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MAJOR HISTOCOMPATIBILITY COMPLEX POLYMORPHISM IN REPTILE CONSERVATION

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Abstract.—**Genes of the major histocompatibility complex (MHC) are closely related to disease resistance and immune response in vertebrates. Although many groups are well represented in the MHC literature, less attention has been given to reptiles. Here we summarize reptile MHC studies, review published accounts investigating links between MHC polymorphism, parasite resistance, and mate choice, evaluate current limitations, and discuss prospects of new technologies for future research. MHC polymorphism appears to be extensive in reptile populations, and current evidence suggests MHC polymorphism may influence parasite resistance and mate choice as in other vertebrates. Prior research strategies have been limited by the type of molecular markers available, the nature of the sequences being amplified, the number of individuals and populations analyzed, the immunology and biology of the host-parasite relationship, and the conditions under which subjects are studied. Finally, reptiles offer special challenges: as ectotherms their susceptibility to pathogens may change dramatically depending on the time of year due to seasonal variations in their immunity. Including a temperature and/or seasonal variable may thus provide new insights into the genetic mechanisms of disease resistance. New technologies and techniques should help to alleviate problems of MHC gene characterization and multi-locus amplification associated with past research and contribute to our understanding of MHC polymorphism in reptile conservation.**

Key Words.—immunogenetics; mate choice; MHC; non-avian reptiles; parasite resistance

INTRODUCTION

Understanding how genetic variation influences individual fitness and population viability allows conservation biologists to develop and implement successful management plans for species of conservation concern. While molecular markers such as microsatellites, minisatellites, and single nucleotide polymorphisms are useful to estimate genetic variation (reviewed in Sunnucks 2000; Brumfield et al. 2003), these markers are not functional genes, and therefore may not accurately estimate fitness (Meyers and Bull 2002; van Tienderen et al. 2002). Major histocompatibility complex (MHC) genes code for proteins with a central role in disease resistance and immune response in vertebrates (Hedrick 1999) and thus, are excellent candidates for the study of adaptive genetic variation (Burri et al. 2008).

The two classical MHC gene groups, class I and class II, encode cell surface glycoproteins that interact with foreign or self-peptides in a peptide binding region (PBR) and present them to T cells. While class I molecules are present on nearly all somatic cells and present peptides derived from intracellular pathogens to cytotoxic T cells, class II molecules occur mainly on antigen presenting cells (APCs) such as B cells and macrophages and present peptides derived from extracellular pathogens to helper T cells (Klein 1986). Multiple copies of MHC genes may be present as a result of gene duplication (reviewed in Edwards and Hedrick 1998), and these copies may have acquired different functions, lower levels of polymorphism, tissue-dependent expression patterns, or no function as is the case with pseudogenes (Janeway et al. 2001).

Both types of classical MHC molecules are composed of protein chains divided into distinct cytoplasmic, transmembrane, and extracellular domains that function in immune signalling, structural anchoring, and peptide presentation respectively (Fig. 1). Class I molecules consist of a single α chain non-covalently bonded to a β_2-microglobulin molecule (Janeway et al. 2001; Fig. 1A). The α_1 and α_2 domains of class I molecules make up the PBR and are encoded by exons two and three respectively of class I genes. Unlike class I molecules, class II molecules are formed by paired α and β chains (Janeway et al. 2001; Fig. 1B). The α_1 and β_1 domains form the PBR and are encoded by exon two of separate class II α and β genes.

Changes in the PBR coding exons of MHC genes can alter the molecule repertoire of an individual and affect the capacity to recognize foreign pathogens and parasites. Individuals may be resilient to pathogens because they possess specific MHC molecules (rare allele advantage hypothesis, Clarke and Kirby 1966) and/or possess several distinct molecules (heterozygote advantage hypothesis, Doherty and Zinkernagel 1975). Both hypotheses are supported by empirical studies. MHC heterozygosity has been shown to predict

