



# Acknowledging more biodiversity without more species

Christophe Dufresnes<sup>a,1</sup> , Nikolay Poyarkov<sup>b,c</sup> , and Daniel Jablonski<sup>d</sup> 

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Delimiting and naming biodiversity is a vital step toward wildlife conservation and research. However, species delimitation must be consistent across biota so that the limited resources available for nature protection can be spent effectively and objectively. To date, newly discovered lineages typically are either left undescribed and thus remain unprotected or are being erroneously proposed as new species despite mixed evidence for completed speciation, in turn contributing to the emerging problem of taxonomic inflation. Inspired by recent conceptual and methodological progress, we propose a standardized workflow for species delimitation that combines phylogenetic and hybrid zone analyses of genomic datasets ("genomic taxonomy"), in which phylogeographic lineages that do not freely admix are ranked as species, while those that have remained fully genetically compatible are ranked as subspecies. In both cases, we encourage their formal taxonomic naming, diagnosis, and description to promote social awareness toward biodiversity. The use of loci throughout the genome overcomes the unreliability of widely used barcoding genes when phylogeographic patterns are complex, while the evaluation of divergence and reproductive isolation unifies the long-opposed concepts of lineage species and biological species. We suggest that a shift in conservation assessments from a single level (species) toward a two-level hierarchy (species and subspecies) will lead to a more balanced perception of biodiversity in which both intraspecific and interspecific diversity are valued and more adequately protected.

conservation | genomics | hybrid zones

Earth's biodiversity remains largely under-appreciated (1), including a large proportion of species made of multiple phylogeographic lineages, i.e., geographically and genetically identifiable population lineages within widespread species (2–4). Genetic diversity contributes to the evolutionary capacity of species to respond to the challenges posed by changing environments and thus holds the key to their survival. Intraspecific phylogeographic lineages are evolutionary solutions in the making and should thus be preserved to ensure effective conservation of the relevant species. Even ephemeral lineages play an important role in generating, sharing, and recombining new adaptive alleles through hybridization and fusion (5). Gene flow between phylogeographic lineages is thus an essential aspect of species diversification (6). For example, by bolstering genetic variability (7, 8), gene flow can increase the overall fitness of populations at risk from climate change (9). Reciprocally, genetically impoverished taxa are the ones most under threat of extinction (10).

Preserving biodiversity in all its complexity thus implies protecting phylogeographic lineages independently. The

delimitation of these lineages and society's appreciation of their value both require rigor and standardization for their conservation to be meaningful (11). At present, this is hampered by theoretical discord in the way these lineages are treated in taxonomy and protected with conservation policies. Here, we address the main issues with current practices and argue that phylogeographic lineages should be acknowledged as taxonomic entities to promote their conservation. To this end, we propose an operational workflow to rank them as species or subspecies using genomic methods, notably through the implementation of an emerging approach of species delimitation.

## Taxonomic Neglect Hampers Conservation

Evolutionary biologists have defined intraspecific genetic lineages as "units", the most famous definitions being "Evolutionary Significant Units" (12), "Evolutionary Populations" (13), or "candidate species" (2), which identify unique sets of populations using population genetic (demographic) or phylogenetic criteria. These concepts are useful to formalize the genetic structuring of species complexes and for the discussion of evolutionary hypotheses, but they raise little social awareness and conservation attention (4). Technically labeled lineages (e.g., "clade B", "North lineage") are rarely implemented in international conservation policies, taxonomist lists, and biodiversity accounts that rely upon them (14). This includes the International Union for Conservation of Nature Red List or any naturalist list and resources such as checklists or citizen science databases.

At the national level, most jurisdictions in Europe and Asia, as well as in the United States (e.g., Endangered Species Act, ESA) consider only described taxa for red list assessments. Unnamed lineages are sometimes eligible for red-listing (e.g., differentiated populations in Australia and Russia; Designatable Units in Canada), and in endangered species, coordinated management plans may define ESU-like units (e.g., National Action Plans in France) or make proposals to legally

Author affiliations: <sup>a</sup>Laboratory of Amphibian Systematics and Evolutionary Research, College of Biology and Environment, Nanjing Forestry University, Nanjing 210037, People's Republic of China; <sup>b</sup>Joint Russian-Vietnamese Tropical Research and Technological Center, Hanoi 122000, Vietnam; <sup>c</sup>Department of Vertebrate Zoology, Lomonosov Moscow State University, Moscow 119234, Russia; and <sup>d</sup>Department of Zoology, Comenius University in Bratislava, Bratislava 84215, Slovakia

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<sup>1</sup>To whom correspondence may be addressed. Email: Christophe.Dufresnes@hotmail.fr.

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protect specific populations (e.g., Spain, Greece). In practice, however, these options are sparingly applied between countries, organisms, and operators, and they do not affect unthreatened species—declining lineages will thus remain neglected if other lineages of the same species thrive in parallel and the species is of least concern overall (15). Moreover, when evolutionary units elude legislation, they may cause conservation paradoxes (16). For example, invasive intraspecific lineages are legally protected in countries where native lineages of the same taxon also occur, since both have the same name and conservation status (17).

The absence of taxonomic recognition thus hampers conservation, and unnamed lineages face higher risks of extinction compared to described taxa (18, 19).

## Should All Lineages Be Species?

While evolutionary biologists tend to leave phylogeographic lineages unnamed, taxonomists tend to oversplit them into multiple species (20, 21). In principle, the scientific community largely agrees that species correspond to diverging populations that eventually become biologically isolated (the endpoint of speciation) (22). However, disagreements remain around how to delimit them in practice, i.e., whether to put the emphasis on divergence and evolutionary independence (the lineage species concept, ref. 23), or the process that leads to it, i.e., reproductive isolation (the biological species concept, ref. 24). Diagnosability, i.e., setting up applicable criteria of identification and delimitation, is central to the practice of taxonomy, and because reproductive isolation is hard to assess, most candidate species are preferentially characterized based on their molecular or phenotypic divergence, combining genetic, morphological, behavioral, and ecological evidence (e.g., integrative taxonomy, ref. 25). The overreliance on molecular analyses to define species has progressively led to consider any diagnosable phylogeographic lineage as a “species under construction”, and by extension, to their recognition as species (26). This shift of paradigm now reaches its climax, with the species rank being frequently claimed to be the only relevant terminal rank in alpha-taxonomy (27).

The “one lineage = one species” ideology has triggered a surge of species descriptions in recent years, in turn contributing to the emerging problem of taxonomic inflation (28–30). Taxonomic inflation is counter-productive to the sustainability of newly split species. Oversplitting evolutionary ephemeris discredits taxonomy, Red List assessments, and can erode the limited trust the general public has in science (31). It also diverts inherently limited conservation resources and induces detrimental biases in conservation practices, for instance by isolating conspecific populations in captive breeding or in meta-population managements (32). In addition, oversplitting favors “taxonomic vandalism”, when unscrupulous authors describe species for their own posterity without substantial evidence for speciation (33).

