

POINTS OF VIEW

Herpetological Review, 2022, 53(1), 47–53.
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Species, Clades, and their Relationship to Paraphyly and Monophyly: Examples from the *Pantherophis obsoletus* Complex

“BECAUSE EVOLUTIONARY DIVERGENCE BELOW THE LEVEL OF SPECIES IS LIABLE TO BE RETICULATE AND POPULATION DEPENDENT, STRICTLY LINEAR HIERARCHICAL SETS OF RELATIONSHIPS ARE UNLIKELY TO BE FOUND. THESE PROBLEMS ARE PARTICULARLY INHERENT WHEN BOTH THE DERIVATIVE AND ANCESTRAL POPULATIONS ARE SIDE BY SIDE AND WHEN EXTINCTION HAS NOT YET CREATED CLEAN-CUT ASSOCIATIONS OF POPULATIONS INTO MONOPHYLETIC ASSEMBLAGES. THIS SITUATION WILL BE EXACERBATED WHEN THE TIME BETWEEN DIVERGENCE EVENTS IS SHORT AND IF SUBSEQUENT HYBRIDIZATION EVENTS INFLUENCE THE MAKEUP OF ONE OR BOTH DIVERGENT ENTITIES. BECAUSE MORE DETAILED UNDERSTANDING OF RELATIONSHIPS AMONG POPULATIONS IS LIKELY TO COME FROM MOLECULAR DATA (SUCH AS DNA SEQUENCES), THERE WILL BE A COMPOUNDING EFFECT OF ASSOCIATION BY HISTORY (DIFFERENTIAL LINEAGE SORTING AND/OR RETENTION OF ANCESTRAL POLYMORPHISMS) AND THE NECESSARY DISTINCTION BETWEEN PHYLOGENIES OF GENES AND THOSE OF ORGANISMS. THUS, IN SOME WAYS, THE MORE WE KNOW, THE MORE DIFFICULT IT MAY BE TO UTILIZE ANY SPECIES CONCEPT AS A FORMAL AND RIGID CONSTRUCT.”

—PATTON AND SMITH (1994). *SYST. BIOL.* 43:23–24

Recent discussions of the taxonomy of the *Pantherophis obsoletus* complex (Burbrink et al. 2020, 2021; Hillis and Wüster 2021) have highlighted a need for a clarification of the terms *monophyly* and *paraphyly* as they are commonly applied to species and higher taxa, respectively. These definitions, and their application to biological taxonomy, are critical to delimitation and recognition of species within this complex of snakes (as well as many other biological taxa). Although these concepts have been the subject of much previous discussion (e.g., Niegel and Avise 1986; Pamilio and Nei 1988; McKittrick and Zink 1988; Patton and Smith 1994; Baum and Shaw 1995), a brief review is needed to respond to the arguments of Burbrink et al. (2021).

Applying Paraphyly and Monophyly to Lineages and Clades.— Before we consider application of the terms *monophyly* and *paraphyly*, it is important to distinguish the two major kinds of biological taxa that are recognized in biological taxonomy:

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lineages and clades. The boundary between these entities is the boundary between reticulate genealogy and divergent phylogeny, or between population genetics and phylogenetics (Baum and Shaw 1995). A genealogical lineage (or sexual plexus) consists of a reproductively connected population of organisms, extending through time across many generations (Wiley 1978; Frost and Hillis 1990; Baum and Shaw 1995). Recognition of a genealogical lineage at any particular point in time involves examination (directly or through genetic inference) of the genealogical connections among individuals. Recognition of a lineage through time involves examination or drawing inferences about reticulating relationships among individuals in successive generations of the extended population. As reproductive barriers arise between lineages, the lineages begin to evolve independently of one another, and we call such independently evolving lineages species (Wiley 1978; Frost and Hillis 1990; Baum and Shaw 1995; Mayden 1997; de Queiroz 1998). We may also identify partially and geographically differentiated sublineages of a species, that nonetheless retain continuous reproductive connections and hence do not evolve independently of one another, as subspecies (Mallet 2013; Hillis 2020). Intraspecific geographic variation (which leads to subspecies designations) is typically maintained by selection gradients within species (Endler 1986).

In contrast, clades are historical groups of taxa that evolved from a common ancestral species. Note that the two kinds of biological taxa, lineages and clades, constitute the two kinds of natural entities that are commonly represented on a phylogenetic tree. The genealogical lineages are represented by the individual segments (lines) that make up the tree, whereas clades represent historical groups of lineages that descend from a single ancestral lineage. Thus, a clade is a specific historical group of lineages that share a common ancestry. Although biologists sometimes include individual organisms on a phylogenetic tree, strictly diverging phylogenetic trees cannot correctly represent genealogical relationships of individuals within sexual species (Morrison 2016).