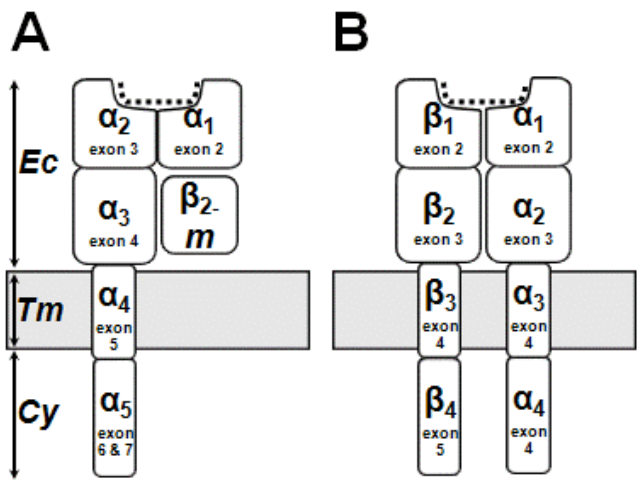

FIGURE 1. General structure and coding exons for classical (A) class I and (B) class II major histocompatibility complex molecules on a cell membrane (shaded gray). The peptide binding region for each molecule is depicted by dotted lines. Abbreviations are *Ec* for extracellular; *Tm* for transmembrane; *Cy* for cytoplasmic domains; and β_2-*m* for β_2-microglobulin molecule.

pathogen and parasite resistance in fish (e.g., Hedrick et al. 2001), amphibians (e.g., Savage and Zamudio 2011), birds (e.g., Westerdahl et al. 2005), and mammals (e.g., Penn et al. 2002). For example, Carrington et al. (1999) demonstrated that AIDS development is delayed in humans with maximum heterozygosity at MHC class I loci compared to homozygotes with fewer alleles. Likewise, particular MHC alleles are associated with higher survivorship and/or lower levels of parasitism in Atlantic Salmon (*Salmo salar*; Langefors et al. 2001), Lowland Leopard Frogs (*Lithobates yavapaiensis*; Savage and Zamudio 2011), House Sparrows (*Passer domesticus*; Bonneaud et al. 2006), and Soay Sheep (*Ovis aries*; Paterson et al. 1998).

Given the fitness advantages of particular MHC alleles or heterozygosity, individuals may preferentially select mates based on their MHC genes (Landry et al. 2001). Mates may be chosen with advantageous genes that confer favorable traits to offspring such as disease resistance (good genes hypothesis, Hamilton and Zuk 1982), to avoid closely related individuals to minimize inbreeding (inbreeding avoidance hypothesis, Potts and Wakeland 1990), or improve compatibility (genetic compatibility hypothesis, Brown and Eklund 1994). Support for MHC-dependent mate choice comes from a variety of vertebrates including Three-spine Sticklebacks (*Gasterosteus aculeatus*; Reusch et al. 2001), Tiger Salamanders (*Ambystoma tigrinum*; Bos et al. 2009), Ring-necked Pheasants (*Phasianus colchicus*; Von Schantz et al. 1996), and White-tailed Deer (*Odocoileus virginianus*; Ditchkoff et al. 2001).

Although many groups are well represented in the MHC literature, reptiles have received less attention. Given that 15% of reptile species are experiencing population declines (IUCN 2012) due to factors such as disease (Gibbons et al. 2000), understanding the implications of MHC polymorphism on parasite resistance and mate choice are critical to the conservation of reptiles. In this review, we summarize the available knowledge regarding MHC polymorphism in reptile populations and review published studies investigating links between MHC polymorphism, parasite resistance, and mate choice. We conclude by discussing the limitations of prior research strategies, what new technologies and techniques may bring, and where future research is needed.

MHC POLYMORPHISM, PARASITE RESISTANCE, AND MATE CHOICE IN REPTILE POPULATIONS

Levels of MHC polymorphism in reptile populations.—Through an exhaustive literature search we identified 48 publications on MHC polymorphism studies in reptiles: 45 peer-reviewed publications and three unpublished theses/dissertations (Appendix 1). We discuss half of these publications in the sections that follow because the remaining publications had small sample sizes ($n < 10$), which were not conducive for inferring population-level variation. Even though the first MHC sequences from reptiles were published in the early 1990s (see Grossberger and Parham 1992), the first report on population-level MHC polymorphism in reptiles came several years later using a restriction

fragment length polymorphism (RFLP) technique developed by Radtkey et al. (1996), who compared MHC class I exon three polymorphism in parthenogenic and non-parthenogenic reproducing gecko species. Other research teams modified the RFLP technique of Radtkey et al. (1996) for use with other reptiles, illustrating high MHC polymorphism in Water Pythons (*Liasis fuscus*; Wittzell et al. 1999; Madsen and Újvári 2006), Sand Lizards (*Lacerta agilis*; Madsen et al. 2000), in some (Madsen et al. 2000) but not all European Adders (*Vipera berus*; Madsen et al. 1999), but not in Hungarian Meadow Vipers (*Vipera ursinii rakosiensis*; Újvári et al. 2002).