Besides theoretical considerations, describing new species from genetic and/or phenotypic data presents methodological shortcomings that are not sufficiently appreciated (34, 35). Single gene evidence, especially fragments of mitochondrial DNA (mtDNA), are often used to suggest that a particular population or range segment is distinct from a well-known,

wide-range species. This is simply not enough information (36), especially given the unreliability of mtDNA in phylogeography due to cytonuclear discordance (37–39) (Box 1). For example, so-called “ghost” mitochondrial lineages, which do not exist in the nuclear genes, are sometimes wrongly associated with taxa (e.g., ref. 40). In parallel, widely used species delimitation algorithms (such as the multispecies coalescent) might statistically define every population as a distinct “species” provided sufficient genetic resolution, even those that clearly lack reproductive isolation (41–43). Furthermore, morphological or ecological differences may reflect processes other than evolutionary divergence and speciation, as they are greatly influenced by phenotypic plasticity and local adaptation (44, 45). The reported differences may not even be meaningful. Taxonomic studies frequently identify candidate taxa by overemphasizing statistically significant but nondiagnostic phenotypic differences, or alternatively, diagnostic differences that are based on very small samples that hardly represent the whole intragroup variation (46, 47)—and in many cases, involving traits that do not convey reproductive isolation.

If species are necessarily lineages, lineages are not necessarily species. Defining all the terminal branches of phylogenetic trees as species is thus either a methodological gamble or a subjective overestimation, and many new “species” will eventually require a reevaluation, causing taxonomic instability.

## Unifying Lineage and Biological Species with Genomic Taxonomy

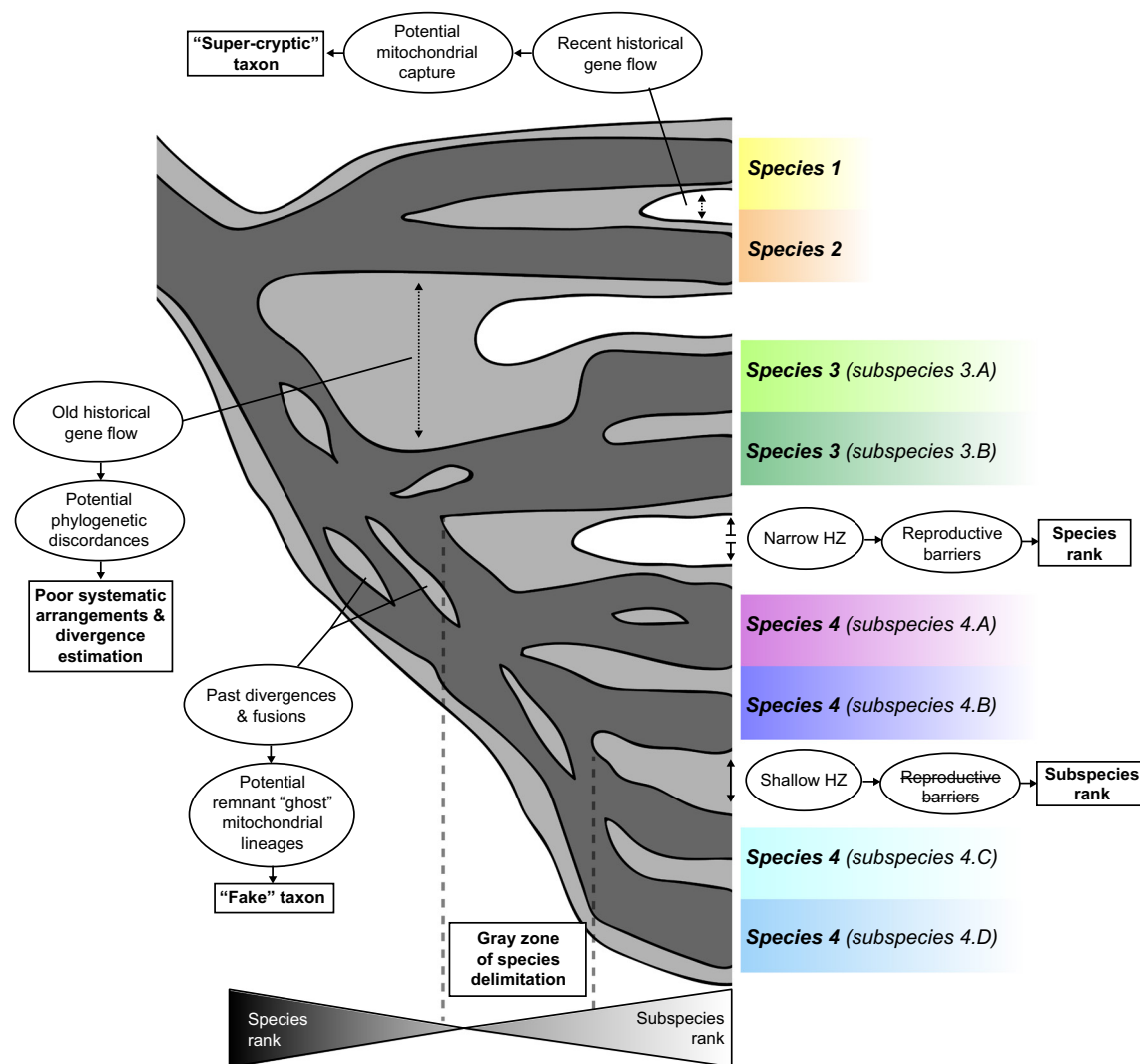
The many species definitions have been channeled into unified concepts and operating workflow that can be effectively applied in taxonomic practices (22, 25, 49–53). Further advances may come from insights into the speciation process itself. Recent research on species complexes has suggested that reproductive isolation often evolves alongside molecular divergence, as genomes gradually become incompatible (the “mass of genes” model) and lineages adapt locally (54, 55). Once postzygotic barriers have become sufficient, lineages no longer merge in the face of hybridization and continue to diverge until complete reproductive isolation is reached (56, 57). The formation of new species thus relates to the formation of lineages and clades, i.e., the two major kinds of biological entities treated in taxonomy. Lineages, i.e., genealogically connected organisms, keep reticulate relationships due to reproductive connections and gene flow. As reproductive barriers arise and gene flow ceases, lineages begin to evolve independently of one another and their relationships bifurcate; these largest lineages are what we call species (23, 58–60). As species lineages become entirely disconnected, they continue to evolve into distinct clades, i.e., ancestral lineages and all their descendants, which are defined as higher taxa (61). The dividing line for species thus lies at the interplay between lineages and clades (61), which corresponds to the boundary between population genetics and phylogenetics, respectively (60).

Though disagreement between their practitioners has fueled the so-called “clado-wars” (62), the competing views of biological vs lineage species thus often converge toward the same taxonomic conclusions. Besides, both face the same dilemma: how to translate the continuous nature of speciation

## Box 1. Phylogeographic patterns and their interpretation in species delimitation

Phylogeographic lineages (dark gray areas) retain mostly reticulate relationships (light gray areas) until reproductive barriers restrict gene flow (white areas). Patterns of introgression across hybrid zones (HZ), which reflect the strength of reproductive barriers, allow delimiting lineages as species (characterized by narrow HZ) or subspecies (characterized by shallow HZ). Their corresponding divergences in turn help defining the “gray zone of species delimitation” and eventually predict the status of lineages for which no hybrid zone exists.