Now consider the meanings of the terms *monophyly* and *paraphyly* with respect to lineages and clades. The general meanings of these terms are relatively uncontroversial. A monophyletic group is generally defined as “an ancestor and all of its descendants” (e.g., in introductory biology textbooks,

e.g., Hillis et al. 2020:460; and in textbooks on phylogenetics, e.g., Baum and Smith 2013:44, 124). A paraphyletic group, in turn, is defined as “an ancestor and some, but not all, of its descendants.” In some contexts, such as the definition of clade names, the application and meaning are both clear. In that context, “ancestor” refers to an ancestral species lineage, and “descendants” refers to all the species that diverged from that ancestral species. Because monophyletic groups (= clades) represent historically connected groups of species, most biologists insist that biological classifications of higher taxa (groups such as genera, families, orders, etc.) be restricted to monophyletic groups—taxa that share a common evolutionary history.

In contrast to the clear application of the terms *monophyly* and *paraphyly* to clades, their application to biological lineages is a muddier issue. Because a lineage (a population extended through time) is composed of many individuals, then perhaps one might try to apply the terms *ancestor* and *descendant* (in the definitions of *monophyly* and *paraphyly*) to individual organisms. However, the relationships of a genealogical lineage of individuals cannot be correctly represented in a strictly diverging phylogenetic tree—at least in sexual species—because every individual has two parents. The relationship of individuals within a lineage do not diverge through time; individuals recombine with one another to produce descendants. Moreover, the only lineages that could ever be “monophyletic” with respect to individuals are those that are founded by a single individual—an impossibility in the usual case of sexual species. In fact, when any biological lineage undergoes a split, even if it instantly resulted in complete reproductive isolation of two descendant lineages, some of the individuals in one lineage have a more recent common ancestor among individuals in the other lineage than to individuals in their own lineage. For this reason, many biologists (e.g., McKittrick and Zink 1988; Patton and Smith 1994; Baum and Shaw 1995) have noted that the concept of monophyly clearly does not, and often cannot, apply to genealogical lineages, at least with respect to the individual organisms those lineages contain. If we consider individuals as the components of species, then all or virtually all species (and other lineage-based taxa, such as subspecies) are clearly paraphyletic, at least at their origins and for many future generations. Genealogical lineages are often not monophyletic groups of individual organisms, nor are they expected to be.

Although a species lineage is virtually never monophyletic in the sense noted above, a sample of individuals from one species taken at a particular point in time may indeed all be more closely related to one another than they are to any individuals in another species. Evidence of such groups, in combination with evidence regarding diagnostic traits that distinguish the sample, is often used to infer that a given sample represents a distinct species. Note, however, that samples of individuals within a species will only be monophyletic in this sense if there is no ongoing hybridization or gene flow from other species. Thus, monophyly of a sample of individuals within a species is only expected under a very limited set of conditions where there is absolutely no hybridization with any other species, and even then only after the lineage has existed in isolation from other lineages for many generations.

There is a second way that we could define *monophyly* and *paraphyly* with respect to lineages. In addition to the historical relationships of individuals, we can also follow the evolutionary relationships of genes within individuals. Unlike reticulate

genealogies of individuals, gene trees do represent diverging relationships through time, which can be correctly represented in a phylogenetic tree (at least if the genes have not undergone recombination). In this case, the terms *ancestor* and *descendant* would refer to particular copies of genes that are present in individuals in the population (Pamilo and Nei 1988). We could then examine the evolutionary relationships of individual genes at one point in the history of a lineage and trace those relationships back through time to the point that all the genes shared the same ancestral copy of the gene. This is the basis of coalescent theory (Hudson 1990). However, although genealogical lineages constrain the ancestor–descendant relationships of genes (in other words, every gene copy must be descended from another gene copy from within an historically connected genealogical lineage), there is no reason that a common ancestral gene must be found in the same lineage segment (= species) as are its descendants. Moreover, different genes will coalesce across many different lineage segments. Coalescent theory shows that we can expect any two copies of a gene in a population of diploid organisms to coalesce on average over about $2N_e$ generations, where N_e = the effective population size of species (although the distribution of expected coalescent times has a large variance; Hudson 1990). A large collection of genes in a population of diploid organisms are expected to converge on the order of $4N_e$ generations (Hudson 1990). Therefore, in a widely distributed common species with an effective population size of a few hundred thousand individuals and a generation time of several years, gene trees are expected to take over a million years (on average) to coalesce, with many taking much longer. This means that many gene trees are expected to be paraphyletic within most large species lineages, especially when speciation events are shallow. Moreover, the coalescent times for different genes will happen at many different points in time, both before and after speciation events. This is the basis for the common observation of Incomplete Lineage Sorting, which refers to the fact that gene trees can have histories that differ from the species tree (Maddison 1997).

