



## Review

## Shedding subspecies: The influence of genetics on reptile subspecies taxonomy

Shannon M. Torstrom<sup>a,b,\*</sup>, Kevin L. Pangle<sup>a</sup>, Bradley J. Swanson<sup>a,b</sup><sup>a</sup> Department of Biology, Central Michigan University, Mount Pleasant, MI 48859, United States<sup>b</sup> Applied Technologies in Conservation Genetics Laboratory, Central Michigan University, Mount Pleasant, MI 48859, United States

## ARTICLE INFO

## Article history:

Received 19 November 2013

Revised 8 March 2014

Accepted 11 March 2014

Available online 22 March 2014

## Keywords:

Genetic distance

Genetic techniques

Integrative taxonomic species concept

Subspecies concepts

## ABSTRACT

The subspecies concept influences multiple aspects of biology and management. The 'molecular revolution' altered traditional methods (morphological traits) of subspecies classification by applying genetic analyses resulting in alternative or contradictory classifications. We evaluated recent reptile literature for bias in the recommendations regarding subspecies status when genetic data were included. Reviewing characteristics of the study, genetic variables, genetic distance values and noting the species concepts, we found that subspecies were more likely elevated to species when using genetic analysis. However, there was no predictive relationship between variables used and taxonomic recommendation. There was a significant difference between the median genetic distance values when researchers elevated or collapsed a subspecies. Our review found nine different concepts of species used when recommending taxonomic change, and studies incorporating multiple species concepts were more likely to recommend a taxonomic change. Since using genetic techniques significantly alter reptile taxonomy there is a need to establish a standard method to determine the species–subspecies boundary in order to effectively use the subspecies classification for research and conservation purposes.

© 2014 Elsevier Inc. All rights reserved.

## Contents

1. Introduction .....	134
2. Methods .....	136
2.1. Statistical analysis .....	136
3. Results .....	136
4. Discussion .....	137
5. Conclusions .....	138
Acknowledgments .....	139
Appendix A .....	139
Appendix B. Supplementary material .....	140
References .....	140

## 1. Introduction

The subspecies classification may reflect evolutionary relationships or only the human need to categorize, but the influence of

subspecies on other facets of biology and management demands that the factors affecting their designation be clearly understood (Starrett, 1958; Groves, 2012). The subspecies category was developed to enhance understanding of geographic variation, speciation, and to refine taxonomic distinction. However, since the establishment of subspecies there has been controversy regarding its necessity (Mayr, 1982; Patten and Unitt, 2002; Hawlitschek et al., 2012) showcased by Mayr (1970) reclassifying 315 species to subspecies,

\* Corresponding author at: Central Michigan University, Brooks Hall Rm 217, Mount Pleasant, MI 48859, United States.

E-mail addresses: [shtorstrom@gmail.com](mailto:shtorstrom@gmail.com) (S.M. Torstrom), [pangl1k@cmich.edu](mailto:pangl1k@cmich.edu) (K.L. Pangle), [swans1bj@cmich.edu](mailto:swans1bj@cmich.edu) (B.J. Swanson).

**Table 1**

List of the species concepts/criteria used in the studies evaluated in this paper.

Species concept/criteria	Concept	Reference
Biological species concept <sup>1</sup>	'Groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups'	Mayr (1942)
Bayesian phylogenetic and phylogeographic method <sup>2</sup>	'This method assesses species-tree uncertainty due to the coalescent using multiple gene trees to identify independently evolving lineages'	Yang and Rannala (2010)
Cohesion species concept	'A species is an evolutionary lineage through the mechanisms that limit the populational boundaries for the action of such basic microevolutionary forces as gene flow, natural selection, and genetic drift'	Templeton (1989)
Evolutionary species concept <sup>1</sup>	'A species is a lineage of ancestral descent which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate'	Wiley (1978)
General lineage species concept <sup>3</sup>	'Species are separately evolving metapopulation lineages, or more specifically, with segments of such lineages'	de Queiroz (1998)
Integrative Taxonomic Species Concept	'This concept rejects the superiority of any particular set of characters (morphological, behavioral, molecular, etc.) over others during the process of recognizing and diagnosing a species, and advocates the combined and integrated use of various such methods.'	Miralles et al. (2010)
Operational species delimitation criteria (aka. Tree Based Delimitation) <sup>4</sup>	'Set of criteria for concluding if mitochondrial lineages represent species: (1) ingroup lineages must be exclusive of the outgroup (2) lineages within the species complex must be geographically exclusive of one another'	Wiens and Penkrot (2002)
Phenetic cluster criterion <sup>5</sup>	'Distinguishes species by their allocation to separate clusters in multivariate analyses'	Sokal and Sneath (1963)
Phylogenetic species concept <sup>6</sup>	A species is an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent	Cracraft (1983)

1 = From Frankham et al., 2012; 2 = Burbrink et al., 2011; 3 = de Queiroz, 2007 4 = Burbrink, 2002; 5 = Guo et al., 2009; 6 = Cracraft, 1989.

reducing the number of North American bird species by 51% (607–292).

Given the multitude of ways used to define a species (Table 1), it is not surprising that this confusion extends down to subspecies as well; some disciplines, such as herpetology have many authors rejecting the subspecies classification (Reiserer et al., 2013) while others, like ornithology, favor the classification for understanding evolutionary divergence and conservation (Hawlitschek et al., 2012). The differences between the disciplines is evident when examining the ratio of species to subspecies within the vertebrate groups: mammals have an approximate 1:2 ratio (Reeder et al., 2007), aves is approximately 1:2.2 ratio (Lepage, 2014), while reptiles are approximately 1:0.3 ratio (Uetz, 2010). Even using the same criteria for subspecies designation could result in a different number of species if different species designations are used (Rodríguez-Robles and De Jesús-Escobar, 2000). Despite the confusion, the term “subspecies” continues to be used in many aspects of biological research and currently influences conservation efforts (Zink, 2004) and legislation (Haig et al., 2006), making it essential to identify and understand any biases in the application of the subspecies concept.

“Subspecies” replaced the term “variety” in zoology, inheriting the meaning of geographic race (variant populations of a species based on geographic location), in the late nineteenth century (Mayr, 1963). Simply replacing the term, the subspecies concept remained an ambiguous classification since species characteristics were not necessarily dependent on geographic variation (Wilson and Brown, 1953; Mayr, 1963; Mayr and Ashlock, 1991). Mayr (1963) redefined subspecies, as “an aggregate of local populations of a species, inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species.” This concept, while more specific, remains ambiguous in meaning and application since “differing taxonomically” has multiple interpretations.

Repeated suggestions have been made to standardize “differing taxonomically, with statistical quantification of subspecies such as the 75% rule (Amadon, 1949). In this case, a population was considered a subspecies if 75% of the population’s morphological characteristics lay outside of 99% of the range of other populations (Amadon, 1949). The percentage, while initially accepted, was eventually argued to be too low because subspecies should be diagnostically distinct thus, the criteria then increased to 90% or

100% separation of defining characteristics between populations (Marshall, 1967; Amadon and Short, 1992). More recently, Patten and Unitt (2002) argued for a 95% rule in order to parallel the standard alpha value of 0.05 in other statistical tests.

Originally the 75% rule applied only toward morphological features using the Biological Species definition, but the “molecular systematics revolution” integrated genetics into the analysis of subspecific designation (Rodríguez-Robles and De Jesús-Escobar, 2000; Patten and Unitt, 2002). The genetic techniques altered the rules for subspecific designation by revising the traditional ranked-based taxonomy to apply a phylogenetic-based taxonomy (Mulcahy, 2008). Currently, the common methods of analysis involve sequencing mitochondrial or nuclear genes to then analyze the genetic distance between the subspecies in order to determine their genetic distinctiveness, or to evaluate the evolutionary lineages between the recognized subspecies (Burbrink, 2002; Fritz et al., 2007). If subspecies have high genetic distance values, or represent distinct evolutionary lineages, they are raised to full species rank, but if the subspecies have low genetic distance values or lack a distinct evolutionary lineage scientists either maintain the subspecies classification or collapse the subspecies to a single species with no subspecies (Burbrink et al., 2000; Fritz et al., 2007; Makowsky et al., 2010). However, the application of genetics raises additional questions about the validity of subspecies as the genetic results often disagree with the recognized subspecies based on morphological data (Ball and Avise, 1992; Burbrink et al., 2000; Phillimore and Owens, 2006). This lack of congruence can lead to alterations in the taxonomy including rejecting subspecies (e.g., Macey et al., 1998; Daniels et al., 2010), combining subspecies (e.g., Podnar et al., 2004), establishing new subspecies (e.g., Guicking et al., 2008), or elevating subspecies to full species (e.g., Leaché and Reeder, 2002; Bryson et al., 2007).

Avian subspecies have been a particular focus for genetic evaluation since Mayr’s reevaluation decreased the number of species by 51% (Mayr, 1970). More recently, Zink (2004) surveyed 41 avian species using mtDNA analyses and found that only 3% of avian subspecies qualified as distinct evolutionary entities. He found that the average bird species had only 1.9 independent evolving groups, based on DNA analysis instead of his surveyed average of 5.5 based on morphological and geographical data. Zink (2004) concluded that there were too many avian subspecies, and suggested that the number of avian subspecies should be collapsed while the

number of species should remain the same. However, this conclusion may be refuted by methods other than mtDNA clades, which may not produce monophyletic groups (Mulcahy, 2008; Genner and Turner, 2012).