The hybrid zone approach of species delimitation is best implemented from genomic phylogeography, notably in the cases of cytonuclear discordance. Relying on mitochondrial DNA may then lead to the misidentification of lineages and to the misestimation of their relative divergence. Among extreme cases, cryptic lineages that lack a mitochondrial identity due to recent hybridization and mitochondrial capture may be overlooked (“super-cryptic” taxon) (48). On the other hand, genetic traces of past events of divergence may still persist in the mitochondrion (“ghost” lineages) even though they no longer correspond to any extant species or subspecies (40).



and of its two central aspects (reproductive isolation and divergence) into a dichotomous classification (species vs nonspecies)? The “gray zone” of speciation (63) de facto implies a “gray zone” of species delimitation: For occasionally hybridizing and/or moderately diverged lineages, species hypotheses will differ depending on given criteria and thresholds. The “relativity of species” (52) has more recently inspired a probabilistic approach of species delimitation (64). In principle, speciation

probabilities could be empirically calculated by testing reproductive isolation (e.g., absent or restricted gene flow, assortative mating) with respect to evolutionary divergence (e.g., divergence time or % of substitution at barcoding genes), which requires comparative analyses across multiple pairs of candidate species.

Hybrid zone analyses offer ad hoc opportunities for such assessments and are increasingly studied in the context of



species delimitation (43, 65, 66) (Box 1). Extensive introgression across secondary contact zones implies overwhelming hybridization and compatibility between the admixing genomes, hence few reproductive barriers—the corresponding lineages are conspecific. In contrast, null or reduced introgression, with respect to the capabilities and opportunities for dispersal, suggests prezygotic (absence of hybrids) and/or postzygotic (genomic incompatibilities) isolation—the corresponding lineages represent incipient species. Empirical studies in amphibians showed that the extent of admixture in hybrid zones (the hybrid zone width) broadly correlates with molecular divergence and notably divergence time in time-calibrated phylogenies (55). Theoretically, the relationship may thus serve to probabilistically predict the status of lineages based only on divergence—for instance, when no contact exists (allopatric lineages, ref. 67; see Box 2). This emerging “hybrid zone approach of species delimitation” thus integrates diagnosable criteria pertaining to both the biological and lineage species concepts and is consequently gaining momentum with taxonomic authorities (e.g., ref. 68).

By providing information on thousands of genetic markers in nonmodel organisms, high-throughput sequencing technologies are revolutionizing species delimitation, making assessments more accessible and more accurate (34, 43, 65). Phylogenomics alleviates the shortcomings of single-gene trees in detecting and measuring the divergence of lineages, accounting for their genome-wide differentiation. Hybrid zone genomics reliably quantifies gene flow and illuminates both the geographic and genomic landscapes of introgression (55) even with modest population sample sizes (69). “Genomic taxonomy”, i.e., taxonomy based on genomic data (a term initially coined for prokaryotes, ref. 70) thus allows researchers to implement more integrative approaches of species delimitation that shall yield better accepted and more stable taxonomic arrangements.

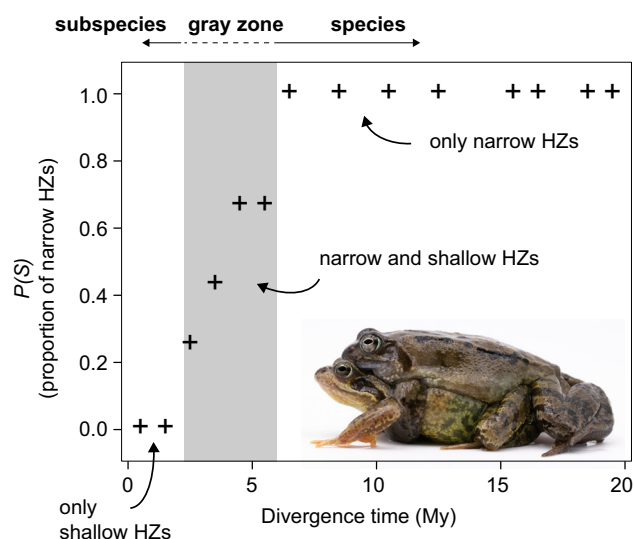
## Divide and Preserve with Subspecies

While the largest lineages can unequivocally be described as species, what to do with their conspecific sublineages (i.e., lineages within a species)? Given the importance of phylogeographic diversity for conservation, we advocate for their taxonomic recognition as well, just not as species. Although recently becoming unpopular due to its numerous conflicting definitions (27, 71), the subspecies rank—the only taxonomic rank below the species category recognized by the International Code of Zoological Nomenclature—offers a much-needed compromise to name lineages for which the speciation process is still incomplete (59, 62, 72–77).

Subspecies were initially intended to acknowledge the geographic variation of polymorphic species, in terms of ecology, behavior, coloration, or morphology, with the notion of intergradation at geographic boundaries (78). The category was eventually abused to name biologically trivial entities with primitive taxonomic methods (79), even promoting racism in humans (80). Its definition then evolved to incorporate molecular differentiation, which has been conceptualized as “phylogeographic subspecies” (*sensu* O'Brien & Mayr, ref. 81). Many historical subspecies were subsequently abandoned because they were not genetically divergent

### Box 2. Speciation probabilities $P(S)$ to guide species delimitation in Palearctic anuran amphibians. Adapted from ref. 55

When hybrid zones cannot be studied, lineages can still be delimited using divergence as a proxy given the link between divergence and hybrid zone (HZ) steepness. In practice, we can compute the probability  $P(S)$  for a lineage to have become a distinct species according to the proportion of narrow HZs (i.e., maintained by reproductive isolation) per unit of divergence. In Palearctic anurans, empirical data show that lineages younger than 2My only form shallow HZs, so  $P(S) = 0$ , and all are ranked as subspecies; lineages older than 6My always form narrow HZs, so  $P(S) = 1$ , and all are ranked as species. Lineages in between alternatively form narrow or shallow HZs (“gray zone”). Note that  $P(S)$  must be computed independently between clades, i.e., here the chart is relevant to Palearctic anurans only. Comparative studies suggest that the gray zone is better defined from genome-average measures of divergence or their derivatives (e.g., divergence time) rather than divergence at single barcoding genes (67). Photo: R. Schmidt.



(74, 82), e.g., two-thirds of the >170 subspecies listed on the US ESA do not have a clear molecular identity (79). Conversely, phylogeographic lineages can be millions of years old while lacking obvious external differentiation (“cryptic” lineages), although careful examinations often flag diagnostic characters. Hence, these are rarely considered eligible for the subspecies category.

Subspecies are convenient for designating geographically differentiated lineages that retain continuous reproductive connections, even without clear external differences (77, 78). Therefore, we argue that the concept of “phylogeographic subspecies” should be extended to any divergent phylogeographic sublineages, including (presumably) cryptic ones. Subspecies may then often correspond to ESUs (75). However, contrary to the operational criteria defined by Moritz (12), wherein ESUs require two lines of evidence for

differentiation (monophyly for mtDNA alleles and allele frequency divergence at nuclear loci), genome-wide divergence alone should ultimately suffice. Notably, this would also account for lineages that experienced mitochondrial capture and lineages of hybrid origin—two situations more pervasive than appreciated (39, 83). To date, ESU-like phylogeographic subspecies already exist, as explicitly proposed for tigers (84) and grass snakes (75), or implicitly adopted in many terrestrial vertebrates (e.g., refs. 48 and 85–88).

