



Molecular Markers and Taxonomic Explosion in Herpetology: More or Less Robust Taxonomy? True and False Advantages of DNA Markers

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Conceptual Paper

Volume 7 Issue 2

Received Date: March 04, 2024

Published Date: March 26, 2024

DOI: 10.23880/izab-16000569

Abstract

Despite the increasing deficit of taxonomic expertise, the number of newly described species since the early 2010s has grown exponentially. This growth is related to the increased use of DNA markers in taxonomic descriptions. However, routine use of DNA markers in taxonomy did not bring practical taxonomy closer to the theory. Species are unique lineages with irreversible evolutionary pathways, and only the presence of distinct populations within the same geographic range, or at least the presence of narrow hybrid zones between the parapatric ranges is a conclusive evidence of evolutionary irreversibility. In the case of allopatric populations, only very high genetic distances, suggesting several tens of millions of years of independent evolution, can be used for validation of species status. This problem cannot be solved by the broader introduction of genomic phylogenies, which also fail to provide robust criteria for evolutionary irreversibility. We can hardly suppose that robust validation of species status is applicable to all or most of hundreds of thousands of animal species, including 20,000 amphibians and reptiles. Instead, practical taxonomy should concentrate on describing recognizable species, maintaining a trade-off between sufficiently detailed descriptions of world biodiversity and the applicability of these descriptions for practical use and meta-analyses, not pretending that formally described species reflect real lineages with independent and irreversible evolutionary pathways. Simultaneously, the non-critical elevation of the taxonomic status of individual geographic populations, contrary to the declared purpose of better-focusing conservation efforts, often has the opposite effect, leaving many formally described taxa outside the conservation umbrella.

Keywords: Taxonomy; New Species; Herpetology

Introduction

The invention of cheap PCR-based sequence technologies, established in the middle 1980s, triggered a revolution in taxonomy [1]. Discovery and description

of new species since the time of Linnaeus and Pallas, were based on the individual experience of the naturalists. Long-lasting discussions were going in attempts to identify the most informative phenotypic characters that could help to separate “real” species from less meaningful traits that could

vary among conspecific populations or morphs or make taxonomy more objective by introducing numerical analysis [2-9]. By the XX century, a generation of taxonomists arose, specialized on smaller organismal groups, such as individual families of beetles or butterflies. It generally is considered that years of experience are required for a person to become a real expert on a certain group being able to make a reliable diagnosis of a species.

Generating molecular genetic data was supposed to make taxonomic practices more objective [10-12]. General biological knowledge is sufficient for seeing the DNA sequence differences between two phenotypically similar specimens. A zoologist working on a specific group of animals can easily convince a non-expert in his taxonomic suggestion, supporting his findings with some molecular genetic information. The new tool became widespread in taxonomic practices in the late 1990s; however, in original species descriptions of the 1990s and early 2000s, genetic

data are rarely reported. In 2005, only in one [13] out of 31 descriptions of new lizard species from 67 most speciose genera the authors provided mitochondrial sequence information. It does not rule out that molecular data were used in the practical taxonomy of these years; however, this usually was done for already described species or elevation of taxonomic status of the earlier described subspecies [14-17]. From this time, the inclusion of molecular data into species descriptions increased exponentially. In 2015, 65% of new lizard species descriptions referred to some molecular data (usually mitochondrial DNA sequences), and in 2023 this proportion increased to 98% [18].

Simultaneously, the number of annually described species increased sharply (Figure 1). In general, 50-100 species of lizards from 67 speciose genera were described within a period of five years since the middle XIX century until the end of the XX century; since the early 2000s, this number increased five times.