The expectation, then, for species and other lineage-based biological taxa, is that they will be paraphyletic with respect to both individual genealogies as well as many gene histories that they contain. Only after a species has been completely reproductively isolated from other species for many generations would we expect a sample of individuals (or genes) to all be more closely related to one another than they are to any individuals (or genes) in other species. For this reason, biologists typically identify genealogical lineages based on the boundaries of reproductive connections among individuals in populations (tokogenesis, or parent–offspring relationships; Frost and Hillis 1990). Species are the largest of such genealogical lineages—the upper bounds of tokogenetic relationships among individuals—and hence they evolve as an individual thing (Ghiselin 1974; Frost and Hillis 1990; Baum and Shaw 1995). But there is no expectation for genealogical lineages to be monophyletic. In contrast, clades are composed of historical groups of these genealogical lineages, each of which evolves on its own evolutionary path. In the case of clades, we rely on monophyly to identify historical groups of species that are all descended from a common ancestral species.

Distinguishing Intraspecific from Interspecific Variation.—As noted above, the boundary between species and clades of higher taxa is also the boundary between tokogenetic relationships (the parent–offspring relationships that define a population

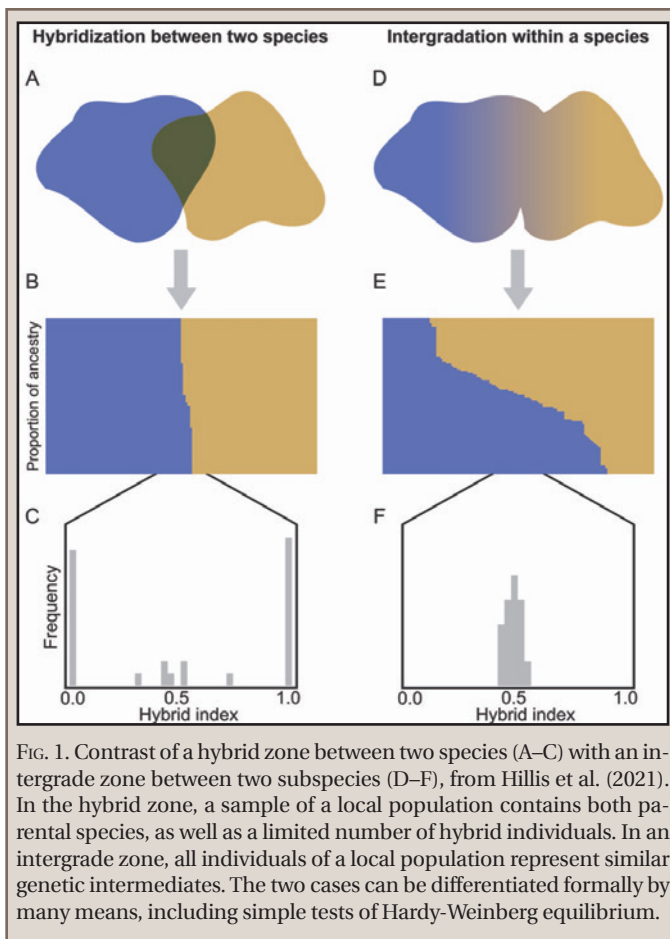


FIG. 1. Contrast of a hybrid zone between two species (A–C) with an intergrade zone between two subspecies (D–F), from Hillis et al. (2021). In the hybrid zone, a sample of a local population contains both parental species, as well as a limited number of hybrid individuals. In an intergrade zone, all individuals of a local population represent similar genetic intermediates. The two cases can be differentiated formally by many means, including simple tests of Hardy-Weinberg equilibrium.

or its extension through time—a genealogical lineage) and phylogenetic relationships (the evolutionary relationships among independently evolving lineages). In practice, how can we identify this boundary, and thus delimit species?

Many species exhibit geographic variation among individuals that are found in different parts of the species range. Thus, the goal of species delimitation comes down to deciding if this variation is associated with some level of reproductive isolation, which is what separates reticulating genealogical relationships from diverging phylogenetic relationships (Hillis et al. 2021; Fig. 1). Evidence of reproductive isolation (as in Fig. 1A–C) can be used to argue that the candidate taxa in question are diverging independently, and hence are separate species. Note that Fig. 1C shows just one way of demonstrating this. There are other ways, such as identifying the specific reproductive isolating mechanisms between the species (Hillis 1981) or showing that the two species are found in sympatry and yet maintain their distinctiveness (Olson 1977). However, biologists need to offer positive evidence that the variation in question is not continuous across a gradient. A continuous gradient (as in Fig. 1D–F) indicates intraspecific (within-lineage), rather than interspecific (between-lineage), variation, as there is clear evidence that the entire continuum is connected through tokogenetic relationships (e.g., represents a single evolving, geographically variable lineage).