Using MHC sequences, researchers find that many reptile populations possess extensive MHC class I polymorphism. For example, 26 Tuataras (*Sphenodon punctatus*) from a large population on Stephen's Island, New Zealand have at least 21 highly divergent (i.e., nucleotide diversity, $\pi = 0.193$) MHC class I exon two alleles (Miller et al. 2007). As these alleles come from multiple loci, however, polymorphism is likely overestimated. Other reptiles such as Loggerhead Sea Turtles (*Caretta caretta*) from the Cape Verde Islands (Stiebens et al. 2013a,b), Saltwater Crocodiles (*Crocodilus porosus*) from Australia (Jaratlerdsiri et al. 2012), Skyros Wall Lizards (*Podarcis gaigeae*) from the Aegean Sea (Runemark 2012), invasive Brown Anoles (*Anolis sagrei*) from Florida (Hung 2013), and additional Tuatara from other islands around New Zealand (Miller et al. 2010) also possess high MHC class I polymorphism though these sequences come from multiple and potentially different types of MHC loci. In contrast, there are exceptional reptile populations that lack MHC class I polymorphism, potentially as a result of population bottlenecks. For instance, Brother's Island Tuataras (*Sphenodon guntheri*), which survive only on North Brother's Island, New Zealand, may have only three MHC class I exon two alleles based on analysis of 27 individuals (Miller et al. 2008). Furthermore, 59 Diamond-backed Terrapins (*Malaclemys terrapin*) from three populations in Cape Cod, Massachusetts share a single allele at a particular MHC class I exon three locus (McCafferty et al. 2013).

As few studies have investigated MHC class II polymorphism in reptile populations, a general pattern of MHC class II polymorphism is not clear in reptiles, and population data are currently only available from two crocodilians (Appendix 1). For example, 45 Nile Crocodiles (*Crocodylus niloticus*) from Botswana and South Africa possess at least 28 MHC class II β exon two alleles, but only nine highly divergent alleles come from at least two classical loci (Badenhorst 2008). In another crocodilian, the Chinese Alligator (*Alligator sinensis*), analysis of 21 individuals suggests at least 16 weakly divergent ($\pi = 0.031$) MHC class II β exon three alleles (Nie et al. 2013), but low polymorphism is not surprising as exon three does not form the PBR of class II molecules (Fig. 1B) and is thus less variable than exon two of class II β genes.

Parasite resistance.—Three published accounts have investigated a link between MHC polymorphism and parasite resistance in reptiles. These accounts assessed MHC class I exon three variation using RFLP analysis and found specific MHC genotypes influenced parasite loads (Olsson et al. 2005a, b; Madsen and Újvári 2006). Olsson et al. (2005a) assessed the relationship between tick load and levels of the physiological stress hormone corticosterone in male Sand Lizards and found that tick loads and corticosterone levels are positively correlated in some but not all individuals. The exceptions are males with a particular RFLP-determined MHC genotype (termed O-males) that have decreased tick loads with increasing corticosterone levels, are more successful in mate acquisition and guarding, and also sire more young. As corticosterone suppresses the immune system, O-males should have increased susceptibility to parasites; however, O-males may invest heavily in combating ticks and reproduction early in the breeding season at the expense of being more susceptible and having more haemoprotozoan blood parasites transmitted by ticks later (Olsson et al. 2005b). Water Pythons with an intermediate number of RFLP bands have the lowest levels of haemoprotozoan blood parasites (*Hepatozoon* sp.), and specific RFLP bands are also correlated with lower blood parasite loads (Madsen and Újvári 2006). In both Sand Lizards and Water Pythons, however, it is not apparent whether seasonal variations in temperature and hormone levels may also influence susceptibility to parasites and potentially bias results (see Discussion). Finally, haemoprotozoan blood parasites were examined, most of which are not considered pathogenic in reptiles (Jacobson 2007). These parasites cause intracellular infections of erythrocytes, which may not express MHC class I molecules. Without MHC class I presentation, infected erythrocytes would need to be phagocytized by APCs such as macrophages, and parasite-derived antigens would need to be loaded onto MHC class II molecules of APCs. Considering these limitations, it is not clear if MHC polymorphism influences parasite loads in reptiles.