The extent of genetic influence on non-avian subspecies classification remains unknown since most studies evaluate species on a case-by-case basis. In this study we reviewed the literature to evaluate the impact of genetic analysis on subspecies classification in reptiles. We focused on the class Reptilia due to the relatively large number of species and subspecies - approximately 9547 species and 3200 subspecies (Uetz, 2010). Our objectives were to determine (1) if genetic analyses resulted in alteration in the number of subspecies within the class Reptilia and if the alterations were consistent across the orders and suborders, (2) if characteristics of the study or genetic variables influenced taxonomic change, (3) if genetic distance values differed between authors elevating subspecies to a species or collapsing subspecies, and (4) the influence of the species concepts applied when analyzing subspecies.

## 2. Methods

We addressed the objectives via a literature review using Web of Science and JSTOR databases and the following search terms: reptile subspecies; snake, turtle, or lizard genetics; and snake, turtle, or lizard subspecies. Studies chosen needed to include genetic sequencing, evaluation of  $\geq 2$  subspecies (or an evaluation of single species lacking any subspecies), and specific discussion of the taxonomic implications.

To determine if the suggested number of subspecies changed after the study we noted each study for the number of subspecies examined and the number of subspecies recommended in the conclusion. We also noted the type of DNA used (mitochondrial, nuclear, or both), number of loci, total base-pair length, sample size, number of sample locations, the study's published year and the species concept applied.

When authors recommended an elevation of a subspecies to species rank or collapsed the subspecies we noted the minimum and maximum genetic distance values and then recorded the midpoint values based on the range. If authors only provided the maximum value we assumed the minimum value was 0. Studies that only provided the mean genetic distance values were excluded from analysis since the mean value does not approximate the median value under non-normal conditions. We excluded studies that did not make a taxonomic recommendation because there were multiple reasons why authors did not alter the taxonomy such as insufficient sample size (e.g., Rocha et al., 2006) and genetic data (e.g., Silva et al., 2010), needing to re-examine morphology (e.g., Burbrink et al., 2000; Linkem et al., 2010), or extending geographic sampling (e.g., Vargas-Ramírez et al., 2010).

### 2.1. Statistical analysis

We used the nonparametric Wilcoxon Paired Signed Rank Test to examine if genetic analyses altered the taxonomic status of a significant number of reptiles. The pairs consisted of the number of subspecies before the genetic analysis (representing the number of subspecies based on morphology) and the number of recommended subspecies after the genetic analysis (representing the number of subspecies based on genetic analysis). We also compared the number of species before and after the study. We are aware that the number of species will not decrease since each study begins with 1 species and will either remain the same or increase after the genetic analysis, but we wanted to determine if there was a significant increase in the number of species after genetic analysis.

Logistic regression was used to evaluate the effect of different study variables on taxonomic recommendation. Variables were considered significant if the alpha value was  $\leq 0.05$ . Models that included different combinations of the variables were ranked using AICc (corrected Akaike's information criteria). The models that had a delta AIC value  $\leq 2$  were considered competitive and evaluated (Burnham and Anderson, 2002). Exact binomial tests and chi-square tests were used to evaluate if the different species concepts influenced altering the species taxonomy.

To compare the genetic distance values when authors elevated or collapsed a subspecies we used the nonparametric Mann-Whitney *U*-test. To make the comparison we used the midpoint value of each study based on its minimum and maximum values. We also compared the minimum genetic distance values reported to elevate subspecies to species level to the maximum genetic distance values reported for collapsing subspecies into a single species. All of our analyses were conducted using the R program version 2.13.1 (R Development Core Team, 2011).

## 3. Results

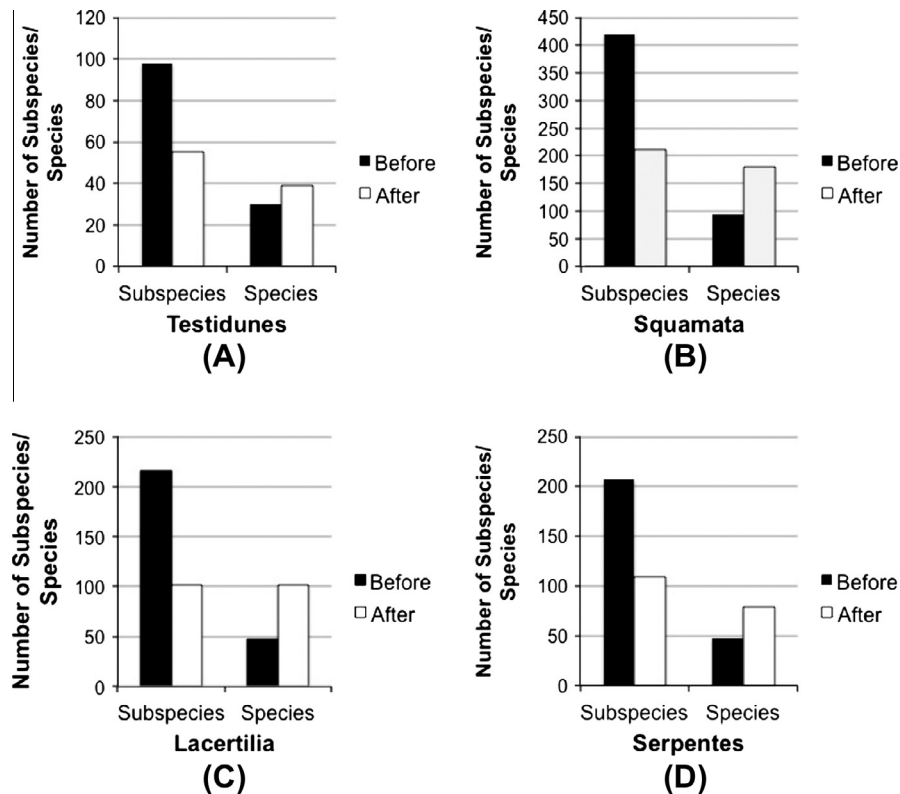
We found 124 studies that met our requirements: 30 Testudines and 94 Squamata (47 Lacertilia, 47 Serpentes; A.1). Testudines were not evaluated by suborder (Cryptodira, Pleurodira) due to the small number of studies we found. Similarly, Crocodylia and Sphenodontida were not included in this review as we did not find a sufficient number of studies for evaluation.

We found that Testudines and Squamata significantly decreased in the number of subspecies ( $T = 162$ ,  $P < 0.05$ ,  $T = 2261$ ,  $P < 0.0001$ ) and increased in the number of species ( $T = 15$ ,  $P < 0.05$ ,  $T = 820$ ,  $P < 0.0001$  Fig. 1A and B) following genetic evaluation. When dividing Squamata into suborders, both Lacertilia and Serpentes significantly decreased in the number of subspecies ( $T = 709$ ,  $P < 0.0001$ ,  $T = 451$ ,  $P < 0.0001$  respectively; Fig. 1C and D) and significantly increased in the number of species ( $T = 231$ ,  $P < 0.0001$ ,  $T = 190$ ,  $P < 0.0001$  respectively; Fig. 1C and D).

Our study character variables varied greatly in range: the year of the published study (1996–2012), sample size (3–480), and number of sample locations (2–207). The genetic variables also exhibited a large range: number of loci (1–20), total base pair length (253–4842), type of DNA ( $n_{\text{mtDNA}} = 86$ ,  $n_{\text{nDNA}} = 1$ ,  $n_{\text{both}} = 37$ ). However, the logistic regressions failed to find any models with predictive power based on the variables we examined (see Supplementary Tables 1–4).

We found that there was a significant difference ( $H = 35.045$ ,  $df = 1$ ,  $P < 0.0001$ ) between the midpoint genetic distance values when authors elevated (median = 6.4%, range = 1.0%–19.4%) a subspecies to when they recommended a collapse (median = 1.0%, range = 0.05%–4.3%) of the subspecies. There was also a significant difference ( $H = 8.922$ ,  $df = 1$ ,  $P < 0.01$ ) between the minimum elevated genetic distance values (median = 4.9%, range = 0%–13%) when compared to the maximum collapsed genetic distance values (median = 2.05%, range = 0.1%–5.6%).

Nine different species concepts were used when evaluating the focal subspecies in the reptile orders. The concepts applied (Table 1) were the phylogenetic species concept ( $n = 64$ ), evolutionary species concept ( $n = 26$ ), general lineage concept ( $n = 6$ ), integrative taxonomic concept ( $n = 3$ ), operational species delimitation criteria/tree based delimitation ( $n = 3$ ), Bayesian phylogenetic and phylogeographic concept ( $n = 1$ ), phenetic cluster criterion ( $n = 1$ ), cohesion species concept ( $n = 1$ ), and biological species concept ( $n = 1$ ). Some authors applied two or more species concepts including phylogenetic and biological species concept ( $n = 12$ ), evolutionary species concept and operational species delimitation criteria ( $n = 2$ ), general lineage species concept and operational species



**Fig. 1.** A comparison between the number of subspecies and species prior to the genetic analysis to the number of subspecies and species after the genetic analysis for the two reptile orders. (A) Testudines. (B) Squamata and for the two Squamata suborders. (C) Lacertilia. (D) Serpentes.

delimitation criteria ( $n = 2$ ), evolutionary species concept and biological species concept ( $n = 1$ ), and phylogenetic species concept and cohesion species concept ( $n = 1$ ).