If there are no barriers to gene flow (Fig. 1D–F), then there is no reason to posit a break in the genealogical lineage, and the variation is intraspecific. We might still be interested in the distinct parts of the species range and wish to recognize those parts as subspecific taxa (e.g., Hillis 2020), but the subspecies

category would clearly indicate that the taxa in question are part of a larger, reproductively connected lineage that is held together by tokogenetic relationships.

Careful examination and study of contact zones is generally viewed as the critical step in distinguishing cases of intraspecific variation from cases of interspecific divergence (Endler 1977; Chambers and Hillis 2020; Hillis et al. 2021). For example, Burbrink et al. (2020) cited studies by Carneiro et al. (2013), Nosil et al. (2009), and Stankowski et al. (2019), each of which examined contact zones (in a variety of taxonomic groups) that share many similarities to the contact zones between the subspecies of *Pantherophis obsoletus*. Notably, in each of those cited studies, the authors retained the subspecies designations of the respective taxa after analyzing the respective contact zones.

The Taxonomy of the Pantherophis obsoletus Complex.—Hillis and Wüster (2021) recently corrected the nomenclature associated with taxa of the *Pantherophis obsoletus* complex delimited by Burbrink et al. (2020). Burbrink et al. (2021) accepted these corrections and updated the online supplement of Burbrink et al. (2020) to reflect the proposed corrections by Hillis and Wüster (2021). Therefore, there does not seem to be any remaining controversy about which names properly apply to the names of the taxa delimited by Burbrink et al. (2020) if those taxa are recognized as valid.

The subspecies or species status of the taxa within the *Pantherophis obsoletus* complex is still under debate, however. Hillis and Wüster (2021) noted that the continuous and gradual intergradation between most of the taxa recognized by Burbrink et al. (2020), and the lack of any evidence of reproductive or genetic barriers among these taxa, strongly supported the traditional view of intraspecific variation, or subspecies (e.g., as represented in Conant 1975). In particular, the broad intergrade zone between *P. obsoletus alleghaniensis* and *P. obsoletus quadrivittatus* is a classic example of the situation shown in Fig. 1D. In the middle of the intergrade zone, all individuals are a similar mix of the two subspecies, with no evidence of sympatry or isolation of the two parental forms (Figure 2). Burbrink et al. (2020) estimated the median cline width between these taxa at 297 km, although they did not indicate where in the >1,200-km-long intergrade zone, along the Fall Line from Florida to Virginia, this estimate applies. It is highly unlikely that the width of the cline is constant across its length.

Burbrink et al. (2020) also estimated the expected cline width under the assumption of neutrality and argued that if there were no selection for the observed variation, then we might expect the cline to be even wider. They identified nine loci that showed cline widths (in the intergrade zone between *P. o. alleghaniensis* and *P. o. quadrivittatus*) that exhibited narrower clines than predicted under their neutral model. There are two important points to consider about this analysis. First, the expectations of neutrality are highly dependent on model parameters that are unknown or are estimated with little confidence, such as the lifetime dispersal distance of snakes, generation time, and the time of divergence of the taxa. For example, the lifetime dispersal estimate used for the calculation was based on a single study of movements between hibernacula and nest sites in the extreme northern range of *P. obsoletus* (Blouin-Demers and Weatherhead 2002). Second, and more importantly, the test of deviation from neutrality is unrelated to the question of distinguishing interspecific from intraspecific variation. It is well established that selection gradients for different genes often exist across

