delimiting the two geographic races of *Agkistrodon contortrix* as "species" ranged from 1.0 to 0.0 in the BPP analyses conducted by Burbrink and Guiher (2015), depending largely on how many hybrids were included or excluded from the analysis. But excluding admixed individuals from the analysis is precisely one of the situations that leads to incorrect species delimitation under BPP (Barley et al. 2018). I understand that many of the papers that discuss the limitations and problems of BPP for species delimitation have been published since Burbrink and Guiher (2015) made their taxonomic recommendations on *Agkistrodon*, so their perspectives on this issue may have changed as well. See Hillis (2019) and Chambers and Hillis (2020) for further discussion of using MSC models for taxonomic decisions.

In Frost and Hillis (1990), we acknowledged a role for subspecies in cases like that of *Agkistrodon* discussed above. In contrast, our primary objection to the application of subspecies was the slicing of a continuous geographic cline into named taxa, which would then not represent any meaningful biological lineage. In recent decades, a different approach has been used to subdivide geographically variable species. Many former subspecies names have been raised to species status on the basis of mitochondrial DNA lineages, especially in snakes (e.g., Burbrink 2001, 2002; Pyron and Burbrink 2009; Krysko et al. 2017). But even though all species are lineages, all lineages (including gene lineages) are not species. Mitochondrial genomes represent maternally inherited gene lineages that often diverge *within* species, and sometimes are even transferred across species boundaries (e.g., Sullivan et al. 2004; Linnen and Farrell 2007; Hailer et al. 2012; Chambers and Hillis 2020). Virtually all widespread species exhibit considerable intraspecific mitochondrial divergence, and offspring of a single father (from different mothers) can have deeply divergent mitochondrial haplotypes, even within a panmictic population (e.g., Morgan-Richards et al. 2017). The geographic boundaries of mitochondrial DNA groups can be quite different from the patterns observed in nuclear genes, including the genes responsible for morphology. Therefore, species delimitation based solely on mitochondrial clades, without attempting to examine nuclear genes or other evidence of reproductive interactions among the mitochondrial haplotype groups, can result in highly misleading species boundaries. Mitochondrial haplotype groups also can be split into smaller and smaller units, thus dividing reproductively connected lineages into ever smaller taxa that may exhibit no barriers to nuclear gene flow or any other evidence of being independent evolutionary species.

Lampropeltis getula is an example of a species that was divided into increasingly smaller taxonomic "species" on the basis of mitochondrial haplotype groups in recent decades. Populations of *L. getula* exhibit a gradual change in phenotype across a broad swath of central Texas (and adjacent states), between what have traditionally been called Speckled and Desert Kingsnakes, or *L. getula holbrooki* and *L. getula splendida* (Blaney 1977; Werler and Dixon 2000). As there is no obvious

way of drawing a distinct line between these named subspecies on the basis of morphology, much of the range of *L. getula* in central Texas is commonly depicted or described as a zone of intergradation (e.g., see map in Conant 1975, and discussion in Blaney 1977 and Werler and Dixon 2000). Nonetheless, Pyron and Burbrink (2009) raised these two gradually intergrading subspecies to species status on the basis of a geographic split in mitochondrial haplotype groups between the east and west ends of the cline. Furthermore, they placed the border between these taxa in Texas more than 500 km west of where it had been depicted by Blaney (1977) or Werler and Dixon (2000) on the basis of morphology. But the gradual cline in color pattern from east to west in Texas (in a region largely unsampled by Pyron and Burbrink 2009) suggests that there is no reproductive break in the connected populations between these nominal taxa, which is not refuted (or even addressed) by the mitochondrial DNA data.

If we rely solely on mitochondrial haplotype groups to define species boundaries, without considering reproductive interactions or nuclear gene flow among haplotype groups, where does the splitting end? Because of the uniparental inheritance of mitochondrial DNA, we can identify ever-smaller mitochondrial clades within species without ever observing reticulation in the mitochondrial tree. If we use mitochondrial haplotype groups, rather than reproductive boundaries, as the sole basis for species delimitation, then we can end up recognizing more and more "species" within a reproductively connected species lineage. We would not expect many of these mitochondrial groups to be consistent with or predictive of historical biparental lineages, nuclear gene histories, morphology, or most other organismal attributes.

Over the past two decades, many taxonomic studies have divided species of amphibians and reptiles into ever-smaller subdivisions based largely or entirely on mitochondrial haplotype groups. For example, Pyron and Burbrink (2009) subdivided *L. getula* into five species on the basis of five mitochondrial haplotype groups that they estimate diverged about 2–5 million years ago (with 95% confidence limits of 0.75–7.32 million years). More recently, Krysko et al. (2017) further subdivided these five haplotype groups into eight species, the most closely related of which they estimate diverged about 0.4 million years ago. Notably, Krysko et al. (2017) examined the contact zones of three of their nominal species in Florida, and because they included one nuclear marker in addition to the mitochondrial genes in their study, they were able to show extensive "hybridization" among the forms where they come into contact. This demonstrated what traditionally would have been called regions of intergradation among subspecies and suggests that the haplotype groups identified by Krysko et al. (2017) are not independently evolving species lineages, but rather represent local sublineages within a larger species lineage.

Fortunately, the period of subdividing wide-ranging species entirely on the basis of mitochondrial haplotype groups appears to be ending, and the taxonomic changes that were made on this basis are beginning to be reversed (see discussion in Hillis 2019). Systematists now routinely examine large numbers of nuclear genes, which often show that mitochondrial gene trees can be inconsistent with patterns of nuclear gene flow and exhibit deep divergences *within* reproductively cohesive species lineages. Recently, for example, Myers et al. (2019) used nuclear genes to re-examine the contact zone between two of

the mitochondrial haplotype groups that were recognized as species by Pyron and Burbrink (2009). The results of Myers et al. (2019) supported the same pattern of intergradation and regional gene flow between geographic races that was originally suggested by a morphological investigation (Blaney 1977). Accordingly, Myers et al. (2019) treated *L. californiae* and *L. splendida* (sensu Pyron and Burbrink 2009) as geographic races of *L. getula* (although they did not formally apply any subspecies names to these groups). I welcome these taxonomic reversals, and argue that the subspecies category is a reasonable way of recognizing geographically distinct historical sublineages within species. But now it is necessary to re-examine (and in many cases formally reverse) the widespread over-splitting of geographically variable species that has occurred over the past two decades.

Species are supposed to inform us about the boundaries of the reproductively united, evolving lineages on the Tree of Life. If we start subdividing these evolutionary lineages into ever-smaller slices that we call species (even though they exhibit reproductive continuity), we will lose the biological meaning of species and misinform the rest of biology with the resultant taxonomy. Instead, the subspecies category (or common names) can be used effectively to differentiate geographic races within a species whenever that is practical or important.

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