Statistical power limited us to only evaluating the two most common species concepts (phylogenetic species concept, evolutionary species concept) regarding the influence of species concept on recommending species change. Authors using the phylogenetic species concept were significantly less likely to recommend a taxonomic change ( $n = 23$  recommendations,  $n = 64$  studies;  $P = 0.033$ ). In contrast, when the evolutionary species concept was applied the probability of recommending a taxonomic change did not differ from random ( $n = 11$  recommendations,  $n = 26$  studies;  $P = 0.557$ ). We then compared all studies that used a single species concept ( $n = 44$  recommendations,  $n = 106$  studies) to all the studies that used two species concepts ( $n = 16$  recommendations,  $n = 18$  studies) using a chi-square test. Authors that used a single species concept were less likely to make a recommendation than those that used multiple concepts ( $\chi^2 = 15.338$ ,  $df = 1$ ,  $P < 0.001$ ).

#### 4. Discussion

Genetic sequencing significantly altered reptile taxonomy by increasing the number of species and decreasing the number of subspecies. In particular, 60% of the 60 studies that recommended taxonomic change raised one or more subspecies to full species rank and 40% collapsed the subspecies while retaining a single species. These results suggest that Zink's (2004) conclusions, that there are too many subspecies currently recognized, for avian species taxonomy are also applicable to reptile taxonomy. Similar to the avian species, reptile genetic data often did not agree with the recognized subspecies that were based on morphological

differences (Bryson et al., 2007; Burbrink et al., 2008; Daza et al., 2009; Bellati et al., 2011) although, some studies showed the genetic data supported the recognized subspecies, in which case the authors did not recommend taxonomic changes (Walker et al., 1995; Ursenbacher et al., 2006; Fritz et al., 2012).

We were surprised that no study variables were able to predict the likelihood of a taxonomic change. However, this result further supports a lack of bias by factors that are typically considered important in taxonomic evaluation such as sample size, number of sample sites, loci used, or total number of base pairs sequenced. Typically, increasing the number of characters should increase the resolution and clade support (de Queiroz et al., 2002), but we did not find these variables to be indicative of taxonomic recommendation. For example, *Trimeresurus flavomaculatus* was originally comprised of 3 subspecies, and despite sequencing 4 mitochondrial genes, totaling 2424 bp, the authors still recommended that more data were required in order to recommend any taxonomic change (Slanders et al., 2004). The year of the selected studies ranged from 1996 to 2012, yet even with the changes of species concepts and improvements of technology the year of the study was not able to predict the likelihood of recommending taxonomic change.

Not surprisingly, we found that genetic distance values significantly differed between recommendations for elevating a subspecies and collapsing a subspecies. A method for determining if the percent genetic distance alters the taxonomy is the comparison of the subspecies in question to the closest sister taxon and then determining a threshold value for elevating to species. If the distance value is greater than the sister taxon this supports the elevation of the species whereas if it is less this supports either the collapse of the subspecies or maintaining the subspecies (Stuckas and Fritz, 2011). For example, Fritz et al. (2012) compared the *Trachemys scripta* subspecies (*T. s. subspecies 1* and *T. s. subspecies 2*) to *T. gageae* using the cytochrome B. The genetic distance between

the species (*T. scripta* and *T. gaigeae*) ranged from 1.47%–1.67%, thus they used 1.5% as the threshold value for recognizing different species. The genetic distance between *T. s. subspecies 1* and *T. s. subspecies 2* was considerably lower at 0.57%, thus the subspecies were not elevated. Using the comparative method shows that there is not a universal threshold value for elevating a subspecies, but needs to be done in a case-by-case system within its phylogenetic context. The comparative method agrees well with our results as we found that the midpoint genetic distances used for elevation ranged from 1.0% to 19.4%. However, the large difference between the median genetic distances used to elevate a subspecies (6.4%) compared to collapsing a subspecies (1.0%) shows that there is a consensus between the researchers that a higher genetic distance percentage is necessary for elevating a subspecies.

The species concept used also may complicate the recommendation for taxonomic change due to the variety of concepts. In the 124 studies we reviewed there were nine species concepts applied when evaluating the subspecies taxonomy. Applying different species concepts when evaluating subspecies creates uncertainty about the validity of the recommended change. For example, Rodríguez-Robles and De Jesús-Escobar's (2000) genetic data depicted the Louisiana pine snake (*Pituophis ruthveni*) as a paraphyletic species. Systematists that prefer monophyly would not recognize *P. ruthveni* as a species, but authors that apply the evolutionary species concept may recognize *P. ruthveni* as a species since *P. ruthveni* is an allopatric taxon with distinguishable morphological characteristics (Rodríguez-Robles and De Jesús-Escobar's, 2000).

The practice of raising subspecies to full species rank has occurred throughout other taxa, thus causing a rapid increase in the number of species (Marris, 2007; Gippoliti and Groves, 2013; Zachos, 2013). The species concept applied when recommending a taxonomic change is one of the main causes of taxonomic splitting (Zachos and Lovari, 2013). For example, the phylogenetic species concept has led to a large increase in the number of species within mammalian taxa (Marris, 2007; Zachos and Lovari, 2013) and within Reptilia this concept was the most common single species concept applied. Taxonomic splitting, when supported, provides essential taxonomic information; however, depending on the level of support raising a subspecies may present further complications in regards to their conservation and overall taxonomic status.

Regardless of the validity of the subspecies concept, the subspecies is still utilized in biology, and perhaps more importantly, because it is considered in legislation and enforcement it needs to be retained (Haig et al., 2006). Given that we found that genetic analysis significantly influences subspecies designations, scientists should determine a standard method to discriminate the species–subspecies boundary. De Queiroz (2007) suggested the Unified Species Concept (USC) stating that all species concepts are similar in theory, species are separately evolving metapopulation lineages, and the method to determine the species (i.e. morphological, phylogenetic, geographic, etc.) are considered as operational criteria (lines of evidence). The USC concept does not address subspecies; however, the integrative taxonomic approach is similar to the USC concept and can be applied for discerning species–species boundary (see Padial et al., 2010 for review). This approach incorporates different sets of characters as separate lines of evidence (e.g., molecular, morphological, behavioral, ecological, etc.) and then uses each line of evidence when considering species/subspecies taxonomic status (Miralles et al., 2010). Hawlitschek et al. (2012), used the integrative approach to evaluate *Lycodryas maculatus* (junior synonym, *L. sanctijohannis*), which is an endemic species in the Comoros Archipelago. The authors used mitochondrial and nuclear DNA and morphological data as the three main lines of evidence to diagnose the species. The mtDNA line of evidence supported differentiation between four island populations, the

mtDNA line of evidence had slight support for four distinct populations, and the morphological line of evidence supported two distinct populations. The authors considered all three lines of evidence and decided *Lycodryas* should be considered as two separate species each with two subspecies. The subjective nature of the process is highlighted by none of the three lines of evidence fully supporting each other, but by applying multiple analyses this integrative approach can increase the consensus regarding the subspecies recommendation (Hawlitschek et al., 2012). However, the integrative taxonomic approach remains underdevelopment, thus this concept lacks a consensual concept (see Padial et al., 2010 for review).

The concept of the integrative approach is not new, as scientists have suggested the need for multiple lines of evidence before altering species taxonomy for more than two decades (Ball and Avise, 1992). The importance of, and difficulty in, incorporating multiple data sets is highlighted by the differing rates of morphological and molecular evolution and the different rates of evolution among different loci (Zink, 2004). Historically, subspecies nomenclature was based more heavily on morphological traits, which tend to be polygenic (Schluter, 1984). Polygenic loci have increased additive variance suggesting morphological traits could provide evidence of isolation before it is detected in mtDNA gene trees or  $F_{ST}$  values (Zink, 2004). The integrative approach ensures that morphological, genetic, geographic, and other lines of evidence are all considered before changing taxonomy, thus maximizing the inclusivity of the data when designating the species–subspecies boundary.

The main limitations to the integrative approach are the substantial amounts of data required to effectively evaluate a species and there is no standard regarding the number of lines of evidence needed for analysis. Nevertheless, some form of a standardized method for evaluating subspecies status is desirable given that legislation, such as the Endangered Species Act, can be invoked to protect this taxonomic level (Fitzpatrick, 2010). The debate on the subspecies concept may become moot as the prices and difficulties of sequencing whole genomes becomes more commonplace, allowing scientists to distinguish subspecies based on all the relevant genetic information along with other equally important lines of evidence (morphological, geographical, behavioral, ecological, etc.). Until that time a rigorous and standardized concept will facilitate both biological understanding and management.

## 5. Conclusions

Whether or not the subspecies is merely a reflection of our need to further categorize species, there is a need to reflect on how we determine subspecies. We found evidence that genetic techniques are altering the subspecies taxonomy within reptiles. Instead of applying a single species concept, we recommend using the integrative taxonomic approach for discerning subspecies since multiple lines of evidence incorporates all available data when determining the species–subspecies boundary. The importance of an integrative boundary is highlighted by 79% (49 of 62) of the studies that applied a single species concept, and did not recommend a change in taxonomy, did suggest further analyses prior to changing the taxonomy. Genetic data are important lines of evidence as established by their influence in recommending taxonomic change, but this should not be to the exclusion of other lines of evidence when altering subspecies taxonomy. Determining the validity of the subspecies rank should no longer be argued since this classification is applied in management and legislation; rather, the focus should be on determining the best and most consistently reliable method to discern subspecies. We suggest that the integrative taxonomic approach provides a more objective method that could be effectively applied for research and conservation purposes.