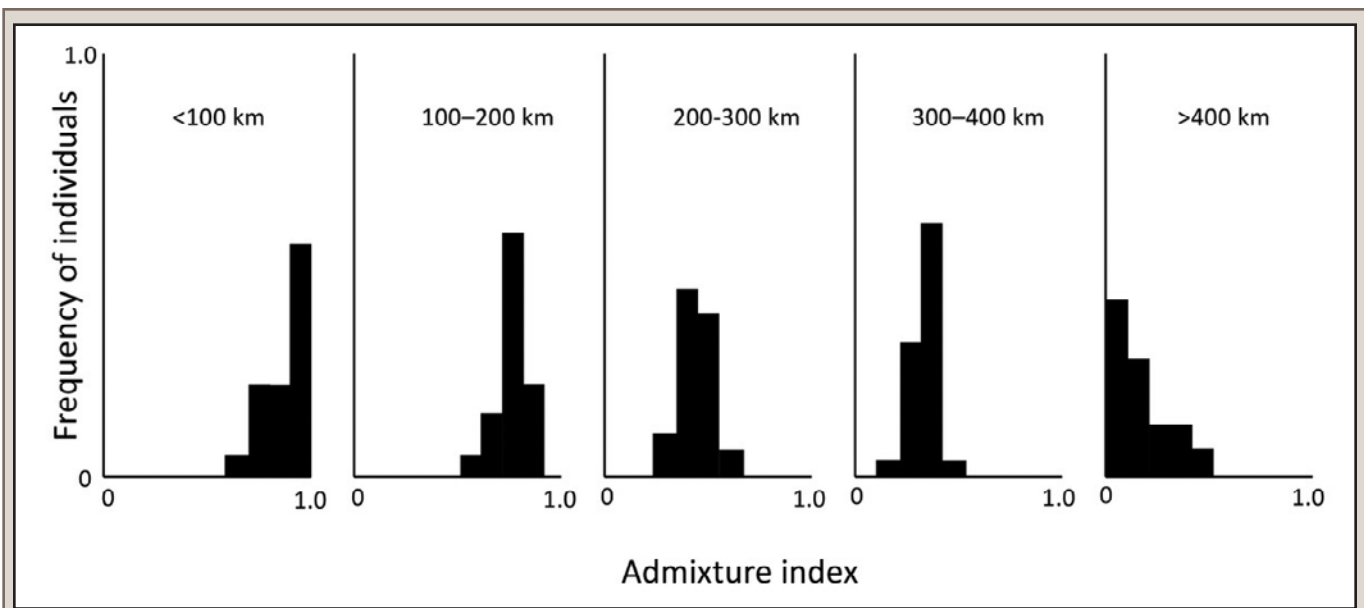


FIG. 2. Frequencies of admixture scores for individuals across the intergrade zone of *Pantherophis obsoletus alleghaniensis* and *P. o. quadrivittatus*, based on data presented in Figure 5 of Burbrink et al. (2020). The five population groups represent 100-km sections of the intergrade zone from west to east, using the distance scale presented by Burbrink et al. (2020). The three central panels (from 100–400 km) represent the identified intergrade zone; note the lack of any parental genotypes in these regions. Admixture scores of 0 represent pure *P. o. quadrivittatus*, and scores of 1.0 represent pure *P. o. alleghaniensis*.

clines within species (e.g., Endler 1977, 1986). There is certainly no reason to expect intraspecific variation to be neutral. Therefore, at best the test suggests that there is selection for the observed clinal intraspecific variation, which is unsurprising for a cline between two subspecies.

In any case, the data of Burbrink et al. (2020) make it clear that *P. o. alleghaniensis* and *P. o. quadrivittatus* gradually and broadly intergrade, in essentially the same pattern that had long been estimated from morphological data. In the middle of the cline between these two taxa, all individuals are of intermediate (mixed) genotypes, and neither parental genotype is present (Fig. 2). The genetic data provide no evidence of any reproductive barriers between the two taxa, and all loci exhibit clinal variation.

Given the broad and gradual intergradation between taxa, what is gained by calling them species rather than subspecies? If they are treated as distinct species, then all snakes within an approximately 300-km-wide zone cannot be identified to species, but rather have to be referred to as “hybrids.” The designation of species also implies that the taxa are independently evolving lineages, not connected by genealogical (parent–offspring) relationships, which is clearly false in this case. In contrast, subspecies designations are always optional, and populations near contact zones are expected to intergrade gradually from one subspecies to another, and to be connected through genealogical relationships of individuals. Thus, subspecies designations are the appropriate way to represent what is known about the biology and evolution of these snakes. Indeed, this appears to be a textbook example of geographic variation within a species.

The contact zone between *P. o. obsoletus* and *P. o. alleghaniensis* is also presented as a gradual cline in Burbrink et al. (2020), although they sampled almost no individuals from the intergrade zone between these taxa. As Burbrink et al. (2020) acknowledged, if large zones of admixture remain unsampled, then estimates of migration will be underestimated and taxon divergence will be overestimated. Despite the lack of sampling through most of this

intergrade zone, Burbrink et al. (2020) nonetheless estimated its width at precisely 147 km. However, they only sampled one pair of snakes of each taxon within 147 km of one another from the entire region north of the mouth of the Mississippi River, across the states of Arkansas, Missouri, Iowa, Minnesota, Wisconsin, Illinois, Kentucky, and Tennessee. Therefore, the extent and nature of most of this intergrade zone is largely unstudied, especially in the region where the two subspecies of Black Ratsnakes are purported to intergrade. Given this lack of sampling, there is no evidence for any reproductive or genetic barrier between these taxa. Without any evidence of reproductive barriers, *P. o. obsoletus* and *P. o. alleghaniensis* are also best viewed as subspecies, if they are recognized as taxa at all.