Mate choice.—Three studies addressed whether MHC polymorphism influences mate choice in reptiles. Olsson et al. (2003) used odor association as a proxy for mate choice and found female Sand Lizards associated more with odors of males with dissimilar MHC genotypes (based on percent RFLP band sharing), but the number of RFLP bands possessed by males did not influence odor preference. In field trials, Olsson et al. (2003) further found that male Sand Lizard body mass is negatively correlated with MHC similarity between

observed mating pairs suggesting that larger males preferentially mate with more MHC-dissimilar females. Whether Sand Lizards can assess quality of mates through MHC-mediated odors is unknown, but mammals appear to discriminate odors based on MHC class I genotypes (reviewed in Penn 2002). Tuataras also show some evidence for MHC-dependent mate choice as the amino acid composition of MHC class I exon two alleles are more dissimilar between mated than randomly chosen pairs within the same study area (Miller et al. 2009); however, this pattern of MHC dissimilarity may be due to social structuring because unrelated individuals generally reside within the same area. Brown Anoles show some evidence of MHC-dependent mate choice as females in the laboratory copulated more frequently with males that possessed different MHC genotypes than their own (Hung 2013). Accordingly, these limited data suggest MHC polymorphism may also influence mate choice in reptiles, but further studies in reptiles with differing mating systems would be fruitful.

DISCUSSION

Limitations of prior research strategies.—Researchers have made great strides in reptile MHC research; however, several important limitations include: (1) the type of molecular markers used; (2) the nature of the sequences being amplified; (3) the number of individuals and populations analyzed; (4) the uncertainty of relying on red blood cell parasitism without understanding how these parasites relate to MHC expression; and, (5) the conditions under which subjects are studied. First, almost one quarter of reptile MHC studies and nearly one half of the studies with suitable sample sizes in Appendix 1 used RFLP markers. RFLPs are difficult to use in inter-specific comparisons and may not accurately estimate polymorphism because probes may hybridize to non-MHC fragments (Wittzell et al. 1999), thus analysis of sequence polymorphism is preferable in comparative studies. Second, amplified reptile MHC sequences may represent products from multiple and/or potentially different types of MHC loci. If multiple loci are amplified for one species but not another, then polymorphism comparisons would not be appropriate. Likewise, polymorphism would be biased if reptile MHC sequences come from a mixture of classical, non-classical, and pseudogene MHC loci. Third, the number of individuals and populations analyzed is problematic as nearly half of the studies in Appendix 1 used fewer than 10 individuals, and only a handful included multiple populations.

Small sample sizes probably result in poor estimates of true population-level variation. Surveying multiple sites would be useful to identify populations that are lowest in MHC diversity and may therefore have reduced population viability and evolutionary potential (Piertney and Oliver 2006). Fourth is the association of reptile MHC class I polymorphism with non-pathogenic red blood cell (RBC) parasites considering RBCs do not have MHC class I expression. For this association to be true, parasitized RBCs would need to be digested by macrophages, pathogen fragments loaded onto MHC class II molecules of macrophages, and then MHC class II alleles be in linkage to MHC class I alleles. Fifth, perhaps of greatest interest but most neglected, are the environmental conditions under which reptiles are studied when investigating the relationship between MHC polymorphism and parasite resistance.

Because reptiles are ectotherms, their immunity and hence susceptibility to pathogens may vary dramatically depending on the time of year (reviewed in Zapata et al. 1992) due partly to temperature changes but also seasonally correlated changes in anatomy and physiology (Origgi 2007). For example, skin graft assays, which assess immune response to foreign tissue grafts from individuals of the same or different species via MHC molecule recognition, are influenced by season and prior exposure in Ocellated Skinks (*Chalcides ocellatus*). Initial signs of rejection appear earlier in skinks receiving a second set of skin grafts than in skinks receiving their first set of skin grafts in winter but not summer (Afifi et al. 1993). How temperature influences MHC-mediated immune response is not clear in reptiles. Studies in fishes suggest temperature may influence generation and activation of immature helper T cells (Bly and Clem 1992). Given the strong correlations between temperature and seasonal variation in reptilian immune responses, reptiles and other ectotherms offer special challenges in studies trying to determine the influence of genetic variation on disease resistance. Estimates of variance in immune response under a temperature and/or seasonal variable may thus provide new insights on the influence of MHC genes.