## Acknowledgments

We thank G. Stamps, K. Nicholson, and two anonymous reviewers for comments, which greatly improved the quality of this manuscript.

## Appendix A

The list of species and studies included for the review.

Species	Citation
<i>Agkistrodon contortrix</i>	Soto et al. (2006)
<i>Atropoides nummifer</i>	Castoe et al. (2003)
<i>Charina bottae</i>	Rodríguez-Robles et al. (2001)
<i>Coluber constrictor</i>	Burbrink et al. (2008)
<i>Coronella austriaca</i>	Santos et al. (2008)
<i>Crotalus horridus</i>	Clark et al. (2003)
<i>Crotalus viridis</i>	Ashton and de Queiroz (2002)
<i>Diadophis punctatus</i>	Fontanella et al. (2008)
<i>Elaphe</i>	Burbrink (2002)
<i>guttata</i> [ <i>Pantherophis guttata</i> ]	
<i>Elaphe obsoleta</i>	Burbrink et al. (2000), Burbrink (2001)
[ <i>Pantherophis obsoleta</i> ]	
<i>Elaphe quatuorlineata</i>	Lenk et al. (2001)
<i>Hierophis viridiflavus</i>	Rato et al. (2009)
<i>Hypsiglena torquata</i>	Mulcahy (2008)
<i>Lachesis muta</i>	Zamudio and Greene (1997)
<i>Lampropeltis getula</i>	Pyron and Burbrink (2009)
<i>Lampropeltis mexicanamexicana</i>	Bryson et al. (2007)
<i>Lampropeltis pyromelana</i>	Burbrink et al. (2011)
<i>Lampropeltis triangulum</i>	Bryson et al. (2007)
<i>Lampropeltis zonata</i>	Rodríguez-Robles et al. (1999)
<i>Leptodeira annulata</i>	Daza et al. (2009)
<i>Leptodeira septentrionalis</i>	Daza et al. (2009)
<i>Leptodeira splendida</i>	Daza et al. (2009)
<i>Lichanura trivirgata</i>	Wood et al. (2008)
<i>Lycodryas sanctijohannis</i>	Hawliitschek et al. (2012)
<i>Macroprotodon brevis</i>	Carranza et al. (2004b)
<i>Macroprotodon cucullatus</i>	Carranza et al. (2004b)
<i>Madagascarophis colubrinus</i>	Nagy et al. (2007)
<i>Malpolon monspessulanus</i>	Carranza et al. (2006a)
<i>Natrix maura</i>	Guicking et al. (2008)
<i>Natrix natrix</i>	Fritz et al. (2012)
<i>Natrix tessellata</i>	Guicking et al. (2009)
<i>Nerodia erythrogaster</i>	Makowsky et al. (2010)
<i>Pituophis deppei</i>	Bryson et al. (2011)
<i>Pituophis lineaticollis</i>	Bryson et al. (2011)
<i>Pituophis melanoleucus</i>	Rodríguez-Robles and De Jesús-Escobar (2000)
<i>Protobothrops jerdonii</i>	Guo et al. (2009)
<i>Python curtus</i>	Keogh et al. (2001)
<i>Rhabdophis tigrinus</i>	Takeuchi et al. (2012)
<i>Sistrurus catenatus</i>	Kubatko et al. (2011)
<i>Sistrurus miliarius</i>	Kubatko et al. (2011)
<i>Thamnophis elegans</i>	Bronikowski and Arnold (2001)
<i>Thamnophis sirtalis</i>	Janzen et al. (2002)
<i>Trimeresurus flavomaculatus</i>	Slanders et al. (2004)
<i>Trimorphodon biscutatus</i>	Devitt (2006)
<i>Viper ammodytes</i>	Ursenbacher et al. (2008)
<i>Vipera aspis</i>	Ursenbacher et al. (2006)

## Appendix A (continued)

Species	Citation
<i>Vipera berus</i>	Ursenbacher et al. (2006)
<i>Acontias lineatus</i>	Daniels et al. (2002)
<i>Acontias meleagris</i>	Daniels et al. (2002)
<i>Ameiva chrysolema</i>	Gifford et al. (2004)
<i>Anolis brevirostris</i>	Glor and Laport (2012)
<i>Anolis distichus</i>	Glor and Laport (2012)
<i>Callisaurus draconoides</i>	Lindell et al. (2005)
<i>Cryptoblepharus boutonii</i>	Rocha et al. (2006)
<i>Diplodactylus granariensis</i>	Oliver et al. (2007)
<i>Egernia (Liopholis) multiscutata</i>	Chapple et al. (2004)
<i>Eremias persica</i>	Rastegar Pouyani et al. (2010)
<i>Eumeces (Plestiodon) gilberti</i>	Richmond and Reeder (2002)
<i>Eumeces (Plestiodon) skiltonianus</i>	Richmond and Reeder (2002)
<i>Gallotia atlantica</i>	González et al. (1996)
<i>Gallotia galloti</i>	González et al. (1996)
<i>Gymnodactylus geckoides</i>	Pellegrino et al. (2005)
<i>Hemidactylus bouvieri</i>	Arnold et al. (2008)
<i>Iberolacerta cyreni</i>	Arribas and Carranza (2004)
<i>Lacerta (Iberolacerta) cyreni</i>	Carranza et al. (2004a)
<i>Lacerta (Scelarcis) perspicillata</i>	Perera et al. (2007)
<i>Lacerta bilineata</i>	Böhme et al. (2007)
<i>Lacerta dugesii (Teira dugesii)</i>	Brehm et al. (2003)
<i>Lacerta lepida (Timon lepida)</i>	Paulo et al. (2008)
<i>Lacerta viridis</i>	Böhme et al. (2007)
<i>Laudakia caucasia</i>	Macey et al. (1998)
<i>Laudakia erythrogastra</i>	Macey et al. (1998)
<i>Liolaemus monticola</i>	Torres-Pérez et al. (2009)
<i>Pachydactylus rugosus</i>	Lamb and Bauer (2000)
<i>Pedioplanis lineocellata</i>	Makokha et al. (2007)
<i>Phrynosoma douglasi</i>	Zamudio et al. (1997)
<i>Platysaurus intermedius</i>	Scott et al. (2004)
<i>Plestiodon brevirostris</i>	Feria-Ortiz et al. (2011)
<i>Podarcis erhardii</i>	Poulakakis et al. (2003)
<i>Podarcis melisellensis</i>	Podnar et al. (2004)
<i>Podarcis muralis</i>	Bellati et al. (2011)
<i>Podarcis taurica</i>	Poulakakis et al. (2005)
<i>Psammmodromus algirus</i>	Carranza et al. (2006b)
<i>Sceloporus jarrovii</i>	Wiens and Penkrot (2002)
<i>Sceloporus magister</i>	Leaché and Mulcahy (2007)
<i>Sceloporus mucronatus</i>	Martínez-Méndez and Méndez-De La Cruz (2007)
<i>Sceloporus serrifer</i>	Martínez-Méndez et al. (2012)
<i>Sceloporus undulatus</i>	Leaché and Reeder (2002)
<i>Sceloporus zosteromus</i>	Leaché and Mulcahy (2007)
<i>Sphaerodactylus fantasticus</i>	Thorpe et al. (2008)
<i>Sphenomorphus (Pinoyscincus) abdictus</i>	Linkem et al. (2010)
<i>Takydromus sexlineatus</i>	Ota et al. (2002)
<i>Uromastyx aegyptia</i>	Wilms et al. (2009)
<i>Uromastyx dispar</i>	Wilms et al. (2009)
<i>Apalone spinifera</i>	McGaugh et al. (2008)
<i>Batagur affinis</i>	Praschag et al. (2009)
<i>Chelydra serpentina</i>	Walker et al. (1998)

(continued on next page)

## Appendix A (continued)

Species	Citation
<i>Chrysemys picta</i>	Starkey et al. (2003)
<i>Cuora galbinifrons</i>	Stuart and Parham (2004)
<i>Emys (Actinemys) marmorata</i>	Spinks and Shaffer (2005)
<i>Emys orbicularis</i>	Fritz et al. (2005)
<i>Hardella thurjii</i>	Praschag et al. (2007)
<i>Homopus signatus</i>	Daniels et al. (2010)
<i>Kinixys belliana</i>	Kindler et al. (2012)
<i>Kinosternon flavescens</i>	Serb et al. (2001)
<i>Lissemys punctata</i>	Praschag et al. (2011)
<i>Mauremys caspica</i>	Fritz et al. (2008)
<i>Mauremys leprosa</i>	Fritz et al. (2006b)
<i>Pangshura smithii</i>	Praschag et al. (2007)
<i>Pangshura tentoria</i>	Praschag et al. (2007)
<i>Pelomedusa subrufa</i>	Vargas-Ramírez et al. (2010)
<i>Pelusios castanoides</i>	Silva et al. (2010)
<i>Pyxis arachnoides</i>	Chiari et al. (2005)
<i>Rhinoclemmys pulcherrima</i>	Le and McCord (2008)
<i>Sternotherus minor</i>	Walker et al. (1995)
<i>Stigmochelys pardalis</i>	Fritz et al. (2010)
<i>Terrapene carolina</i>	Butler et al. (2011)
<i>Testudo graeca</i>	Fritz et al. (2007)
<i>Testudo hermanni</i>	Fritz et al. (2006a)
<i>Testudo horfieldii</i>	Fritz et al. (2009)
<i>Trachemys decussata</i>	Fritz et al. (2012)
<i>Trachemys dorbigni</i>	Fritz et al. (2012)
<i>Trachemys scripta</i>	Fritz et al. (2012)
<i>Trachemys venusta</i>	Fritz et al. (2012)

## Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympve.2014.03.011>.