Although they were not explicitly examined by Burbrink et al. (2020), at least two additional subspecies within *P. obsoletus* are supported by traditional morphological data: *P. obsoletus spiloides* (Gray Ratsnake; the spotted form within southern portion of the range delimited as *alleghaniensis* by Burbrink et al., 2020), and *P. obsoletus lindheimeri* (Texas Ratsnake; the distinctly patterned and colored form within the southern range of western populations of *P. obsoletus*). Based on color patterns, these two subspecies intergrade near the mouth of the Mississippi River, so the 147-km-wide intergrade zone reported by Burbrink et al. (2020) for *P. o. obsoletus* and *P. o. alleghaniensis* apparently applies to the cline between Texas and Gray Ratsnakes. There is not yet sufficient sampling and testing of the other contact zones of these two subspecies to determine their precise taxonomic status, although the morphological and distributional data are consistent with their traditional recognition as additional subspecies within *P. obsoletus*.

On the southwestern end of the range of *P. obsoletus*, the related *P. bairdi* has long been recognized as a distinct species, based on the fact that *P. obsoletus* and *P. bairdi* co-occur in sympatry, and that hybridization between the two species does not result in gradual intergradation and gene flow (Olson 1977;

Vandewege et al. 2012). In other words, there has long been evidence that suggests these two species resemble the case illustrated in Fig. 1A–C.

Burbrink et al. (2021) objected to the recognition of *P. bairdi* and *P. obsoletus* as distinct species (if the latter includes the subspecies *P. o. alleghaniensis* and *P. o. quadrivittatus*, as suggested by Hillis and Wüster 2021) on the basis that this arrangement would render *P. obsoletus* “paraphyletic.” This assertion of paraphyly is based on evidence that at least some genes in *P. bairdi* are more closely related to some genes in western populations of *P. obsoletus*, than they are to some genes in eastern populations of *P. obsoletus*. That is true, but it is also expected, given the geographical distribution of the two species. Indeed, as discussed earlier (and in greater detail by Patton and Smith 1994), paraphyly of genes across species is expected whenever there is a speciation event that involves a wide-ranging species such as *P. obsoletus*. The “cloudogram” of gene trees presented by Burbrink et al. (2020) shows that the only way to avoid paraphyly of genes within species in the group would be to recognize the entire complex, including *P. bairdi*, as a single species, which would then be called *P. obsoletus*. Given the sympatry and evidence of reproductive isolation between *P. bairdi* and *P. obsoletus*, that solution does not seem justified (nor is it necessary).

Nonetheless, Burbrink et al. (2021) expressed concern that *P. obsoletus*, if split from *P. bairdi*, may be considered paraphyletic (based on the paraphyly of some genes, including mtDNA, as well as some hybrid individuals between the two species). This concern seems odd in light of the fact that Burbrink et al.’s preferred solution, of recognizing *P. alleghaniensis* and *P. quadrivittatus* as additional distinct species, creates two additional paraphyletic species (in the same sense that *P. obsoletus* is paraphyletic with respect to *P. bairdi*). Nowhere do Burbrink et al. (2020, 2021) offer evidence of the monophyly of any of their recognized species, and their summary of gene trees shows that these taxa are clearly not monophyletic with respect to the examined genes. Moreover, these taxa are clearly not monophyletic with respect to individuals, either, as Burbrink et al.’s (2020) data show that some individuals they identify as each species (in intergrade zones) are genetically more closely related to individuals of the second taxon involved in the cline, than they are to geographically distant individuals in their own species. Indeed, they split their “species” *P. alleghaniensis* and *P. quadrivittatus* at the point that individuals transition from >50% genes of one taxon to >50% genes of the other taxon, across the broad intergrade zone. Clearly, the individuals identified as each species on either side of this transition point are most closely related to one another by descent, which makes each of the “species” recognized by Burbrink et al. (2021) paraphyletic with respect to both individuals and genes. Therefore, far from “fixing” their stated objection to the paraphyly of *P. obsoletus*, the taxonomy of Burbrink et al. (2021) simply expands the number and extent of paraphyletic taxa.

As noted earlier, paraphyly is a taxonomic concept that is best applied to clades, rather than to species. If it is applied to species, then any degree of hybridization between species will result in paraphyly of species. Moreover, the splitting of lineages (speciation) leads to paraphyly of species, if paraphyly is defined on the basis of either individuals or their constituent genes.

As outlined by Hillis et al. (2021), the problem faced by systematists in species delimitation of widespread taxa is in distinguishing intraspecific from interspecific variation. This requires careful examination of contact zones to distinguish

hybrid zones (where there is evidence of reproductive barriers) from intergrade zones (where there is no evidence of such barriers). Biologists may still argue over the evidence and conclusions from careful analysis of contact zones, but the arguments should at least be based on examination of the relevant evidence and dense sampling of contact zones. Without clear evidence of barriers to gene flow, as in the contact zones of subspecies of *P. obsoletus*, the taxa are most appropriately considered subspecies.