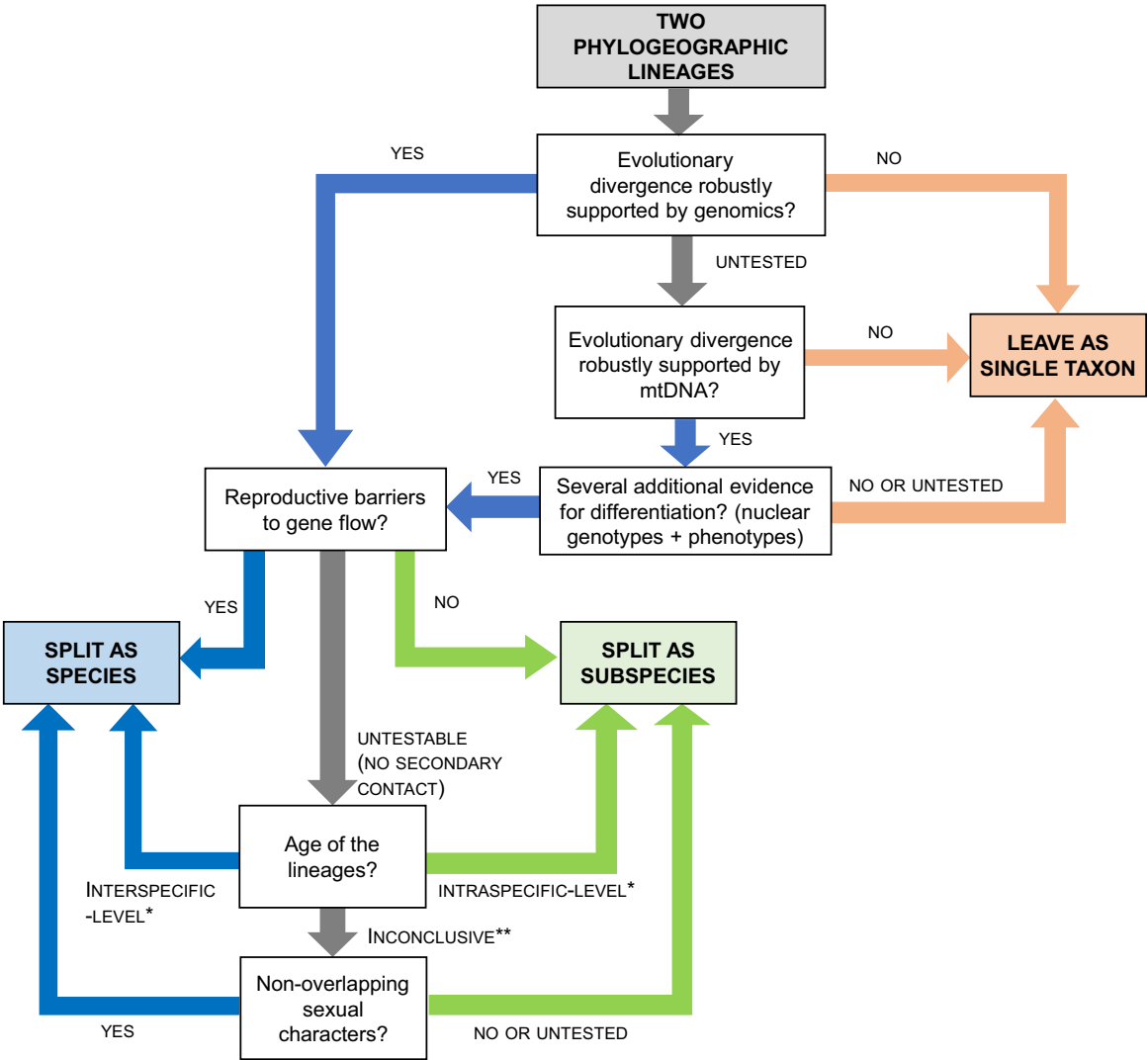
In practical terms, deciding the species and subspecies rank may thus rely on direct (hybrid zone width) or indirect (the corresponding genetic divergence) measures of genetic compatibility. Updated taxonomies may be enforced by new descriptions, by resurrecting available historical nomina, or

by revising existing arrangements to raise or sink currently recognized subspecies and species. Finally, taxa that do not represent distinct evolutionary lineages (i.e., no genetic divergence) should be synonymized, i.e., kept under the same name according to the nomenclatural rules of priority. Box 3 provides a practical workflow to apply this approach with respect to the available evidence.

Subspecies are increasingly represented in national red lists (89, 90), although they are exceptions, especially in Asia (e.g., China, Vietnam, Thailand). Characterizing the intraspecific variation of endangered species into consistent taxonomic units is the premise of successful recovery plans (91, 92) as it contributes to evidence-based policy decisions, e.g., when codifying translocation actions, fighting illegal trafficking (93),

### Box 3. Operational workflow to delimit phylogeographic lineages into species and subspecies

The workflow begins by investigating the evolutionary significance of lineages and then deals with ranking them as species (blue) or subspecies (green).