## References

- Amadon, D., Short, L.L., 1992. Taxonomy of lower categories—suggested guidelines. *Bull. Br. Ornithol. Club.* 112A, 11–38.
- Amadon, D., 1949. The seventy-five per cent rule for subspecies. *Condor* 51, 250–258.
- Arnold, E.N., Vasconcelos, R., Harris, D.J., Mateo, J.A., Carranza, S., 2008. Systematics, biogeography and evolution of the endemic *Hemidactylus* geckos (Reptilia, Squamata, Gekkonidae) of the Cape Verde Islands: based on morphology and mitochondrial and nuclear DNA sequences. *Zool. Scripta*, 37, 619–636.
- Arribas, O., Carranza, S., 2004. Morphological and genetic evidence of the full species status of *Iberolacerta cyreni* *martinezricai* (Arribas 1996). *Zootaxa* 634, 1–24.
- Ashton, K.G., de Queiroz, A., 2002. Molecular systematics of the Western Rattlesnake, *Crotalus viridis* (Viperidae), with comments on the utility of the d-loop in phylogenetic studies of snakes. *Mol. Phylogeny. Evol.* 21, 176–189.
- Ball, R.M., Avise, J.C., 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* 109, 626–636.
- Bellati, A., Pellitteri-Rosa, D., Sacchi, R., Nistri, A., Galimberti, A., Casiraghi, M., Fasola, M., Galeotti, P., 2011. Molecular survey of morphological subspecies reveals new mitochondrial lineages in *Podarcis muralis* (Squamata: Lacertidae) from the Tuscan archipelago (Italy). *J. Zool. Syst. Evol.* 49, 240–250.
- Böhme, M.U., Fritz, U., Kotenko, T., Dzukic, G., Ljubisavljevic, K., Tzankov, N., Berendonk, T.U., 2007. Phylogeography and cryptic variation within the *Lacerta viridis* complex (Lacertidae, Reptilia). *Zool. Scripta*, 36, 119–131.
- Brehm, A., Jesus, J., Spinola, H., Alves, C., Vicente, L., Harris, D.J., 2003. Phylogeography of Madeiran endemic lizard *Lacerta dugesii* inferred from mtDNA sequences. *Mol. Phylogeny. Evol.* 26, 222–230.
- Bronikowski, A.M., Arnold, S.J., 2001. Cytochrome b phylogeny does not match subspecific classification in the western terrestrial garter snake, *Thamnophis elegans*. *Copeia* 2, 508–513.
- Bryson Jr., R.W., Pastorini, J., Burbrink, F.T., Forstner, M.R.J., 2007. A phylogeny of the *Lampropeltis mexicana* complex (Serpentes: Colubridae) based on mitochondrial DNA sequences suggests evidence for species-level polyphyly within *Lampropeltis*. *Mol. Phylogeny. Evol.* 43, 674–684.
- Bryson Jr., R.W., García-Vázquez, U.O., Riddle, B.R., 2011. Phylogeography of middle American gophersnakes: mixed responses to biogeographical barriers across the Mexican transition zone. *J. Biogeogr.* 38, 1570–1584.
- Burbrink, F.T., Lawson, R., Slowinski, J.B., 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* 54, 2107–2118.
- Burbrink, F.T., Fontanella, F., Pyron, A., Guiher, T.J., Jimenez, C., 2008. Phylogeography across a continent: the evolutionary & demographic history of the North American racer. *Mol. Phylogeny. Evol.* 47, 274–288.
- Burbrink, F.T., Yao, H., Ingrassi, M., Bryson Jr, R.W., Guiher, T.J., Ruane, S., 2011. Speciation at the Mogollon Rim in the Arizona mountain kingsnake (*Lampropeltis pyromelana*). *Mol. Phylogeny. Evol.* 60, 445–454.
- Burbrink, F., 2001. Systematics of the eastern ratsnake complex (*Elaphe obsoleta*). *Herpetol. Monogr.* 15, 1–53.
- Burbrink, F.T., 2002. Phylogeographic analysis of the cornsnake (*Elaphe guttata*) complex as inferred from maximum likelihood and Bayesian analyses. *Mol. Phylogeny. Evol.* 25, 465–476.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference*, second ed. Springer, New York.
- Butler, J.M., Dodd Jr, C.K., Aresco, M., Austin, J.D., 2011. Morphological and molecular evidence indicates that the gulf coast box turtle (*Terrapene carolina major*) is not distinct evolutionary lineage in the Florida panhandle. *Biol. J. Linn. Soc.* 102, 889–901.
- Carranza, S., Arnold, E.N., Amat, F., 2004a. DNA phylogeny of *Lacerta (Iberolacerta)* and other lacertine lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction. *Syst. Biodivers.* 2, 57–77.
- Carranza, S., Arnold, E.N., Wade, E., Fahd, S., 2004b. Phylogeography of the false smooth snakes, *Macroprotodon* (Serpentes, Colubridae): mitochondrial DNA sequences show European populations arrived recently from northwest Africa. *Mol. Phylogeny. Evol.* 33, 523–532.
- Carranza, S., Arnold, E.N., Pleguezuelos, J.M., 2006a. Phylogeny, biogeography, and evolution of two Mediterranean snakes, *Malpolon monspessulanus* and *Hemorrhhois hippocrepis* (Squamata, Colubridae), using mtDNA sequences. *Mol. Phylogeny. Evol.* 40, 532–546.
- Carranza, S., Harris, D.J., Arnold, E.N., Batista, V., Gonzalez de la Vega, J.P., 2006b. Phylogeography of the lacertid lizard, *Psammotromus algirus*, in Iberia and across the Strait of Gibraltar. *J. Biogeogr.* 33, 1279–1288.
- Castoe, T.A., Chippindale, P.T., Campbell, J.A., Ammerman, L.K., Parkinson, C.L., 2003. Molecular systematics of the middle American jumping pitvipers (Genus *Atropoides*) and phylogeography of the *Atropoides nummifer* complex. *Herpetologica* 59, 420–431.
- Chapple, D.G., Keogh, J.S., Hutchinson, M.N., 2004. Molecular phylogeography and systematics of the arid-zone members of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Mol. Phylogeny. Evol.* 33, 549–561.
- Chiari, Y., Thomas, M., Pedrono, M., Vieites, D.R., 2005. Preliminary data on genetic differentiation within the Madagascar spider tortoise, *Pyxis arachnoides*. *Salamandra* 41, 35–43.
- Clark, A.M., Moler, P.E., Possardt, E.E., Savitzky, A.H., Brown, W.S., Bowen, B.W., 2003. Phylogeography of the Timber Rattlesnake (*Crotalus horridus*) based on mtDNA sequences. *J. Herpetol.* 37, 145–154.
- Cracraft, J., 1983. Species concepts and speciation analysis. *Curr. Ornithol.* 1, 159–187.
- Cracraft, J., 1989. Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. *Spec. Consequences* 1989, 28–59.
- Daniels, S.R., Heideman, N., Hendricks, M., Wilson, B., 2002. A molecular phylogeny for south Africa limbless lizard taxa of the subfamily Acontina (Sauria: Scincidae) with special emphasis on relationships within *Acontias*. *Mol. Phylogeny. Evol.* 24, 315–323.
- Daniels, S.R., Hofmeyr, M.D., Henen, B.T., Baard, E.H., 2010. Systematics and phylogeography of a threatened tortoise, the speckled padloper. *Anim. Conserv.* 13, 237–246.
- Daza, J.M., Smith, E.N., Páez, V.P., Parkinson, C.L., 2009. Complex evolution in the neotropics: the origin and diversification of the widespread genus *Leptodeira* (Serpentes: Colubridae). *Mol. Phylogeny. Evol.* 53, 653–667.
- de Queiroz, A., Lawson, R., Lemos-Espinal, J.A., 2002. Phylogenetic relationships of north American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much dna sequence is enough? *Mol. Phylogeny. Evol.* 22, 315–329.
- de Queiroz, K., 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: Howard, D.J., Berlocher, S.H. (Eds.), *Endless Forms: Species and Speciation*. Oxford University Press, New York, pp. 57–75.
- de Queiroz, K., 2007. Species concepts and species delimitation. *Syst. Biol.* 56, 879–886.
- Devitt, T.J., 2006. Phylogeography of the western lyresnake (*Trimorphodon biscutatus*): testing aridland biogeographical hypotheses across the Nearctic–Neotropical transition. *Mol. Ecol.* 15, 4387–4407.
- Feria-Ortiz, M., Manríquez-Morán, N.L., Nieto-Montes de Oca, A., 2011. Species limits based on mtDNA and morphological data in the polytypic species *Plestiodon brevirostris* (Squamata: Scincidae). *Herpetol. Monogr.* 25, 25–51.
- Fitzpatrick, J.W., 2010. Chapter 5: Subspecies are for Convenience. *Ornithol. Monogr.* 67, 54–61.
- Fontanella, F.M., Feldman, C.R., Siddall, M.E., Burbrink, F.T., 2008. Phylogeography of *Diadophis punctatus*: extensive lineage diversity and repeated patterns of