Gibbs et al. (2006) explicitly tested for genetic barriers between the eastern and central mitochondrial lineages identified by Burbrink et al. (2000), and they found no evidence for any barriers to gene flow, or even association between mtDNA haplotypes and nuclear markers. Their findings could not have been more definitive or clear:

“WITHIN THE HYBRID REGION, WE FOUND HIGHLY VARIABLE FREQUENCIES OF mtDNA HAPLOTYPES AMONG ISOLATED SUB-POPULATIONS, NO ASSOCIATION BETWEEN VARIATION IN CYTONUCLEAR (mtDNA) AND NUCLEAR (MICROSATELLITE DNA) MARKERS, NO DIFFERENCE IN SURVIVAL OR REPRODUCTIVE SUCCESS AMONG SNAKES WITH DIFFERENT mtDNA HAPLOTYPES, AND NO EFFECT OF MATE SIMILARITY IN mtDNA ON FEMALE CLUTCH SIZE. THESE RESULTS ARGUE THAT THE EASTERN AND CENTRAL PHYLOGROUPS HAVE MERGED IN THIS REGION, LIKELY DUE TO A LACK OF ADAPTIVE DIFFERENTIATION BETWEEN INDIVIDUALS IN EACH LINEAGE.”

—GIBBS ET AL. 2006. MOL. ECOL. 15:3755.

The data presented by Burbrink et al. (2020) appear to be completely consistent with this conclusion, suggesting that the proposed species split is unjustified. The Gibbs et al. (2006) results also show that the mtDNA lineages have nothing to do with species boundaries in the group, providing further reason for taxonomists to be unconcerned about paraphyly of mtDNA lineages within *P. obsoletus*. It also shows that the mitochondrial-based taxonomy that was assumed and tested by Burbrink et al. (2020) does not correspond to species boundaries.

When Are Taxonomic Changes Warranted?—Burbrink et al. (2021) took my general recommendations regarding nomenclatural changes (Hillis 2019) out of context when they say that I claimed we should “leave nomenclature the #@%& alone!” That statement was the final step in a flow chart that made suggestions about when and if nomenclatural changes were warranted. It was a final step in a series of decisions to keep nomenclature unchanged, when such changes are unwarranted, as well as the final step after actions have been taken to correct nomenclature, when such changes are warranted. The point of the flow chart is that we should not make changes in nomenclature unless they are necessitated by data, and that we should be careful to make as few changes necessary to align nomenclature to the totality of our current understanding—as was done, for example, by recognizing *P. bairdi* as a distinct species. Once nomenclatural problems are fixed, additional nomenclatural changes should not be made unless they, too, are warranted by data. Premature or poorly supported nomenclatural changes should be avoided, especially when they are inconsistent across data sets. This stability of names is important because names of taxa are the way that biologists and the public communicate about biodiversity, and changing names of well-known species and subspecies, without adequate justification, leads to confusion in the literature and in public understanding.

It is instructive, for example, to imagine what changes would have been made in this millennium to the nomenclature surrounding the *P. obsoletus* complex if the recommendations in my flow chart (Hillis 2019) had been followed. What have we learned about the complex, compared to what we knew in the late 20th century? In the 1990s, the complex was viewed as consisting of two species, then called *Elaphe obsoleta* and *Elaphe bairdi*. *Elaphe obsoleta* was considered a polytypic species, meaning that it was known to exhibit considerable geographic variation across its range. Various subspecies were recognized to account for this variation. Wherever two subspecies come into contact, it is clear that they intergrade broadly, so historically there was little debate that the taxa are best called subspecies. In contrast, Olson (1977) documented that *Elaphe obsoleta* and *Elaphe bairdi* occurred sympatrically, and each species maintained its distinctiveness in areas of overlap, so *Elaphe bairdi* was recognized as a distinct species from *E. obsoleta*.

Since then, the widely distributed genus *Elaphe*, as it was applied in the 1990s, has been shown to be polyphyletic (Ultiger et al. 2002). According to my flow chart for nomenclatural change, fixing this problem was an appropriate reason to change the generic name. Thus, the straightforward change to *Pantherophis* for the North American ratsnakes was adopted. However, shortly thereafter, Burbrink and Lawson (2007) proposed synonymizing *Pantherophis* with *Pituophis*, based on weak support from a few regions of mtDNA, and one nuclear gene. Other authors (e.g., Collins and Taggart 2008), based on the same evidence used by Burbrink and Lawson (2007), instead divided *Pantherophis* into multiple smaller genera. Then, Pyron and Burbrink (2009) added additional genes to the analysis, and achieved strong statistical support for a monophyletic *Pantherophis*, and returned the situation to what had initially been proposed by Ultiger et al. (2002). The initial change by Ultiger et al. (2002) was clearly warranted by all analyses, but the subsequent multiple taxonomic changes were unwarranted by the weak evidence presented, and they caused unnecessary taxonomic confusion. This history clearly did not follow the principle of making taxonomic changes only when strongly compelled by evidence, and then to “fix it in the simplest way possible, and then leave it the #@%& alone!” Fortunately, most North American snake systematists have now returned to recognizing *Pantherophis* as a monophyletic genus, so the taxonomic turmoil with regard to the generic names appears to have abated.