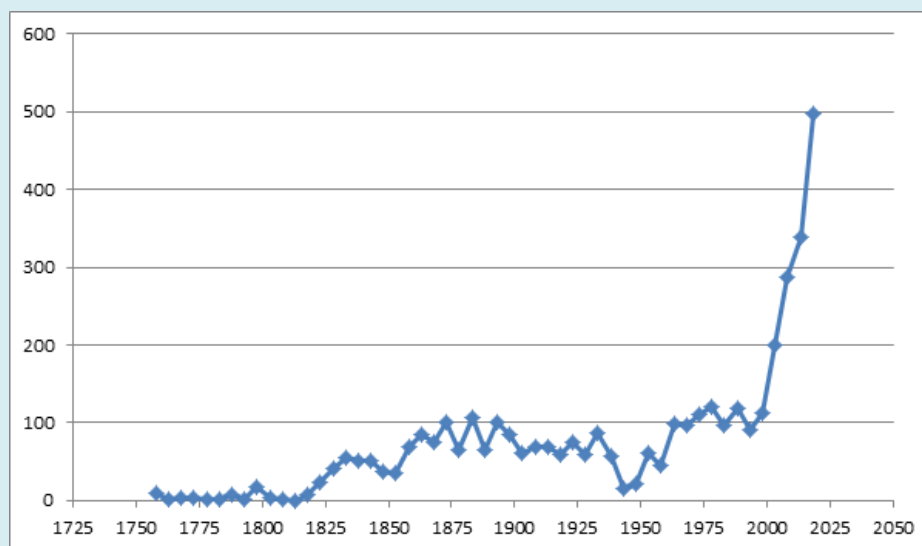


Figure 1: The number of new species of lizards described per five year periods, since middle XVIII century to the present day. The diagram is based on the analysis of 67 lizard genera that include 20 or more described species, according to Uetz, et al. [18]. Only newly described taxa are considered; infraspecific taxa with elevated status or re-described species are ignored.

This taxonomic explosion may look surprising because it happened concurrently with the general decline of taxonomic expertise [19-21]. This fact suggests that the wide use of DNA sequencing was likely decisive for the fast growth of the nominal species' number. The development of Barcoding of Life projects [22-26] was an additional factor for the taxonomic explosion. These projects accelerated efforts towards the documentation of biodiversity, and revealed the presence of a "barcoding gap", i.e. some level of mitochondrial DNA divergence, which is in line with species boundaries described by experienced taxonomists [27].

Limitations of Mitochondrial DNA: Positive and Negative Sides

In a living cell, mitochondrial genes produce thousands of copies, different from most nuclear genes [28,29]. This makes mitochondrial DNA (mtDNA) fragments easier to amplify than any other parts of the genome [30,31]. As a result, mtDNA sequencing has become a routine method for phylogenetic and taxonomic studies since the late 1980s. In Figure 2, the growth of the amphibian and reptile studies using mtDNA analysis is shown between 1980 and 2023.

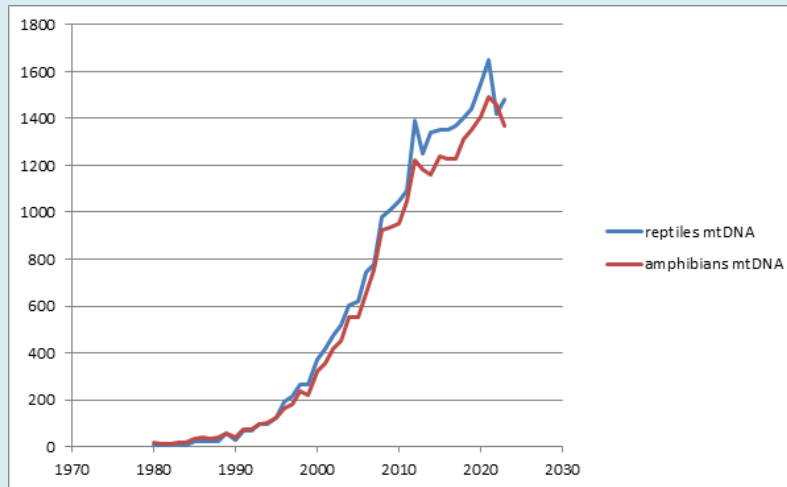


Figure 2: Growth of the number of publications including words “mitochondrial DNA”, “reptiles” and “amphibians” between 1980 and 2023, according to Google Scholar records.