- historical demography in a trans-continental snake. *Mol. Phylogen. Evol.* 46, 1049–1070.
- Frankham, R., Ballou, J.D., Dudash, M.R., Eldridge, M.D.B., Fenster, C.B., Lacy, R.C., Mendelson III, J.R., Porton, I.J., Ralls, K., Ryder, O.A., 2012. Implications of different species concepts for conserving biodiversity. *Biol. Con.* 153, 25–31.
- Fritz, U., Fattizzo, T., Guicking, D., Tripepi, S., Pennisi, M.G., Lenk, P., Joger, U., Wink, M., 2005. A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys* (Reptilia, Testudines, Emydidae). *Zool. Scr.* 34, 351–371.
- Fritz, U., Auer, M., Bertolero, A., Cheylan, M., Fattizzo, T., Hundsdörfer, A.K., Sampayo, M.M., Pretus, J.L., Široký, P., Wink, M., 2006a. A rangewide phylogeography of Hermann's tortoise, *Testudo hermanni* (Reptilia: Testudines: Testudinidae): implications for taxonomy. *Zool. Scr.* 35, 531–543.
- Fritz, U., Barata, M., Busack, S.D., Fritzsche, G., Castilho, R., 2006b. Impact of mountain chains, sea straits and peripheral populations on genetic and taxonomic structure of a freshwater turtle, *Mauremys leprosa* (Reptilia, Testudines, Geomydidae). *Zool. Scr.* 35, 97–108.
- Fritz, U., Hundsdörfer, A.K., Široký, P., Auer, M., Kami, H., Lehmann, J., Mazanaeva, L.F., Türkozan, O., Wink, M., 2007. Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western palaearctic tortoises (*Testudo graeca* complex; Testudines, Testudinidae). *Amphibia-Reptilia* 28, 97–121.
- Fritz, U., Ayaz, D., Buschbom, J., Kami, H.G., Mazanaeva, L.F., Aloufi, A.A., Auer, M., Rifai, L., Silic, T., Hundsdörfer, A.K., 2008. Go east: phylogeographies of *Mauremys caspica* and *M. rivulata* – discordance of morphology, mitochondrial and nuclear genomic markers and rare hybridization. *J. Evol. Biol.* 21, 527–740.
- Fritz, U., Auer, M., Chirikova, M.A., Duysbayeva, T.N., Eremchenko, V.K., Kami, H.G., Kashkarov, R.D., Masroor, R., Moodley, Y., Pindrani, A., Široký, P., Hundsdörfer, A.K., 2009. Mitochondrial diversity of the widespread central Asian steppe tortoise (*Testudo horsfieldii* Gray, 1844): implications for taxonomy and relocation of confiscated tortoises. *Amphibia-Reptilia* 30, 245–257.
- Fritz, U., Daniels, S.R., Hofmeyer, M.D., González, J., Barrio-Amorós, C.L., Široký, P., Hundsdörfer, A.K., Stuckas, H., 2010. Mitochondrial phylogeography and subspecies of the wide-ranging sub-Saharan leopard tortoise *Stigmochelys pardalis* (Testudines: Testudinidae) – a case study for the pitfalls of pseudogenes and GenBank sequences. *J. Zool. Syst. Evol. Res.* 48, 348–359.
- Fritz, U., Stuckas, H., Vargas-Ramírez, M., Hundsdörfer, A.K., Maran, J., Päckert, M., 2012. Molecular phylogeny of central and south American slider turtles: implications for biogeography and systematics (Testudines: Emydidae: *Trachemys*). *J. Zool. Syst. Evol. Res.* 50, 125–136.
- Genner, M.J., Turner, G.F., 2012. Ancient hybridization and phenotypic novelty within Lake Malawi's cichlid fish radiation. *Mol. Biol. Evol.* 29, 195–206.
- Gifford, M.E., Powell, R., Larson, A., Gutberlet Jr, R.L., 2004. Population structure of a phenotypically variable teiid lizard (*Ameiva chrysolaelma*) from Hispaniola: the influence of a geologically complex island. *Mol. Phylogen. Evol.* 32, 735–748.
- Gippoliti, S., Groves, C.P., 2013. "Taxonomic inflation" in the historical context of mammalogy and conservation. *Hystrix, Ital. J. Mammal.* doi: 10.4404/hystrix-23.2-8685.
- Glor, R.E., Laport, R.G., 2012. Are subspecies of Anolis lizards that differ in dewlap color and pattern also genetically distinct? A mitochondrial analysis. *Mol. Phylogen. Evol.* 64, 255–260.
- González, P., Pinto, F., Nogales, M., Jiménez-Asensio, J., Hernández, M., Cabrera, V.M., 1996. Phylogenetic relationships of the Canary Islands endemic lizard genus *Gallotia* (Sauria: Lacertidae), inferred from mitochondrial DNA sequences. *Mol. Phylogen. Evol.* 6, 63–71.
- Groves, C., 2012. Species concept in primates. *Am. J. Primatol.* 74, 687–691.
- Guicking, D., Joger, U., Wink, M., 2008. Molecular phylogeography of the viperine snake *Natrix maura* (Serpentes: Colubridae): evidence for strong intraspecific differentiation. *Org. Divers. Evol.* 8, 130–145.
- Guicking, D., Joger, U., Wink, M., 2009. Cryptic diversity in an Eurasian water snake (*Natrix tessellata*, Serpentes: Colubridae): Evidence from mitochondrial sequence data and nuclear ISSR-PCR fingerprinting. *Org. Divers. Evol.* 9, 201–214.
- Guo, P., Malhotra, A., Li, C., Creer, S., Pook, C.E., Wen, T., 2009. Systematics of the *Protobothrops jerdonii* complex (Serpentes, Viperidae, Crotalinae) inferred from morphometric data and molecular phylogeny. *Herpetol. J.* 19, 85–96.
- Haig, S.M., Beaver, E.A., Chambers, S.M., Draheim, H.M., Dugger, B.D., Dunham, S., Elliott-Smith, E., Fontaine, J.B., Kesler, D.C., Knaus, B.J., Lopes, I.F., Loschl, P., Mullins, T.D., Sheffield, L.M., 2006. Taxonomic considerations in listing subspecies under the U.S. endangered species act. *Biology* 20, 1584–1594.
- Hawltitschek, O., Nagy, N.T., Glaw, F., 2012. Island evolution and systematic revision of comoran snakes: why and when subspecies still make sense. *PLoS ONE* 7, e42970.
- Janzen, F.J., Krenz, J.G., Haselkorn, T.S., Brodie, E.D., Brodie, E.D., 2002. Molecular phylogeography of common garter snakes (*Thamnophis sirtalis*) in western North America: implications for regional historical forces. *Mol. Ecol.* 11, 1739–1751.
- Keogh, J.S., Barker, D.G., Shine, R., 2001. Heavily exploited but poorly known: systematics and biogeography of commercially harvested pythons (*Python curtus* group) in Southeast Asia. *Biol. J. Linn. Soc.* 73, 113–129.
- Kindler, C., Branch, W.R., Hofmeyer, M.D., Maran, J., Široký, P., Vences, M., Harvey, J., Hauswaldt, J.S., Schleicher, A., Stuckas, H., Fritz, U., 2012. Molecular phylogeny of African hinge-back tortoises (*Kinixys*): implications for phylogeography and taxonomy (Testudines: Testudinidae). *J. Zool. Syst. Evol. Res.* 50, 192–201.
- Kubatko, L.S., Gibbs, H.L., Bloomquist, E.W., 2011. Inferring species-level phylogenies and taxonomic distinctiveness using multilocus data in *Sistrurus* rattlesnakes. *Syst. Biol.* 60, 393–409.
- Lamb, T., Bauer, A.M., 2000. Relationships of the *Pachydactylus rugosus* group of geckos (Reptilia: Squamata: Geckkonidae). *Afr. Zool.* 35, 55–67.
- Le, M., McCord, W.P., 2008. Phylogenetic relationships and biogeographical history of the genus *Rhinoclemmys* Fitzinger, 1835 and the monophyly of the turtle family Geomydidae (Testudines: Testudinoidea). *Zool. J. Linn. Soc.* 153, 751–767.
- Leaché, A.D., Mulcahy, D.G., 2007. Phylogeny, divergence times and species limits of spiny lizards (*Sceloporus magister* species group) in western north American deserts and Baja California. *Mol. Ecol.* 16, 5216–5233.
- Leaché, A.D., Reeder, T.W., 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Syst. Biol.* 51, 44–68.
- Lenk, P., Joger, U., Wink, M., 2001. Phylogenetic relationships among European ratsnakes of the genus *Elaphe* Fitzinger based on mitochondrial DNA sequence comparisons. *Amphibia-Reptilia* 22, 329–339.
- Lepage, D., 2014. *Avibase – the World Bird Database*.
- Lindell, J., Méndez-de la Cruz, F.R., Murphy, R.W., 2005. Deep genealogical history without population differentiation: discordance between mtDNA and allozyme divergence in the zebra-tailed lizard (*Callisaurus draconoides*). *Mol. Phylogen. Evol.* 36, 682–694.
- Linkem, C.W., Hesed, K.M., Diesmos, A.C., Brown, R.M., 2010. Species boundaries and cryptic lineage diversity in a Philippine forest skink complex (Reptilia: Squamata; Scincidae: *Lygosominae*). *Mol. Phylogen. Evol.* 56, 572–585.
- Macey, J.R., Schulte II, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shammakov, S.M., Papenfuss, T.J., 1998. Phylogenetic relationships among agamid lizards of the *Laudakia caucasia* species group: testing hypotheses of biogeographic fragmentation and an area cladogram for the Iranian plateau. *Mol. Phylogen. Evol.* 10, 118–131.
- Makokha, J.S., Bauer, A.M., Mayer, W., Matthee, C.A., 2007. Nuclear and mtDNA-based phylogeny of southern African sand lizards, *Pedioplanis* (Sauria: Lacertidae). *Mol. Phylogen. Evol.* 44, 622–633.
- Makowsky, R., Marshall Jr, J.C., McVay, J., Chippindale, P.T., Rissler, L.J., 2010. Phylogeographic analysis and environmental niche modeling of the plain-bellied watersnake (*Nerodia erythrogaster*) reveals low levels of genetic and ecological differentiation. *Mol. Phylogen. Evol.* 55, 985–995.
- Marris, E., 2007. Linnaeus at 300: The species and the specious. *Nature* 446 (7133), 250–253.
- Marshall, J.T., 1967. Parallel variation in North and Middle American screech-owls. *Monogr. West. Found. Vertebr. Zool.* 1, 1–72.
- Martínez-Méndez, N., Méndez-De La Cruz, F.R., 2007. Molecular phylogeny of the *Sceloporus torquatus* species-group (Squamata: Phrynosomatidae). *Zootaxa* 1609, 73–68.
- Martínez-Méndez, N., Meja, O., Rocha-Gómez, A., Méndez-De La Cruz, F.R., 2012. Morphological convergence and molecular divergence: the taxonomic status of *Sceloporus serrifer* (squamata, phrynosomatidae) subspecies. *Zool. Scr.* 41, 97–108.
- Mayr, E., Ashlock, P.D., 1991. Principles of systematic zoology, second ed. McGraw-Hill Inc., New York.
- Mayr, E., 1942. Systematics and the Origin of Species. Columbia University Press, New York, NY.
- Mayr, E., 1963. Animal Species and Evolution. The Belknap Press of Harvard University Press, Massachusetts.
- Mayr, E., 1970. Populations, Species and Evolution. The Belknap Press of Harvard University, Cambridge, Massachusetts.
- Mayr, E., 1982. Of what use are subspecies? *Auk* 3, 593–595.
- McGaugh, S.E., Eckerman, C.M., Janzen, F.J., 2008. Molecular phylogeography of *Apalone spinifer* (Reptilia, Trionychidae). *Zool. Scr.* 37, 289–304.
- Miralles, A., Vasconcelos, R., Perera, A., Harris, D.J., Carranza, S., 2010. An integrative taxonomic revision of the cape Verdean skinks (Squamata, Scincidae). *Zool. Scr.* 40, 16–44.
- Mulcahy, D.G., 2008. Phylogeography and species boundaries of the western north American nightsnake (*Hypsiglena torquata*): revisiting the subspecies concept. *Mol. Phylogen. Evol.* 46, 1095–1115.
- Nagy, Z.T., Glaw, F., Andreone, F., Wink, M., Vences, M., 2007. Species boundaries in Malagasy snakes of the genus *Madagascrophis* (Serpentes: Colubridae sensu lato) assessed by nuclear and mitochondrial markers. *Org. Divers. Evol.* 7, 241–251.
- Oliver, P., Hugall, A., Adams, M., Cooper, S.J.B., Hutchinson, M., 2007. Genetic elucidation of cryptic and ancient diversity in a group of Australian dipodactyline geckos: the *Diplodactylus vittatus* complex. *Mol. Phylogen. Evol.* 44, 77–88.
- Ota, H., Honda, M., Chen, S.L., Hikida, T., Panha, S., Oh, H.S., Matsui, M., 2002. Phylogenetic relationships, taxonomy, character evolution and biogeography of the lacertid lizards of the genus *Takydromus* (Reptilia: Squamata): a molecular perspective. *Biol. J. Linn. Soc.* 76, 493–509.
- Padial, J.M., Miralles, A., De la Riva, I., Vences, M., 2010. The integrative future of taxonomy. *Front. Zool.* 7, 16.
- Patten, M.A., Unitt, P., 2002. Diagnosability versus mean differences of sage sparrow subspecies. *Auk* 119, 26–35.