In contrast to the now stable generic name, it has been difficult to follow all the proposed name changes for the *P. obsoletus* complex, in various parts of its range, that have been proposed in a series of papers from the Burbrink lab. In just the past two decades, names in some areas have changed from *Elaphe* or *Pituophis* or *Pantherophis o. obsoletus* to *P. spiloides* to *P. alleghaniensis*, whereas in other areas the names have changed from *P. o. quadrivittatus* to *P. alleghaniensis* and then to *P. quadrivittatus*. The proposed boundaries among the recognized species have continued to change, even though there is little sampling (and there are no reported diagnostic characters) to identify where some of these boundaries are, or if they are justified. Have we actually moved forward in how the nomenclature informs the public, or other biologists, about these common snakes? Very little new information has emerged about the limits or interactions of the geographical races of this polytypic species. And yet, the names and proposed distributions have changed multiple times, adding considerable confusion. All of the geographical races of *P. obsoletus* intergrade with one

another over vast areas, so in many places it is impossible to identify a snake in this complex beyond calling it *P. obsoletus*.

Now it appears we are back to where we were in the late 1900s with regard to the species boundaries. There is good evidence to split one species from the rest of the complex: *P. bairdi*, on the basis of its narrow sympatry with *P. obsoletus*, with only rare hybridization (Olson 1977; Vandewege et al. 2012). The genetic data collected over the past twenty years are all consistent with the idea that the rest of *P. obsoletus* is a polytypic species, with several distinct subspecies that broadly intergrade over large areas of contact. The genetic data suggest an additional split of the former subspecies *P. o. obsoletus* into two areas, which could be called *P. o. obsoletus* (the Western Black Ratsnake), and *P. o. alleghaniensis* (the Eastern Black Ratsnake). Exactly where these two forms intergrade, and how they might be distinguished without extensive gene sequencing, have not yet been clarified. Previous morphometric assessments were based on the mitochondrial groupings, which do not correspond closely to the more recent divisions of the taxa by Burbrink et al. (2020) based on nuclear genes. According to the molecular data, the morphologically distinctive subspecies *P. o. quadrivittatus* (Yellow Ratsnake) is distributed where it was always thought to occur, on the southeastern Coastal Plain, and it broadly intergrades with *P. o. alleghaniensis* along the Fall Line, consistent with the geographic distribution of color patterns. There has not yet been an explicit analysis of the contact zone between *P. o. lindheimeri* (Texas Ratsnake) and *P. o. obsoletus*, but the morphology suggests that these subspecies intergrade near the Texas/Oklahoma border. Likewise, the intergrade zones between *P. o. spiloides* (Gray Ratsnake) and the other subspecies of *P. obsoletus* require additional study to determine their exact location and the extent of intergradation. One or more additional subspecies with limited distributions (e.g., *P. o. williamsi*, Gulf Hammock Ratsnake) may warrant recognition as well, but sampling to date has not been sufficient to evaluate their taxonomic status. Therefore, there is considerable work to be done to understand the geographic variation in *P. obsoletus* and the nature and interactions of its various subspecies. This suggests a wealth of future studies that would help illuminate the taxonomy of the group.

Far from being a “straightjacket” for future nomenclatural and taxonomic studies, my recommendations (Hillis 2019) provide a roadmap for the return to careful systematic analyses of species delimitation and geographic variation. Those recommendations also encourage taxonomic stability, in recognition of the diverse community of users served by systematics. As Hillis et al. (2021) made clear, the evidence needed to propose taxonomic and nomenclatural changes will differ greatly in different regions, depending on how well they are studied and what is known about the fauna. In regions where we are still in the age of initial discovery of new taxa, the data needed to propose new species are quite different than they are for one of the most common and well-known snakes in North America (*P. obsoletus*). In the latter case, we should expect careful analysis and sampling of contact zones, integration of information across multiple data sets, and clear evidence of reproductive barriers before elevating subspecies to species status.

Acknowledgements.—I thank E. Anne Chambers, Craig Guyer, Andrew Holycross, Harry Greene, Kenneth Wray, Brian Sullivan, and Wolfgang Wüster for discussions and comments about species, paralogy, and the *Pantherophis obsoletus* complex.

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