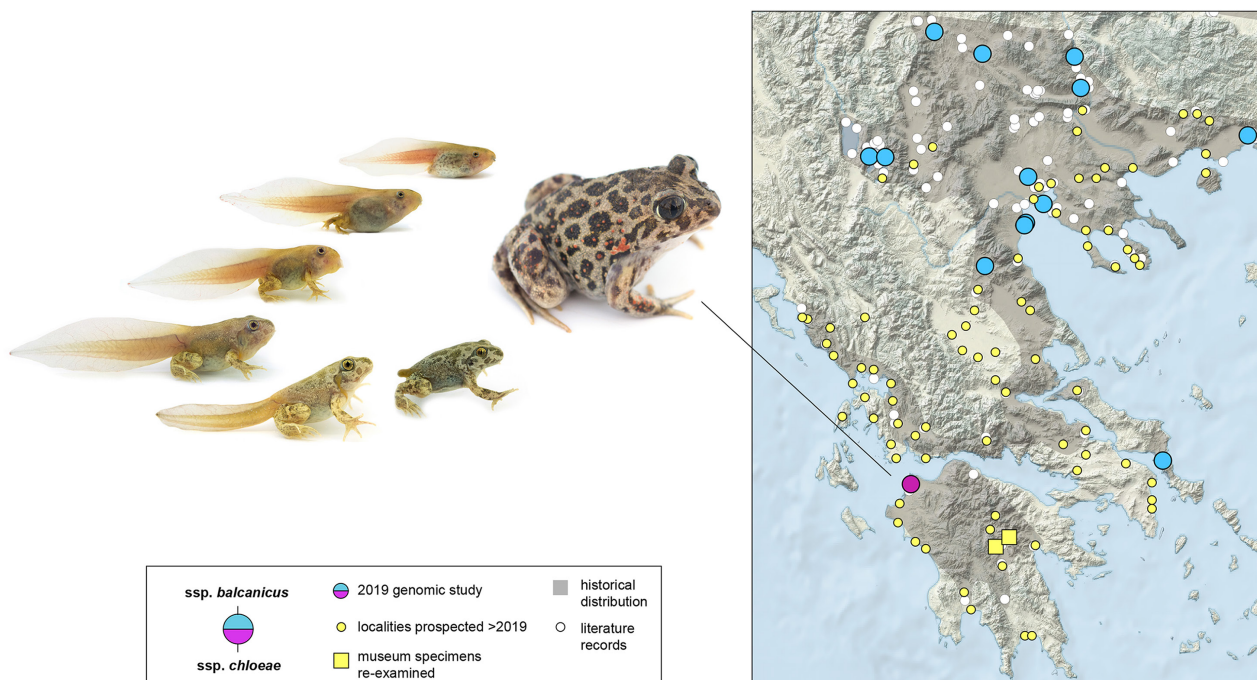


\*as inferred from the age of confirmed species and subspecies  
 \*\* the relationship between divergence and reproductive isolation could not be established

#### Box 4. How the description of phylogeographic subspecies may spark conservation interests: the case of Greek spadefoot toads

The Balkan spadefoot toad (*Pelobates balcanicus*) is a widespread amphibian from southeastern Europe. In Greece, populations have been declining for decades, but they received no specific attention (the last detailed account dates back to the 1980s) because the species is not facing an immediate risk of extinction due to its relatively large range at the global scale.

Despite little resources for zoological research and few active naturalists in Greece, the genomic identification and taxonomic description of a microendemic subspecies in 2019 (*P. b. chloeeae*) (88) have motivated a nationwide prospection effort (yellow dots on the map) combined with a conservation genetics project piloted by a Greek PhD student (I. Strachinis) since 2020. New populations were discovered, museum specimens from extinct populations were examined (yellow squares), and local amateur herpetologists have started to document life history and population trends, e.g., the photomontage depicting larval development is the work of E. Tzoras, a Peloponnese resident. This new knowledge will be the touchstone of the species' conservation in Greece in the years to come.



and managing biological invasions (17). The description of new phylogeographic subspecies can also breathe life into the conservation of otherwise neglected declining populations, for instance by bringing forward the uniqueness of regional biodiversity in an intelligible way (75). Box 4 provides an empirical example of an endemic amphibian from the Balkans.

Using the subspecies category to recognize intraspecific lineages thus kills three birds with one stone. First, it attracts much-needed attention for their independent conservation by offering them a universal designation (e.g., a taxonomic name) that shall facilitate legislation and management and raise awareness among the general public. Second, it helps mitigate the species-level taxonomic inflation by offering an alternative to naming all lineages as species. Third, it circumvents the controversy of phenotypic subspecies (79), which may be considered invalid or referred to as morphotypes or ecotypes by interested parties.

#### General Recommendations

Genomic taxonomy should be implemented with care (53). When screening for candidate lineages for taxonomic recognition (first part of our workflow, Box 3), common pitfalls must be avoided, such as including hybrids in phylogenomic analyses (53, 83), or confounding phylogeographic breaks with isolation-by-distance (94) and recent population structure (67). As emphasized for ESUs, identifying subspecies should consider the evolutionary pattern rather than a given extent of divergence (12, 95), since the latter is dataset-dependent. For instance, instead of applying a fixed threshold of minimal divergence, algorithms that partition phylogenetic trees into groups of genetically similar individuals (e.g., the multispecies coalescent, ref. 96; ASAP, ref. 97) could be used to define divergent lineages. When ranking those lineages with the hybrid zone approach (second part of our workflow, Box 3), one should keep in mind that



introgression patterns do not only reflect selection against hybrids. For the same level of reproductive isolation, the structure of hybrid zone may vary due to demographic factors such as dispersal opportunities (98), the history of range shifts (99), or the age since first contact (100). This appears to affect young incipient species more than old ones, however, for which strong hybrid incompatibilities always warrant restricted gene flow and thus steep hybrid zones (101).

The tempo and mode of speciation are expected to differ between and within animal clades (102). Thus, speciation thresholds and probabilities taken from the hybrid zone literature may not be portable to unrelated organisms. Instead, the gray zone of speciation must be evaluated independently for each focal group (Box 2) and rely on age estimates obtained from the same (or at least comparable) time-calibrated phylogenies, as it could otherwise result in drastically different predictions of the gray zone (see, e.g., refs. 103, 104 in slow worms). Importantly, divergence can only be a reliably proxy if reproductive isolation evolves through the gradual accumulation of molecular differences between diverging lineages (the “mass of genes” model, ref. 55). If speciation relies on behavioral or ecological cues without extensive molecular divergence (e.g., rapid adaptive radiation or ecological speciation, ref. 105), it might be more relevant to quantify differentiation in the signals themselves (106). In any case, when newly documented lineages cannot be reliably ranked, a conservative approach to initially consider them as subspecies would help in maintaining taxonomic stability. Finally, speciation is not always gradual and there will be situations where older lineages appear reproductively more compatible than younger ones. Applying the hybrid zone approach may thus result in delimiting “paraphyletic species”, a conundrum that divides taxonomists (107). However, the issue of paraphyletic vs monophyletic species is irrelevant if species boundaries are defined by reproductive connections (population genetics) rather than phylogenetics (61, 108).

The safeguarding of wildlife relies on the inherently limited protection and management resources being spent effectively and objectively. Channeling those resources for defensible endangered taxa would gain from more consistency in the way intra- and interspecific diversity is delimited within and between organismal groups (109). Today, species are the main currency to define biodiversity in existing legislation and thus remain more popular among scientists (e.g., in the ESA). As a result, conservation issues sometimes bias taxonomic assessments. For instance, some endangered conspecific lineages are erected as separate species (e.g., ref. 67), and some subspecies are deliberately maintained despite

evidence for synonymy (e.g., ref. 110), all to increase their value. Instead, we would rather encourage legislators to improve existing policies, so subspecies are treated as independent conservation units worldwide.

Taxonomic proposals are hypotheses about the biological boundaries of species, and ultimately, it is up to the wider taxonomic community to test, and thereby accept, or refute these hypotheses (111). In principle, taxonomic committees offer the opportunity to reevaluate controversial species and subspecies (i.e., previously delimited based on unconvincing data), but these reevaluations must be objective and based strictly on the scientific evidence and nomenclatural rules (68, 106, 112). In practice, however, taxonomic authorities can be contentious due to the social conventions, conservation priorities, and operational criteria they adhere to (113). In this fractured context, our workflow should contribute to science-based taxonomy and thus more stable taxon boundaries, which are both necessary for global conservation (114, 115).