Mitochondrial gene sequences contain important information on species evolution. Mitochondrial phylogenies help to reconstruct patterns of geographic expansion of a taxon. Projecting phylogenies on geographic maps became a specific field of science called phylogeography [32-36]. Comparing mtDNA sequences helps to infer the geological time when the ancestral lineages of two populations started to diverge. The molecular clock hypothesis assumes that substitutions in individual genes are aggregated with a

predictable speed [37,38]. Molecular clocks can be calibrated by known and well-dated geological events (such as the separation of two islands by sea) [39,40], or by comparing DNA sequences of current specimens with fossil DNA [41,42]. Molecular clock hypothesis was repeatedly criticized, because fixation rates of new substitutions may depend on the population size, and can substantially differ among taxa [43-46], but proper statistical analysis helps to estimate reasonable confidence limits [47-51].

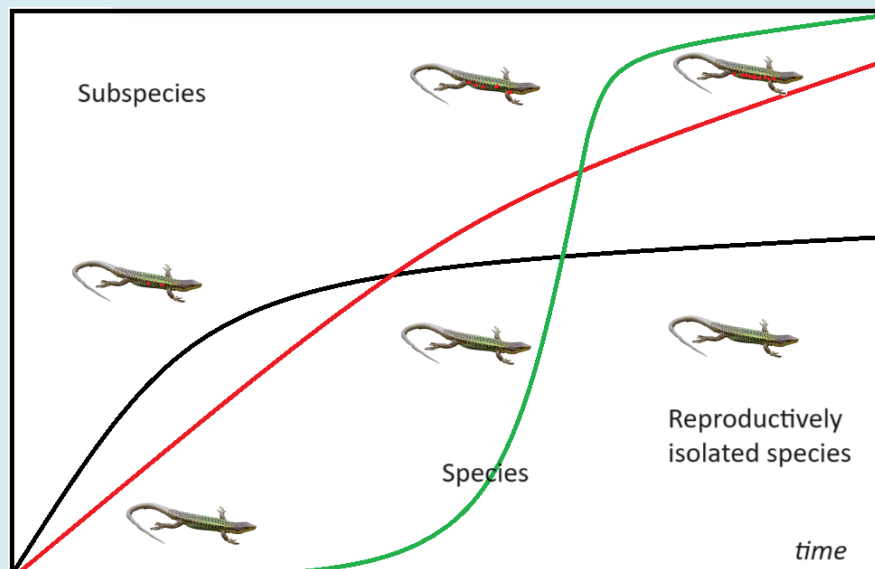


Figure 3: Speciation process. Black line shows growth of fitness related with interbreeding of once isolated lineages due to the heterozygote advantage. Red line shows the effect of outbreeding depression. When the lines cross, speciation process becomes irreversible. Green line shows growth of the effectiveness of pre-zygotic isolation due to reinforcement.

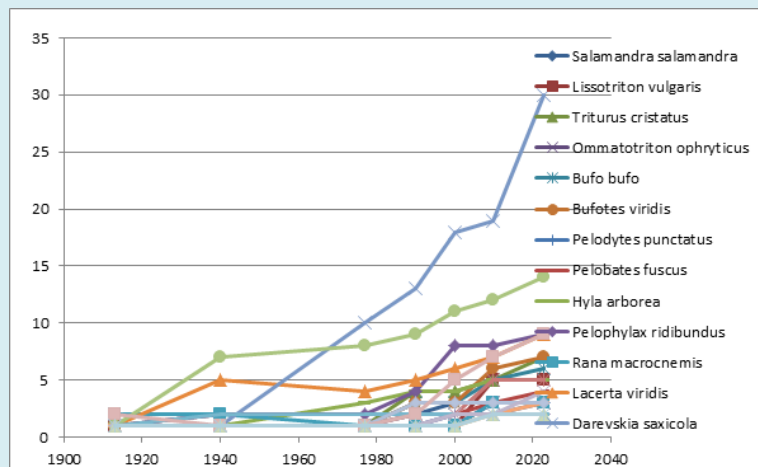


Figure 4: Splitting of some species of amphibians and reptiles from West Eurasia between the early XX century and present time. The names in the legend are those currently applied to species that were later split into two or more “derivative” species.