What new technologies and techniques may bring.—New technologies and techniques promise to greatly expand our knowledge and understanding of MHC polymorphism in reptile and vertebrate populations. Here we consider the potential application of genome walking, genome assemblies, genome libraries, and next-generation sequencing technologies to MHC studies in reptiles. Genome walking is an all-encompassing term for PCR-based strategies used to extend characterized genomic regions that has only recently become widely used in MHC characterization (Babik 2010). By extending partial MHC exon sequences, genome walking can facilitate development of locus-specific primers for MHC alleles through careful placement of primers in the introns flanking the exon of interest and help researchers eliminate multi-locus amplification (e.g., Kiemnec-Tyburczy and Zamudio 2013). MHC sequences derived from genome assemblies will also be valuable in

characterizing reptile MHC genes. For example, the genome assembly for the Green Anole (*Anolis carolinensis*) has already been leveraged for MHC primer development in other Squamates (e.g., Murphy et al. 2009; Runemark 2012). Genome assemblies may further help to reduce multiple locus amplification and differentiate the type of MHC locus isolated in reptile MHC studies but only if the assemblies contain abundant MHC sequence data, which is not always the case as early drafts of genome assemblies often contain gaps in the MHC region. Genome library screening using MHC sequences as probes can also improve MHC locus characterization especially if large DNA fragment libraries (i.e., cosmid, fosmid, or bacterial artificial chromosome) are generated and screened.

Next-generation sequencing technologies (NGS) will not only facilitate the characterization of MHC genes by making it economically possible to sequence reptile genomes and genome libraries but will also help to increase the number of individuals and populations assessed. For example, amplicon sequencing allows the MHC variants of hundreds of individuals to be simultaneously sequenced without extensive cloning of PCR products, an approach that significantly lowers costs and labor. Nevertheless, the resulting data analyses can prove challenging even when using 454 pyrosequencing or Illumina MiSeq sequencing by synthesis, which are well suited for MHC genotyping given their long read lengths (Babik 2010). Target enrichment, or sequence capture, (reviewed in Mamanova et al. 2010) is another NGS technology of great value to MHC studies, especially considering multiple MHC loci as well as others can be targeted simultaneously. For example, Elbers and Taylor (2015) sequenced all known immune response genes among 16 Gopher Tortoises (*Gopherus polyphemus*) using target enrichment with baits developed by filtering the Western Painted Turtle (*Chrysemys picta bellii*) genome by the gene ontology term Immune Response and found polymorphic single nucleotide polymorphisms in the coding regions of 491 immune genes and 12 MHC genes. Sequencing transcriptomes (e.g., Wang et al. 2012) from reptiles that do not currently have a closely related reference genome available is another NGS procedure of great value to MHC studies. Researchers could sequence transcriptomes from reptile lymphoid tissues including the thymus, spleen, gut-associated lymphoid tissue (GALT) and bone marrow (Zimmerman et al. 2010), mine the transcriptomes for MHC and other immune genes, develop baits to use in target enrichment experiments, and sequence desired immune genes.

Where future research is needed.—Knowledge of MHC polymorphism would be especially useful in those reptiles that are species of conservation concern and are susceptible to infectious diseases. For instance, North American tortoises (*Gopherus* sp.) can contract an infectious upper respiratory tract disease associated with pathogens such as the bacteria *Mycoplasma agassizii*, and the disease has been implicated as a potential cause of tortoise die-offs (Brown et al. 1994). In Eastern Box Turtles (*Terrapene carolina carolina*), even though the prevalence of *Ranavirus* may be low (Allender et al. 2011a), infection outbreaks still pose a significant threat as *Ranavirus* may have caused 27 deaths over two years in a Maryland population (Scott Farnsworth, pers. comm.). In another chelonian, the Green Sea Turtle (*Chelonia mydas*), tumors containing chelonid fibropapilloma-associated herpesvirus (Herbst et al. 2004) can occlude the eyes and mouth, eventually leading to starvation, which may jeopardize long-term persistence. North American rattlesnakes such as the Eastern Massasauga (*Sistrurus c. catenatus*) can succumb to a flesh-eating *Chrysosporium* fungus (Allender et al. 2011b). Eight *Chrysosporium*-attributed mortalities occurred in a southern Illinois population of 50–60 snakes (Choquette 2012). Although these examples are restricted to North America due to a lack of data collected elsewhere, they demonstrate that disease in reptiles is common enough to warrant concern about factors affecting disease susceptibility.