- Paulo, O.S., Pinheiro, J., Miraldo, A., Bruford, M.W., Jordan, W.C., Nichols, R.A., 2008. The role of vicariance vs. dispersal in shaping genetic patterns in ocellated lizard species in the western Mediterranean. *Mol. Ecol.* 17, 1535–1551.
- Pellegrino, K.C., Rodrigues, M.T., Waite, A.N., Morando, M., Yassuda, Y.Y., Sites Jr., J.W., 2005. Phylogeography and species limits in the *Gymnodactylus darwini* complex (Gekkonidae, Squamata): genetic structure coincides with river systems in the Brazilian Atlantic forest. *Biol. J. Linn. Soc.* 85, 13–26.
- Perera, A., Vasconcelos, R., Harris, D.J., Brown, R.P., Carretero, M.A., Pérez-Mellado, V., 2007. Complex patterns of morphological and mtDNA variation in *Lacerta perspicillata* (Reptilia; Lacertidae). *Biol. J. Linn. Soc.* 90, 479–490.
- Phillimore, A.B., Owens, I.P.F., 2006. Are subspecies useful in evolutionary and conservation biology? *Proc. R. Soc. B.* 273, 1049–1053.
- Podnar, M., Mayer, W., Trvrković, N., 2004. Mitochondrial phylogeography of the Dalmatian wall lizard, *Podarcis melisellensis* (Lacertidae). *Org. Divers. Evol.* 4, 307–317.
- Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E., 2003. Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae). *Mol. Phylog. Evol.* 28, 38–46.
- Poulakakis, N., Lymberakis, P., Valakos, E., Pafilis, P., Zouros, E., Mylonas, M., 2005. Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. *Mol. Ecol.* 14, 2433–2443.
- Praschag, P., Hundsdorfer, A.K., Fritz, U., 2007. Phylogeny and taxonomy of endangered south and south-east Asian freshwater turtles elucidated by mtDNA sequence variation (Testudines: Geoemydidae: *Batagur*, *Callagur*, *Hardella*, *Kachuga*, *Pangshura*). *Zool. Scr.* 36, 429–442.
- Praschag, P., Holloway, R., Georges, A., Packert, M., Hundsdorfer, A.K., Fritz, U., 2009. A new subspecies of *Batagur affinis* (Cantor, 1847), one of the world's most critically endangered chelonians (Testudines: Geoemydidae). *Zootaxa* 2233, 57–68.
- Praschag, P., Stuckas, H., Packert, M., Maran, J., Fritz, U., 2011. Mitochondrial DNA sequences suggest a revised taxonomy of Asian flapshell turtles (*Lissemys*, Smith, 1931) and the validity of previously unrecognized taxa (Testudines: Trionychidae). *Vertebr. Zool.* 61, 147–160.
- Pyron, R.A., Burbrink, F.T., 2009. Systematics of the common kingsnake (*Lampropeltis getula*; Serpentes: Colubridae) and the burden of heritage in taxonomy. *Zootaxa* 2241, 22–23.
- R Development Core Team 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <<http://www.R-project.org/>>.
- Rastegar Pouyani, E., Rastegar Pouyani, N., Kazemi Nourini, S., Joger, U., Wink, U.M., 2010. Molecular phylogeny of the *Eremias persica* complex of the Iranian plateau (Reptilia: Lacertidae), based on mtDNA sequences. *Zool. J. Linn. Soc.* 158, 641–660.
- Rato, C., Zuffi, M.A.L., Corti, C., Fornasiero, S., Gentili, A., Razzetti, E., Scali, S., Carretero, M.A., Harris, D.J., 2009. Phylogeography of the European whip snake, *Hierophis viridiflavus* (Colubridae), using mtDNA and nuclear DNA sequences. *Amphibia-Reptilia* 30, 283–289.
- Reeder, D.M., Helgen, K.M., Wilson, D.E., 2007. Global trends and biases in new mammal species discoveries. *Occasional Papers, Museum of Texas Tech University*. 269, 1–36.
- Reiserer, R.S., Schuett, G.W., Beck, D.D., 2013. Taxonomic reassessment and conservation status of the beaded lizard, *Heloderma horridum* (Squamata: Helodermatidae). *Amphib. Reptil. Conserv.* 7, 74–96.
- Richmond, J.Q., Reeder, T.W., 2002. Evidence for parallel ecological speciation in scincid lizards of the *Eumeces skiltonianus* species group (Squamata: Scincidae). *Evolution* 56, 1498–1513.
- Rocha, S., Carretero, M.A., Vences, M., Glaw, F., Harris, D.J., 2006. Deciphering patterns of transoceanic dispersal: the evolutionary origin and biogeography of coastal lizards (*Cryptoblepharus*) in the western Indian Ocean region. *J. Biogeogr.* 33, 13–22.
- Rodríguez-Robles, J.A., De Jesús-Escobar, J.M., 2000. Molecular systematics of the new world gopher, bull, and pinesnakes (Pituophis: Colubridae), a transcontinental species complex. *Mol. Phylog. Evol.* 14, 35–50.
- Rodríguez-Robles, J.A., Denardo, D.F., Staubs, R.E., 1999. Phylogeography of the California mountain kingsnake, *Lampropeltis zonata* (Colubridae). *Mol. Ecol.* 8, 1923–1934.
- Rodríguez-Robles, J.A., Stewart, G.R., Papenfuss, T.J., 2001. Mitochondrial DNA-based phylogeography of north American rubber boas, *Charina bottae* (Serpentes: Boidae). *Mol. Phylog. Evol.* 18, 227–237.
- Santos, X., Roca, J., Pleguezuelos, J.M., Donaire, D., Carranza, S., 2008. Biogeography and evolution of the smooth snake *Coronella austriaca* (Serpentes: Colubridae) in the Iberian Peninsula: evidence for messinian refuges and Pleistocene range expansions. *Amphibia-Reptilia* 29, 35–47.
- Schluter, D., 1984. Morphological and phylogenetic relations among the Darwin's finches. *Evolution*, 921–930.
- Scott, I.A.W., Keogh, J.S., Whiting, M., 2004. Shifting sands and shifty lizards: molecular phylogeny and biogeography of African flat lizards (*Platysaurus*). *Mol. Phylog. Evol.* 31, 618–629.
- Serb, J.M., Phillips, C.A., Iverson, J.B., 2001. Molecular phylogeny and biogeography of *Kinosternon flavescens* based on complete mitochondrial control region sequences. *Mol. Phylog. Evol.* 18, 149–162.
- Silva, A., Rocha, S., Gerlach, J., Rocamora, G., Dufrenoy, A., Harris, D.J., 2010. Assessment of mtDNA genetic diversity within the terrapins *Pelusios subniger* and *Pelusios castanoides* across the Seychelles islands. *Amphibia-Reptilia* 31, 583–588.
- Slanders, K.L., Malhotra, A., Thorpe, R.S., 2004. Ecological diversification in a group of Indomalayan pitvipers (*Trimeresurus*): convergence in taxonomically important traits has implications for species identification. *J. Evol. Biol.* 17, 721–731.