Simultaneously, the use of mtDNA sequences for the analysis of species boundaries is problematic. One challenge is incomplete lineage sorting [52]. Each of the two populations descending from a common ancestor may be not genetically homogenous, and fixation of alleles at polymorphic loci in diverging populations can occur at different speeds; both the same or different alleles can be fixed, depending on a locus [47,53,54]. As a result, phylogenetic trees based on unlinked genes may not coincide, causing an inconsistency between gene evolution and species evolution [52,55-59]. Different authors suggested combining sequences of mitochondrial and nuclear genes to infer more reliable phylogenies [60-62]. Another problem is a common horizontal transfer of mitochondrial genes among closely related species [63-70]. Probably due to these problems, and the broader invention of genomic technologies, since the early 2010s, the growth rate of the publications presenting mt-DNA data in herpetological studies declined (Figure 2).

Attempts to Overtake Incomplete Lineage Sorting Problem: Right and Wrong Approaches

Since late 1990s, evolutionary biologists and taxonomists invented a routine complementing mitochondrial sequences with sequences of one or more nuclear genes, usually those which produce multiple copies in a genome, such as genes C-mos, RAG-1, BDNF in amphibians and reptiles [71-75]. Coding nuclear genes are far more conservative than mitochondrial DNA, probably because of continually acting stabilizing selection [76]. Nuclear gene sequences usually have too few informative positions for producing reliable, statistically well-supported phylogenetic patterns if closely related species are studied. Some authors use a concatenation of nuclear and mitochondrial genes, trying to increase the

robustness of the generated phylogenies [77-81]. This is a conceptually incorrect approach. Concatenated sequences that include mitochondrial genes with a substantial number of informative positions and nuclear genes with just a few informative sites usually produce a tree simply coinciding with mitochondrial phylogeny. Meanwhile, the very sense of the analysis of incomplete lineage sorting is validation of a null hypothesis suggesting coinciding phylogenies, based on the unlinked loci analyzed separately. Other taxonomic papers provide separate phylogenies or haplotype networks based on individual nuclear genes: in this way, incomplete lineage sorting can indeed be detected [82-87].

Species Concepts: from Dobzhansky and Mayr to the Present Day

The taxonomic explosion of the late 1980s occurred along with the resuming of the discussion about species concepts, which never ended since the publication of Charles Darwin’s “The Origin of Species” [88]. A very logical concept of polytypic biological species to which the majority of scientists appealed for several decades, has an important weakness: it cannot be practically applied to thousands of described taxa [89,90]. Validation of pre- or post-zygotic isolation, a central issue of this concept, requires extensive analysis, which does not apply to most newly described species because of a lack of funds, time, and expertise. In fact, most taxonomists relied on a traditional taxonomic approach based on more or less subjective expert opinions [91]. However, the lack of a firm scientific theory behind the taxonomic practices challenges taxonomic conclusions. For this reason, concepts that were easier to validate, such as the phylogenetic species concept [92-94], replaced the biological species concept. The phylogenetic species concept

considers monophyletic origin as a sufficient precondition for considering a population to be a separate species. The concept of genealogical concordance, based on the idea of incomplete lineage sorting is, in fact, an extended definition of phylogenetic species, because it refers to the ancestral evolutionary pathway and not to the current metapopulation structure of a taxon.

Some other species concepts have become popular in recent decades. The relatively old concept of Evolutionary species does not require reproductive isolation as a necessary precondition of species reality [95-97]. The unified species concept of DeQueiroz, defining species as a separately evolving lineage, accepts ways of validating species status [98,99]. The departure from the biological species concept was largely a result of understanding that introgressive hybridization does not necessarily cause a merger of the evolutionary lineages and may occur concurrently with the ongoing divergent evolution [100,101].

DeQueiroz did not thoroughly explain what “separately evolving lineage” means. Simultaneously, one of the central ideas of the theory of evolution and speciation is the irreversibility of evolutionary changes. In general, the evolutionary pathway is considered to be irreversible [102-104], although this hardly can be applied to ephemeral lineages that occasionally re-merge in case of secondary contact. I would suggest that the evolutionary pathway of a species cannot be reversed, and each species is unique: it can evolve further, split into two daughter species, or extinct, but not lose its distinctness from any other species. The irreversibility criteria is concurrent with the concept of evolutionary species and separately evolving lineage of DeQueiroz, which don't rule out potential hybridization, but consider the presence of stable, species-specific complexes of genotypic and phenotypic characters.