Conservation strategies should be directed toward maintaining the process of evolution, not just preserving its products we perceive as final, i.e., species (116). The best way to protect biodiversity would be to enforce a hierarchical (species > subspecies > populations) rather than a species-oriented binary (species ≠ non-species) system of conservation assessment, using recognized taxonomic categories rather than creating new terminologies. From a geographic perspective, protecting areas that contain high intraspecific genetic diversity, not just high species richness (e.g., ref. 117), must become an important component in evaluating biodiversity hotspots (118, 119). Genomic taxonomy now provides the means to acknowledge more biodiversity without more species, so researchers and conservationists are better armed to unveil those species’ secrets and plan their survival.

**Data, Materials, and Software Availability.** There are no data underlying this work.

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1. C. Mora *et al.*, How many species are there on Earth and in the Ocean. *PLoS Biol.* **9**, e1001127 (2011).
2. D. R. Vieites *et al.*, Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 8267–8272 (2009).
3. C. E. Hinchliff, S. A. Smith, J. F. Allman, K. A. Cranston, Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 12767–12769 (2015).
4. J. V. Freudenstein, M. B. Broe, R. A. Folk, B. T. Sinn, Biodiversity and the species concept – Lineages are not enough. *Syst. Biol.* **66**, 644–656 (2017).
5. R. C. Garrick *et al.*, Extending phylogeography to account for lineage fusion. *J. Biogeogr.* **46**, 268–278 (2019).
6. S. H. Martin *et al.*, Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Res.* **23**, 1817–1828 (2013).
7. T. Schmitt, G. M. Hewitt, The genetic pattern of population threat and loss: A case study of butterflies. *Mol. Ecol.* **13**, 21–31 (2004).
8. R. Frankham, Genetics and extinction. *Biol. Conserv.* **125**, 131–140 (2005).
9. J. P. Sexton, S. Y. Strauss, K. J. Rice, Gene flow increases fitness at the warm edge of a species' range. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11704–11709 (2011).
10. D. Spielman, B. W. Brook, R. Frankham, Most species are not driven to extinction before genetic factors impact them. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15261–15264 (2004).
11. S. T. Garnett, L. Christidis, Taxonomy anarchy hampers conservation. *Nature* **546**, 25–27 (2017).
12. C. Moritz, Defining 'Evolutionarily Significant Units' for conservation. *Trends Ecol. Evol.* **9**, 373–375 (1994).
13. R. S. Waples, O. Gaggiotti, What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.* **15**, 1419–1439 (2006).
14. S. Hoban *et al.*, Global commitments to conserving and monitoring genetic diversity are now necessary and feasible. *Bioscience* **71**, 964–976 (2021).