- Sokal, R.R., Sneath, P.H.A., 1963. Principles of Numerical Taxonomy. WH Freeman, San Francisco.
- Soto, J.G., Powell, R.L., Reyes, S.R., Wolana, L., Swanson, L.J., Sanchez, E.E., Perez, J.C., 2006. Genetic variation of a disintegrin gene found in the American copperhead snake (*Agkistrodon contortrix*). *Gene* 373, 1–7.
- Spinks, P.Q., Shaffer, H.B., 2005. Range-wide molecular analysis of the western pond turtle (*Emys marmorata*): cryptic variation, isolation by distance, and their conservation implications. *Mol. Ecol.* 14, 2047–2064.
- Starkey, D.E., Bradley Shaffer, H., Burke, R.L., Forstner, M.R., Iverson, J.B., Janzen, F.J., Rhodin, A.G.J., Ultsch, G.R., 2003. Molecular systematics, phylogeography, and the effects of pleistocene glaciation in the painted turtle (*Chrysemys picta*) complex. *Evolution* 57, 119–128.
- Starrett, A., 1958. What is the subspecies problem? *Syst. Zool.* 17, 111–115.
- Stuart, B.L., Parham, J.F., 2004. Molecular phylogeny of the critically endangered Indochinese box turtle (*Cuora galbinifrons*). *Mol. Phylog. Evol.* 31, 164–177.
- Stuckas, H., Fritz, U., 2011. Identity of *Pelodiscus sinensis* revealed by DNA sequences of an approximately 180-year-old type specimen and a taxonomic reappraisal of *Pelodiscus* species (Testudines: Trionychidae). *J. Zool. Syst. Evol. Res.* 49, 335–339.
- Takeuchi, H., Ota, H., Oh, H.S., Hikida, T., 2012. Extensive genetic divergence in the east Asian natricine snake, *Rhabdophis tigrinus* (Serpentes: Colubridae), with special reference to prominent geographical differentiation of the mitochondrial cytochrome b gene in Japanese populations. *Biol. J. Linn. Soc.* 105, 395–408.
- Templeton, A.R., 1989. The meaning of species and speciation: a genetic perspective. In: Otte, D., Endler, J.A. (Eds.), *Speciation and its Consequences*. Sinauer Associates, Sunderland, Massachusetts, pp. 3–27.
- Thorpe, R.S., Jones, A.G., Malhotra, A., Surget-Groba, Y., 2008. Adaptive radiation in lesser antillean lizards: molecular phylogenetics and species recognition in the Lesser Antillean dwarf gecko complex, *Sphaerodactylus fantasticus*. *Mol. Ecol.* 17, 1489–1504.
- Torres-Peréz, F., Méndez, M.A., Benavides, E., Moreno, R.A., Lamborot, M., Palma, R.E., Ortiz, J.C., 2009. Systematics and evolutionary relationships of the mountain lizard *Liolaemus monticola* (Liolaemini): how morphological and molecular evidence contributes to reveal hidden species diversity. *Biol. J. Linn. Soc.* 96, 635–650.
- Uetz, P., 2010. The original description of reptiles. *Zootaxa* 2334, 59–68.
- Ursenbacher, S., Carlsson, M., Helfer, V., Tegelstrom, H., Fumagalli, L., 2006. Phylogeography and pleistocene refugia of the adder (*Vipera berus*) as inferred from mitochondrial DNA sequence data. *Mol. Ecol.* 15, 3425–3437.
- Ursenbacher, S., Schweiger, S., Tomović, L., Crnobrnja-Isailović, J., Fumagalli, L., Mayer, W., 2008. Molecular phylogeography of the nose-horned viper (*Vipera ammodytes*, Linnaeus (1758)): evidence for high genetic diversity and multiple refugia in the Balkan peninsula. *Mol. Phylog. Evol.* 46, 1116–1128.
- Vargas-Ramírez, M., Vences, M., Branch, W.R., Daniels, S.R., Glaw, F., Hofmeyr, M.D., Kuchling, G., Maran, J., Papenfuss, T.J., Siroký, P., Vieites, D.R., Fritz, U., 2010. Deep genealogical lineages in the widely distributed African helmeted terrapin: evidence from mitochondrial and nuclear DNA (Testudines: Pelomedusidae: *Pelomedusa subrufa*). *Mol. Phylog. Evol.* 56, 428–440.
- Walker, D., Burke, V.J., Barak, I., Avise, J.C., 1995. A comparison of mtDNA restriction sites vs. control region sequences in phylogenetic assessment of the musk turtle (*Stemotherus minor*). *Mol. Ecol.* 4, 365–373.
- Walker, D., Moler, P.E., Buhlmann, K.A., Avise, J.C., 1998. Phylogeographic uniformity in mitochondrial DNA of the snapping turtle (*Chelydra serpentina*). *Anim. Conserv.* 1, 55–60.
- Wiens, J.J., Penkrot, T.A., 2002. Delimiting species using dna and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Syst. Biol.* 51, 69–91.
- Wiley, E.O., 1978. The evolutionary species concept reconsidered. *Syst. Biol.* 27, 17–26.
- Wilms, T.M., Böhme, W., Wagner, P., Lutzmann, N., Schmitz, A., 2009. On the phylogeny and taxonomy of the genus *Uromastix* Merrem, 1820 (Reptilia: Squamata: Agamidae: Uromastycinae) – resurrection of the genus *Saara* Gray, 1845. *Bonn. Zool. Beitr.* 56, 55–99.
- Wilson, E.O., Brown, W.L., 1953. The subspecies concept and its taxonomic application. *Syst. Zool.* 2, 97–111.
- Wood, D.A., Fisher, R.N., Reeder, T.W., 2008. Novel patterns of historical isolation, dispersal, and secondary contact across Baja California in the rosy boa (*Lichanura trivirgata*). *Mol. Phylog. Evol.* 46, 484–502.
- Yang, Z.H., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci. USA* 107, 9264–9269.
- Zachos, F.E., Lovari, S., 2013. Taxonomic inflation and the poverty of the phylogenetic species concept – a reply to Gippoliti and Groves. *Hystrix, J. Mammal. doi: 10.4404/hystrix-24.1-8849*.
- Zachos, F.E., 2013. Species splitting puts conservation at risk. *Nature* 494, 35.

- Zamudio, K.R., Greene, H.W., 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae: implications for neotropical biogeography, systematics, and conservation. *Biol. J. Linn. Soc.* 62, 421–442.
- Zamudio, K.R., Jones, K.B., Ward, R.H., 1997. Molecular systematics of short-horned lizards: biogeography and taxonomy of a widespread species complex. *Syst. Biol.* 46, 284–305.
- Zink, R.M., 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proc. R. Soc. Land. B.* 271, 561–564.

#### Further reading

- Parham, J.F., Türkozan, O., Stuart, B.L., Arakelyan, M., Shafei, S., Macey, J.R., Papenfuss, T.J., 2006. Genetic evidence for premature taxonomic inflation in Middle Eastern tortoises. *Proc. Calif. Acad. Sci.* 57, 955–964.
- Zusi, R.L., 1982. Intraspecific geographic variation and the subspecies concept. *Auk* 99, 606–608.