Multiple genetic studies conducted in recent decades showed that hybridization and gene flow between diverging incipient species is commonplace [105,106]. Why could this happen? Fixation of differential de novo mutations in two isolated populations, as a result of gene drift and selection, decreases the number of the shared alleles. Interbreeding of individuals with different alleles at the same locus increases heterozygosity, reduces the probability of inbreeding depression, and, consequently, can increase the fitness of the hybrid offspring. Multiple examples suggest superior fitness in hybrids of even formally described different species [107-109]. However, in some cases, heterozygotes have inferior fitness [110-114]. Moreover, over time, the differential mutations are aggregated that change genomic architecture, and cause dysfunction of genes in hybrids, or incompatibility between nuclear and organellar genomes [115]. Inferior fitness of heterozygotes can also be a result

of adaptation of the isolated populations to different environmental conditions [116,117]. These deleterious effects slowly aggregate, and above a certain threshold their effect overweighs the positive effect of heterosis (Figure 3). After this time point, the speciation process becomes irreversible.

Similar to the biological species concept, the irreversibility of speciation (i.e., presence of separate evolutionary pathways) can hardly apply to practical taxonomy. The inferior fitness of hybrids is hard to detect without exhaustive study on the population level. It is impossible to conduct such studies for hundreds of annually described nominal species; therefore, this criterion, albeit theoretically very convincing, can rarely be used as an effective tool. This builds a gap between evolutionary theory and formal taxonomy. Probably, it is counterproductive to apply strict species criteria for describing new species, but even more harmful is an incorrect application of theory. The monophyletic origin does not prove that populations, even with diagnostic phenotypic features, cannot merge in case of secondary contact. Neither the presence of genealogical concordance, nor geographic isolation can be taken as an evidence of irreversible speciation process. The level of differences sufficient for the development of full reproductive isolation varies among animal taxa. It could be taken as an indirect argument of species-level differences and not a conclusive evidence.

Can we give priority to one or another of these concepts? Irrespective of a theory, scientists need to name living beings for inventing terms that help to understand what we are speaking about. Scientific names, such as *Bufo bufo*, *Lacerta agilis*, or *Natrix natrix* save us time: we don't need to write long sentences explaining what kind of animal we are speaking about, whether or not it has a tail and legs and if its body is covered with scales, or how it is colored, replacing long unnecessary explanations by a Latin binomial invented by Linnaeus. Hence, practical taxonomy, first of all, should pursue the nomenclature well describing diagnostic features of a group of organisms, and last but not least, easy to remember by ecologists and evolutionary biologists. Those purposes are rarely considered in recent taxonomic practices. Scientists describe thousands of “cryptic” species with no diagnostic phenotypic characters, based only on the differences in the sequence of a few genes [118]. Based on some sequence differences, they often split “traditional” species into several new ones which are difficult to remember by a non-expert in a specific taxonomic group. How this process of splitting is intensified in recent decades, in the example of the West Eurasian herpetological fauna is shown in Figure 4. In total, 25 species from 22 genera of amphibians and reptiles were split into 47 species by 1977, 86 species before 2000, and into 140 species by 2023.

Introgressive Hybridization Challenges Determination of Species' Boundaries

Although introgressive hybridization may not prevent continuing divergence of incipient species [100], it has a certain impact on the evolution within each of the hybridizing lineages. Some genes may be horizontally transmitted from one lineage to another. Typical cases of DNA transmissions among the species, sometimes interpreted as “reticulate speciation”, is transfer of mitochondrial DNA among the species. In some cases, such as frogs *Rana chensinensis* and *R. kukunoris* [66], toads *Bufo fowleri* and *Bufo americanus* [63], horned lizards of genus *Phrynosoma* [65], skinks of genus *Messalina* [86], Alligator lizards from the genus *Elgaria*, or rock lizards *Darevskia obscura* and *Darevskia portschinskii*, in spite of a distinct phenotypic and allelic boundary between the species, mitochondrial DNA of a related species replaced original mitochondrial lineage in the peripheral part of their ranges. In the Carpathian newt (*Lissotriton montandoni*) the original mitochondrial lineage is completely replaced by the mitochondrial DNA of a more widespread smooth newt (*Lissotriton vulgaris*) [119,120]. The same is true for leaf-tailed geckos of the genus *Uroplatus*, or some rock lizards from the genus *Darevskia*. Genetic studies showed discordance between mitochondrial and nuclear phylogeny of at least four very distinct and reproductively isolated species of this genus: *Darevskia alpina*, *D. derjugini*, *D. praticola*, and *D. parvula*. In the first two species, mitochondrial genes are closer to that of lizards with whom their geographic range overlap than with allopatric species to which they are closer phenotypically and by nuclear genes. In general, the discordance of nuclear and mitochondrial phylogenies is not an exception for natural genetic systems.