15. M. Kuijt *et al.*, The introduction of three cryptic tree frog species in the Dutch coastal dunes challenges conservation paradigms. *Amphib. Reptil.* **44**, 1–10 (2023).
16. M. P. Marchetti, T. Engstrom, The conservation paradox of endangered and invasive species. *Conserv. Biol.* **30**, 434–437 (2016).
17. S. Koster *et al.*, The conservation paradox of an introduced population of a threatened species: Spadefoot toads in the coastal dunes of the Netherlands. *Amphib. Reptil.* **44**, 11–18 (2023).
18. L. J. Pollock *et al.*, Protecting biodiversity (in all its complexity): New models and methods. *Trends Ecol. Evol.* **35**, 1119–1128 (2020).
19. J. Liu, F. Slik, S. Zheng, D. B. Lindenmayer, Undescribed species have higher extinction risk than known species. *Conserv. Lett.* **15**, e12876 (2022).
20. N. J. B. Isaac, J. Mallet, G. M. Mace, Taxonomic inflation: Its influence on macroecology and conservation. *Trends Ecol. Evol.* **19**, 464–469 (2004).
21. P.-M. Agapow *et al.*, The impact of species concept on biodiversity studies. *Q. Rev. Biol.* **79**, 161–179 (2004).
22. S. Stankowski, M. Ravnin, Defining the speciation continuum. *Evolution* **75**, 1256–1273 (2021).
23. K. de Queiroz, "The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations" in *Endless Forms: Species and Speciation*, D. J. Howard, S. H. Berlocher, Eds. (Oxford University Press, 1998), pp. 57–75.
24. E. Mayr, *Systematics and the Origin of Species* (Columbia University Press, 1942).
25. J. M. Padial, A. Miralles, I. De la Riva, M. Vences, The integrative future of taxonomy. *Front. Zool.* **7**, 16 (2010).
26. J. Cracraft, "Speciation and its ontology: The empirical consequences of alternative species concepts for understanding patterns and processes of differentiation" in *Speciation and Its Consequences*, D. Otte, J. A. Endler, Eds. (Sinauer Associates, 1989), pp. 28–59.
27. F. T. Burbink *et al.*, Empirical and philosophical problems with the subspecies rank. *Ecol. Evol.* **12**, e9069 (2022).
28. A. Miralles, M. Vences, New metrics for comparison of taxonomies reveal striking discrepancies among species delimitation methods in *Madascincus* lizards. *PLoS One* **8**, e68242 (2013).
29. F. E. Zachos, Taxonomic inflation, the phylogenetic species concept and lineages in the tree of life – a cautionary comment on species splitting. *J. Zool. Syst. Evol. Res.* **53**, 180–184 (2015).
30. P. Uetz, A. Stylianou, The original descriptions of reptiles and their subspecies. *Zootaxa* **4375**, 257–264 (2018).
31. W. Denzer, H. Kaiser, Naming and gaming: The illicit taxonomic practice of 'nomenclatural harvesting' and how to avoid it. *J. Zool.* **320**, 161–168 (2023).
32. F. E. Zachos, Species splitting puts conservation at risk. *Nature* **494**, 35 (2013).
33. W. Wüster, S. A. Thomson, M. O'Shea, H. Kaiser, Confronting taxonomic vandalism in biology: Conscientious community self-organization can preserve nomenclatural stability. *Biol. J. Linn. Soc.* **133**, 645–670 (2021).
34. A. Georges *et al.*, Genomewide SNP markers breathe new life into phylogeography and species delimitation for the problematic short-necked turtles (Chelidae: *Emydura*) of eastern Australia. *Mol. Ecol.* **27**, 5195–5213 (2017).
35. D. Ahrens *et al.*, Is it time to describe new species without diagnoses? – A comment on Sharkey *et al.* (2021). *Zootaxa* **5027**, 151–159 (2021).
36. N. Gallego-García *et al.*, Where to set the bar? Recent descriptions inflate species number in South American toad-headed turtles (*Mesoclemmys*). *Zootaxa* **5263**, 566–574 (2023).
37. D. P. L. Toews, A. Brelsford, The biogeography of mitochondrial and nuclear discordance in animals. *Mol. Ecol.* **21**, 3030–3907 (2012).
38. T. Bonnet, R. Leblois, R. Rousset, P.-A. Crochet, A reassessment of explanations for discordant introgressions of mitochondrial and nuclear genomes. *Evolution* **71**, 2140–2158 (2017).
39. C. Dufresnes, D. Jablonski, A genomics revolution in amphibian taxonomy. *Science* **377**, 1272 (2022).
40. A. Petzold, A. Hassanin, A comparative approach for species delimitation based on multiple methods of multi-locus DNA sequence analysis: A case study of the genus *Giraffa* (Mammalia, Cetartiodactyla). *PLoS One* **15**, e0217956 (2020).
41. J. Sukumaran, L. L. Knowles, Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 1607–1612 (2017).
42. L. C. Campillo, A. J. Barley, R. C. Thomson, Model-based species delimitation: Are coalescent species reproductively isolated? *Syst. Biol.* **69**, 708–721 (2020).
43. C. Dufresnes *et al.*, Integrating hybrid zone analyses in species delimitation: Lessons from two anuran radiations of the Western Mediterranean. *Heredity* **124**, 423–438 (2020).
44. S. J. Weaver, C. T. Callaghan, J. J. L. Rowley, Anuran accents: Continental-scale citizen science data reveal spatial and temporal patterns of call variability. *Ecol. Evol.* **10**, 12115–12128 (2020).
45. V. J. Piñeros *et al.*, Genome-wide species delimitation analyses of a silverside fish species complex in central Mexico indicate taxonomic over-splitting. *BMC Ecol. Evol.* **22**, 108 (2022).
46. J. Šmid, J. Moravec, M. Zawadzki, M. Van den Berg, One subspecies less for the European herpetofauna: The taxonomic and nomenclatural status of *Hemidactylus turcicus spinalis* (Reptilia: Gekkonidae). *Zootaxa* **3911**, 44–446 (2015).
47. C. Dufresnes *et al.*, Shedding light on taxonomic chaos: Diversity and distribution of South Asian skipper frogs (Anura, Dicroglossidae, *Euphyctis*). *Syst. Biodiv.* **20**, 2102686 (2022).
48. C. Dufresnes, N. M. Probonas, I. Strachinis, A reassessment of the diversity of green toads (*Bufo*) in the circum-Aegean region. *Integr. Zool.* **16**, 420–428 (2021).
49. K. de Queiroz, Species concepts and species delimitation. *Syst. Biol.* **56**, 879–886 (2007).
50. J. S. Wilkins, *Species: A History of the Idea* (University of California Press, 2009).
51. F. E. Zachos, *Species Concepts in Biology* (Springer, 2016).
52. J. Kollár, A. Pouličková, P. Dvořák, On the relativity of species, or the probabilistic solution to the species problem. *Mol. Ecol.* **31**, 411–418 (2022).
53. P. J. Unmack *et al.*, Plotting for change: An analytical framework to aid decisions on which lineages are candidate species in phylogenomic species discovery. *Biol. J. Linn. Soc.* **135**, 117–137 (2022).
54. S. Singhal, C. Moritz, Reproductive isolation between phylogeographic lineages scales with divergence. *Proc. R. Soc. B.* **280**, 20132246 (2013).
55. C. Dufresnes *et al.*, Mass of genes rather than master genes underlie the genomic architecture of amphibian speciation. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2103963118 (2021).
56. C. Dufresnes, P.-A. Crochet, Sex chromosomes as supergenes of speciation: Why amphibians defy the rules? *Philos. Trans. R. Soc. B: Biol. Sci.* **377**, 1856 (2022).
57. S. A. Anderson, J. T. Weir, The role of divergent ecological adaptation during allopatric speciation in vertebrates. *Science* **378**, 1214–1218 (2022).
58. E. O. Wiley, The evolutionary species concept reconsidered. *Syst. Zool.* **27**, 17–26 (1978).
59. D. R. Frost, D. M. Hillis, Species in concept and practice: Herpetological applications. *Herpetologica* **46**, 87–104 (1990).
60. D. A. Baum, K. L. Shaw "Genealogical perspectives on the species problem" in *Experimental and Molecular Approaches to Plant Biosystematics*, P. C. Hoch, A. G. Stephenson, Eds. (Monographs in Systematic Botany from the Missouri Botanical Garden, 1995), vol. 53, pp. 289–303.
61. D. M. Hillis, Species, clades, and their relationship to paraphyly and monophyly: Examples from the *Pantherophis obsoletus* complex. *Herpetol. Rev.* **53**, 47–53 (2022).
62. D. R. Frost, A. G. Kluge, D. M. Hillis, Species in contemporary herpetology: Comments on phylogenetic inference and taxonomy. *Herpetol. Rev.* **23**, 46–54 (1992).
63. C. Roux *et al.*, Shedding light on the grey zone of speciation along a continuum of genomic divergence. *PLoS Biol.* **1**, e2000234 (2016).
64. J. Sukumaran, M. T. Holder, L. L. Knowles, Incorporating the speciation process into species delimitation. *PLoS Comput. Biol.* **17**, e1008924 (2021).
65. E. A. Chambers, T. L. Marshall, D. M. Hillis, The importance of contact zones for distinguishing interspecific from intraspecific geographic variation. *Syst. Biol.* **72**, 357–371 (2023).
66. D. M. Hillis, E. A. Chambers, T. J. Devitt, Contemporary methods and evidence for species delimitation. *Ichthyol. Herpetol.* **109**, 895–903 (2021).
67. C. Dufresnes, S. N. Litvinchuk, Diversity, distribution and molecular species delimitation in frogs and toads from the Eastern Palearctic. *Zool. J. Linn. Soc.* **195**, 695–760 (2022).
68. J. Speybroeck *et al.*, Species list of the European herpetofauna – 2020 update by the taxonomic committee of the societies europaea herpetologica. *Amphib. Reptil.* **41**, 139–189 (2020).
69. C. Dufresnes *et al.*, Inferring genetic structure when there is little: Population genetics versus genomics of the threatened bat *Miniopterus schreibersii* across Europe. *Sci. Rep.* **13**, 1523 (2023).
70. C. C. Thompson *et al.*, Microbial genomic taxonomy. *BMC Genom.* **14**, 913 (2013).
71. K. de Queiroz, An updated concept of subspecies resolves a dispute about the taxonomy of incompletely separated lineages. *Herpetol. Rev.* **51**, 459–461 (2020).
72. E. O. Wilson, W. L. Brown, The subspecies concept and its taxonomic application. *Syst. Biol.* **2**, 97–111 (1953).
73. E. Mayr, Of what use are subspecies? *Auk* **99**, 593–595 (1982).
74. A. B. Phillimore, I. P. F. Owens, Are subspecies useful in evolutionary and conservation biology? *Proc. R. Soc. B.* **273**, 1049–1053 (2006).
75. C. Kindler, U. Fritz, Phylogeography and taxonomy of the barred grass snake (*Natrix helvetica*), with a discussion of the subspecies category in zoology. *Vertebr. Zool.* **68**, 269–281 (2018).
76. D. M. Hillis, Species delimitation in herpetology. *J. Herpetol.* **53**, 3–12 (2019).
77. D. M. Hillis, The detection and naming of geographic variation within species. *Herpetol. Rev.* **51**, 52–56 (2020).
78. J. Mallet, "Subspecies, semispecies, superspecies" in *Encyclopedia of Biodiversity*, S. A. Levin, Ed. (Elsevier Academic Press, 2013), vol. 7, pp. 45–48.
79. R. M. Zink, L. B. Klicka, The taxonomic basis of subspecies listed as threatened and endangered under the endangered species act. *Front. Conserv. Sci.* **3**, 971280 (2022).
80. A. R. Templeton, Biological races in humans. *Stud. Hist. Philos. Biol. Biomed. Sci.* **44**, 26–271 (2013).
81. S. J. O'Brien, E. Mayr, Bureaucratic mischief: Recognizing endangered species and subspecies. *Science* **251**, 1187–1188 (1991).
82. R. M. Ball, J. C. Avise, Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* **109**, 626–636 (1992).
83. J. Ambu *et al.*, Genomic phylogeography illuminates deep cyto-nuclear discordances in midwife toads (*Alytes*). *Mol. Phylogenet. Evol.* **183**, 107783 (2023).
84. S.-J. Luo *et al.*, Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *PLoS Biol.* **2**, e442 (2012).
85. A. Miralles *et al.*, Shedding light on the Imps of Darkness: an integrative taxonomic revision of the Galápagos marine iguanas (genus *Amblyrhynchus*). *Zool. J. Linn. Soc.* **181**, 678–710 (2017).
86. M. Aszlam *et al.*, It takes two to tango – Phylogeography, taxonomy and hybridization in grass snakes and dice snakes (Serpentes: Natricidae: *Natrix* natrix, N. tessellata). *Vertebr. Zool.* **71**, 813–834 (2021).
87. D. Jablonski *et al.*, Morphological and genetic differentiation in the anguid lizard *Pseudopus apodus* supports the existence of an endemic subspecies in the Levant. *Vertebr. Zool.* **71**, 175–200 (2021).
88. C. Dufresnes *et al.*, Call a spade a spade: Taxonomy and distribution of *Pelobates*, with description of a new Balkan endemic. *ZooKeys* **859**, 131–158 (2019).
89. S. M. Haig *et al.*, Taxonomic considerations in listing subspecies under the U.S. Endangered Species Act. *Conserv. Biol.* **20**, 1584–1594 (2006).
90. S. Gippoliti, G. Amori, The problem of subspecies and biased taxonomy in conservation lists: The case of mammals. *Folia Zool.* **56**, 113–117 (2007).
91. M. Braby, R. Eastwood, N. Murray, The subspecies concept in butterflies: Has its application in taxonomy and conservation biology outlived its usefulness? *Biol. J. Linn. Soc.* **106**, 699–716 (2012).
92. A. Wilting *et al.*, Planning tripe recovery: Understanding intraspecific variation for effective conservation. *Sci. Adv.* **1**, e1400175 (2015).
93. S. Prost *et al.*, Genomic analyses show extremely perilous conservation status of African and Asiatic cheetahs (*Acinonyx jubatus*). *Mol. Ecol.* **31**, 4208–4223 (2022).