Actually, we don't know how common the introgressive pattern of mitochondrial DNA in different organismal groups is. Unfortunately, combined analysis of mitochondrial and nuclear genes does not always aim to infer patterns of introgression. As a consequence, it remains a formal procedure pursuing the search of some theoretical ground for nominating species.

Practical and Conservation Outcomes: Taxonomic Inflation

Some authors defined the accelerated description of new species in the first quarter of the XXI century as “taxonomic inflation”. The very idea of this definition is that unlimited descriptions of new species, applying to highly variable arguments and datasets, result in the devaluation of a species as a part of nature deserving attention and conservation. Some negative consequences of taxonomic inflation are discussed by previous authors [121]. Here I would underline the three most important ones from my point of view.

(1) Complication of the task for decision-makers. Governmental bodies, which are in charge of environmental protection, must identify conservation priorities for effectively spending taxpayer's money. A routine part of this procedure is identifying species that need more urgent conservation actions than the others. In national legislation, there are no definitions for upper taxa or “species groups”, and for this reason, the officials require scientists to provide them with scientific names of prioritized species or, at least, subspecies. In private conversations, taxonomists commonly suggest that describing new species is useful for increasing attention to taxa, especially local endemics, which need to be protected. I think that the actual effect is the opposite. Including five taxa in lists of protected species (such as national Red Lists) would not increase the funds that a government will spend for their conservation. Hundreds and thousands of nominal species suggested for conservation actions just frighten decision-makers who often have superficial knowledge of taxonomy. Further, splitting a species, formally protected by law, into several ones often causes a mess, and this “old” species remains protected by national legislation or international agreements only in a part of the range where the nominal form is found. Lizard *Lacerta trilineata*, snakes *Dolichophis jugularis*, *Elaphe quatuorlineata*, *Vipera ammodytes*, *Vipera ursini*, amphibians *Bufo viridis* and *Hyla arborea* are included under Annex II (species requiring special protection) of Bern convention on conservation of Wildlife and Natural Habitats (<https://www.coe.int/en/web/bern-convention/home>). Simultaneously, subspecies of all these species from the Caucasus biodiversity hotspot were re-described as separate species in recent decades [122-152]. None of these “new” species is prioritized in conservation programs supported by the European Commission.

(2) Nominal species commonly interbreed with their close relatives in the contact areas. Gene flow among the populations (either conspecific or formally non-conspecific) is a natural process affecting genetic diversity, exchange of adaptive alleles, and the effective population size. Typically, hybridization is not desirable because it may result in inferior fitness of hybrids. But simultaneously, common introgression of genes among populations may indicate the presence of positive effects of natural hybridization, including transfer of adaptive genes and increasing an effective population size, if gene flow is sufficiently high. Protection of a single nominal taxon and leaving unprotected adjacent populations of even formally different species may substantially reduce the effective size of its population and increase its vulnerability.

(3) For the understanding of the structure and dynamics of global biodiversity, it is important to develop integrated analyses of faunas and floras for large regions of the World, including biogeographic realms. Such analyses preferably cover evolutionarily distant taxonomic groups. Growth

of the number of formally described allopatric species misleads scientists conducting such an analysis, causing overestimation of differences and hiding similarities. For instance, herpetological fauna of central and Mediterranean parts of Europe has several widespread species, such as *Lissotriton vulgaris*, *Triturus cristatus*, *Pelophylax ridibundus*, *Hyla arborea*, *Bufo viridis*, *Bufo bufo*, *Lacerta trilineata*, *Anguis fragilis*, *Vipera ursini*, *Vipera ammodytes*, *Elaphe quatuorlineata*, or *Malpolon monspessulanus*. All these species were assumed to exist in the Caucasus biodiversity hotspot before the status of Caucasian regional populations or subspecies was elevated to species [127]. As a result of taxonomic splitting, the region has now the species *Triturus karelinii*, *Pelophylax bedriagae*, *Hyla orientalis*, *Bufo variabilis*, *Bufo verrucosissimus*, *Anguis colchica*, *Vipera eriwanensis*, *Vipera transcaucasiana*, *Elaphe urartica*, *Malpolon insignitus*. Most of these species are hardly recognizable from their Mediterranean relatives without a detailed study of their morphology or comparison of their DNA markers, and their ecosystem function hardly differs from that of their closest relatives. However, the nomenclatural differences give an impression of higher differences between the Caucasian and European herpetological faunas than in fact, it has. It also inflates the importance of real relict endemics, such as Caucasian salamander (*Mertensiella caucasica*) or Caucasian parsley frog (*Pelodytes caucasicus*), whose separate evolution counts tens of millions of years and who developed unique, highly specific phenotypes and ecological niches [127-129].

Do DNA Markers Make Taxonomy Less Biased?

As said, there is hardly a universal algorithm that could firmly validate species status (i.e., that of an evolutionary lineage whose evolutionary pathway became irreversible). Neither morphological study nor the analysis of mitochondrial DNA or even genomic phylogeny can solve this question without deep analysis, including hybridization experiments or a comparative study of viability for hybrids and purebreds. Such kind of analysis cannot cover hundreds of thousands of described species, including about 20,000 amphibians and reptiles. Practical taxonomists and evolutionary biologists commonly use any argument, based on the DNA marker analysis, to support their nomenclatural suggestions. We could classify these arguments: (1) Use of genetic distance based on the mitochondrial sequence analysis. This approach may be used as an indirect argument for treating an OTU (operative taxonomic unit) as a separate species, assuming that the molecular clock hypothesis works and it is likely that the number of genomic architectural mutations over time inevitably cause reproductive incompatibility. However, this time may substantially vary among the taxa. Besides, in some cases, pre-zygotic isolation may develop before post-zygotic [130]. (2) Mitochondrial (or nuclear) phylogeny as

an argument for attaining species status to geographically separated populations. This argument is commonly used along with a morphological description of a taxon. However, monophyly is a weak argument if not supported by accounted genetic differences. Many local populations, at some stage after their establishment, are monophyletic groups of organisms. They often share common phenotypic features, which is not evident for the irreversibility of their evolution. Non-critical use of phylogenies for species description may cause extreme taxonomic inflation in the case of “nomenclatural harvesting” [131]. (3) Genealogical concordance, i.e., concurrent phylogeny based on unlinked genetic character. It is supposed that the lineages that achieved this stage of differentiation are isolated long enough to achieve an independent evolutionary pathway [132]. However, if the effective size of an isolated population is small, genealogical concordance can be reached in a short time, which may be insufficient for the development of pre- or post-zygotic isolation or irreversibility of evolution. Moreover, in most cases, scientists rely on combined analysis of mitochondrial and one or two nuclear genes, which usually have insufficient signals for building a robust phylogeny. Besides, concordant phylogenies based on very few loci are insufficient for driving conclusions about genealogical concordance throughout the entire genome. (4) The most expensive and informative approaches are those based on the high-throughput sequences of genomes, such as RAD sequencing [133,134]. These approaches help to build more reliable phylogenetic trees and group species into genetic units using Bayesian inference [135]. However, even in this case, one cannot rule out the effect of a small effective population size, when genetic specificity is achieved in a small period of divergent evolution, insufficient for the development of effective reproductive isolation.