94. L. Rancilhac *et al.*, Phylogeographic breaks and how to find them: An empirical attempt at separating vicariance from isolation by distance in a lizard with restricted dispersal. *Peer Community J.*, **3**, e74 (2023).
95. D. J. Fraser, Bernatchez L, Adaptive evolutionary conservation: Towards a unified concept for defining conservation units. *Mol. Ecol.* **10**, 2741–2752 (2001).
96. L. Liu *et al.*, Coalescent methods for estimating phylogenetic trees. *Mol. Phylogenet. Evol.* **53**, 320–328 (2009).
97. N. Puillandre, S. Brouillet, G. Achaz, ASAP: Assemble species by automatic partitioning. *Mol. Ecol. Resour.* **21**, 609–620 (2021).
98. J. P. McEntee, J. G. Burleigh, S. Singhal, Dispersal predicts hybrid zone widths across animal diversity: Implications for species borders under incomplete reproductive isolation. *Am. Nat.* **196**, 9–28 (2020).
99. I. van Riemdsdijk *et al.*, Two transects reveal remarkable variation in gene flow on opposite ends of a European toad hybrid zone. *Heredity* **131**, 15–24 (2023).
100. C. Dufresnes *et al.*, The effect of phylogeographic history on species boundaries: A comparative framework in *Hyla* tree frogs. *Sci. Rep.* **10**, 5502 (2020).
101. C. Dufresnes *et al.*, Revisiting a speciation classic: Comparative analyses support sharp but leaky transitions between *Bombina* toads. *J. Biogeogr.* **48**, 548–560 (2021).
102. S. B. Hedges *et al.*, Tree of life reveals clock-like speciation and diversification. *Mol. Biol. Evol.* **32**, 835–845 (2015).
103. B. R. Lavin, D. J. Girman, Phylogenetic relationships and divergence dating in the Glass Lizards (Anguinae). *Mol. Phylogenet. Evol.* **133**, 128–140 (2019).
104. V. Gvoždík *et al.*, Phylogenomics of *Anguis* and *Pseudopus* (Squamata, Anguillidae) indicates Balkan–Apennine mitochondrial capture associated with the Messinian event. *Mol. Phylogenet. Evol.* **180**, 107674 (2023).
105. R. J. Rundell, T. D. Price, Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol. Evol.* **24**, 394–399 (2009).
106. J. A. Tobias *et al.*, Quantitative criteria for species delimitation. *Ibis* **152**, 724–746 (2010).
107. M. D. Crisp, G. T. Chandler, Paraphyletic species. *Telopea* **6**, 813–838 (1996).
108. J. L. Patton, M. F. Smith, Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (genus *Thomomys*). *Syst. Biol.* **43**, 11–26 (1994).
109. S. M. Torstom, K. L. Pangle, B. J. Swanson, Shedding subspecies: The influence of genetics on reptile subspecies taxonomy. *Mol. Phylogenet. Evol.* **76**, 134–143 (2014).
110. N. Schultze *et al.*, Mitochondrial ghost lineages blur phylogeography and taxonomy of *Natrix helvetica* and *N. natrix* in Italy and Corsica. *Zool. Scr.* **49**, 395–411 (2020).
111. M. Lambert, Taxonomy: Retain scientific autonomy. *Nature* **546**, 600 (2017).
112. A. J. Helbig *et al.*, Guidelines for assigning species rank. *Ibis* **144**, 518–525 (2002).
113. A. Aleixo, On species concepts, species delimitation criteria, taxonomy committees, and biases: A response to Lima (2022a). *Ornithol. Res.* **31**, 62–70 (2023).
114. S. A. Thomson *et al.*, Taxonomy based on science is necessary for global conservation. *PLoS Biol.* **16**, e2005075 (2018).
115. N. Holstein, F. Luebert, Taxonomy: Stable taxon boundaries. *Nature* **548**, 158 (2017).
116. M. Denoël *et al.*, Quantifying rarity of intraspecific diversity at multiple spatial scales by combining fine-grain citizen-based data across national boundaries. *Biol. Conserv.* **280**, 109937 (2023).
117. C. Mi *et al.*, Global protected areas as refuges for amphibians and reptiles under climate change. *Nat. Comm.* **14**, 1389 (2023).
118. Y. Hu *et al.*, Spatial patterns and conservation of genetic and phylogenetic diversity of wildlife in China. *Sci. Adv.* **7**, eabd5725 (2021).
119. C. Schmidt, J. Munshi-South, S. Dray, C. J. Garroway, Determinants of genetic diversity and species richness of North American amphibians. *J. Biogeogr.* **49**, 2005–2015 (2022).