Generally speaking, none of the broadly used methods based on genetic or morphological differences between OTUs can be taken as conclusive evidence for separate evolutionary pathways, i.e. considering a population to be a separate species. The only safe approach remains one defined in early papers of Mayr, et al. [136,137] the presence of two distinct and non-merging taxa within the same geographic range. An additional group of evidence is the presence of a narrow hybrid zone when two species come into contact but remain distinct throughout most of their range, such as in a classical example of fire-bellied toads [138,139]. In the case of geographically isolated populations talking about the presence of different species can only be safe if the lineages are isolated obviously for tens of millions of years and show a very strong phenotypic hiatus – such as in the case of sister species of relict salamanders [140], or parsley frogs.

In all other cases nominating geographically isolated, closely related OTUs cannot be considered an objective

way of describing species diversity if we agree that species are lineages with separate and irreversible evolutionary pathways. But what should we do with the myriads of binomials, which scientists introduced for the last two and a half centuries? Here I would return to “practically useful” taxonomy: it should help scientists, not only, to understand what are we speaking about when using a particular scientific name. In other words, it should keep a balance between a sufficiently detailed description of the observed biological diversity and the ability of a person to memorize the meaning of words.

Both describing more and more species and renaming earlier described taxa (both species and higher order taxonomic groups) do not help to memorize species names. For instance, 30 years ago, most Colubrid snakes from the Caucasus Ecoregion were aggregated into two genera (*Coluber*, *Elaphe*). Currently, species from the clade to which the nominal genus *Coluber* belongs are distributed among ten genera, and only two of them, *Macroprotodon* and *Eirenis*, were not members of this genus in early taxonomic systems. The inclusion of these two genera into *Coluber* would be a more parsimonious taxonomic solution, although taxonomists preferred splitting and turning three former genera into ten new ones.

In some recent papers [141,142] broader use of the concept of geographic subspecies is suggested for allopatric, genetically, and morphologically close population groups. Nominating subspecies may be the way to a detailed description of biodiversity without the infinite complication of biological nomenclature. Understanding diversity within polytypic species is important for experts working on specific taxa, and retaining more inclusive species names would help to understand diversity for the general public, conservation bureaucrats and activists, and scientists analyzing biogeographic patterns throughout large geographic areas. Nominating subspecies does not meet conceptual difficulties that meet species descriptions. The criterion for describing separate subspecies is clear and simple: the ranges of different subspecies cannot coincide or overlap. If they are allopatric, the subspecies can be described based on even little genetic differences or the presence of private (not necessarily diagnostic) phenotypic characters. If the ranges are parapatric, i.e., conspecific individuals may move across the geographic boundaries between populations, the subspecies can still be described based on distinct diagnostic characters but no evidence for the formation of a tension hybrid zone [143-145] or otherwise evidence of selection against the subspecies hybrids. In this case, populations with intermediate characters or polymorphic by diagnostic characters do not belong to any subspecies, and their species name can be used without indication of a subspecies.

Conclusion

Use of Genetic Markers for Better Understanding Biodiversity

Molecular markers are indispensable for various kinds of biodiversity research, potentially including identification of species boundaries, but only rarely are they correctly used for the identification of these boundaries. It is correct to identify species based on the DNA marker analysis of individuals from the same range or contiguous ranges, but in the case of allopatric taxa, mtDNA sequences and even genomic data-based phylogenies could only be used as an indirect argument, in case if the differences are excessive. In all other cases, the description of a new species is still based on the experience and preferences of a taxonomic expert, and molecular markers are used for supporting a pre-defined suggestion and not for objective and unbiased validation of a hypothesis. The appearance of new species names in checklists does not depict “true” species diversity; at least if we assume that species is a real evolutionary lineage that attained an irreversible stage of divergence from its closest relatives (and hence has an independent evolutionary pathway). For practical reasons, however, the description of new species does not, and probably should not pursue strict evolutionary concepts. Instead, it can be accepted that, in most cases, formally named species are more or less subjective entities, helping to better understand the existing diversity. In this latter case, however, taxonomists should not oversplit the existing taxa, remembering potential problems associated with taxonomic inflation. Molecular markers, meanwhile, remain a powerful tool for understanding phylogeographic patterns, gene flow rates among the populations, or speciation process [146-150].

Acknowledgement

The paper was prepared while the author was in acceptance of grant by Shota Rustaveli National Science Foundation, project code FR-23-17324. The author appreciates students of Ecology at Ilia State University and Oleksiy Yanchukov for useful discussions.

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