Climate change may also influence disease susceptibility in reptiles by affecting the distribution, prevalence, and severity of pathogens (Altizer et al. 2001). On the one hand, climate change may influence pathogens and/or their vectors. For example, environmental temperature is positively correlated with prevalence of several avian blood pathogens along an altitudinal gradient in Australia, possibly due to temperature constraints on the developing pathogens whilst in their vectors (Zamora-Vilchis et al. 2012). On the other hand, climate change may affect the host. For instance, fungal disease susceptibility may be influenced by climate change in Timber Rattlesnakes (*Crotalus horridus*) as disease prevalence increased during abnormally wet years but only for inbred populations (Clark et al. 2011). Given the probable impact of climate change, it is becoming increasingly important to understand the mechanisms of disease resistance in reptiles of conservation concern.

CONCLUSIONS

Much progress has been made in 24 y since Grossberger and Parham's (1992) publication of the first reptile MHC sequences: MHC polymorphism appears to be extensive in surveyed reptile populations and to also influence parasite resistance and mate choice as in other vertebrates. Prior studies, however, may have analyzed sequences from multiple MHC loci and not accounted for temperature and/or seasonal variation in reptilian immune responses. Previous research has also examined

parasites that are not considered pathogenic in reptiles. Thus a better understanding of amplified sequences, the influence of temperature on reptilian immune responses, and host-parasite relationships would greatly enhance our understanding of MHC polymorphism in this currently neglected group. Additional MHC surveys could benefit those reptiles threatened by infectious disease by elucidating which populations are at greatest risk and which would be best suited as donors to augment genetic diversity of potentially compromised populations.

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APPENDIX 1. Summary of MHC studies in reptiles.

Species	MHC Class	References
Crocodilia		
Family Alligatoridae		
Alligator mississippiensis	I,IIα,IIβ	Edwards et al. 1995[ac]; Jaratlerdsiri et al. 2014b[ac], c[ac]
Alligator sinensis	I,IIα,IIβ	Shi et al. 2004[ac]; Liu et al. 2007[c]; Li et al. 2008[ac]; Nie et al. 2012[c], 2013[c]; Jaratlerdsiri et al. 2014b[ac], c[ac]
Caiman crocodylus	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Caiman latirostris	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Caiman yacare	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Melanosuchus niger	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Paleosuchus palpebrosus	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Family Crocodylidae		
Crocodylus acutus	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus intermedius	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus jonsoni	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus mindorensis	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus moreletii	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus niloticus	I,IIα,IIβ	Badenhorst 2008[c]; Li et al. 2010[ac]; Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus novaeguineae	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus palustris	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus porosus	I,IIα,IIβ	Jaratlerdsiri et al. 2012[c], 2014a[ac], b[ac], c[ac]
Crocodylus rhombifer	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Crocodylus siamensis	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Mecistops cataphractus	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Osteolaemus tetraspis	I,IIα,IIβ	Jaratlerdsiri et al. 2014b[ac], c[ac]
Rhynchocephalia		
Family Sphenodontidae		
Sphenodon punctatus	I,IIβ	Miller et al. 2005[ac], 2006[ac], 2007[c], 2008[c], 2009[c], 2010[c], 2015[ac]
Sphenodon guntheri	I	Miller et al. 2008[c]
Squamata		
Family Colubridae		
Natrix natrix	IIβ	Marosi et al. 2011[ac]
Natrix tessellate	IIβ	Marosi et al. 2011[ac]
Nerodia sipedon	I	Grossberger & Parham 1992[ac]
Family Gekkonidae		
Hemidactylus frenatus	I	Radtkey et al. 1996[b]
Hemidactylus garnotii	I	Radtkey et al. 1996[b]
Lepidodactylus auerolineatus	I	Radtkey et al. 1996[b]
Lepidodactylus lugubris	I	Radtkey et al. 1996[b]
Lepidodactylus moestus	I	Radtkey et al. 1996[b]
Lepidodactylus sp. *Arno*	I	Radtkey et al. 1996[b]
Lepidodactylus sp. Takapoto	I	Radtkey et al. 1996[b]
Family Iguanidae		
Amblyrhynchus cristatus	I,IIβ	Glaberman & Caccone 2008[ac]; Glaberman et al. 2008[c], 2009[ac]
Conolophus subcristatus	I	Glaberman & Caccone 2008[ac]; Glaberman et al. 2008[c]

Ctenosaura clarki	I	Glaberman et al. 2008[c]
Ctenosaura defensor	I	Glaberman et al. 2008[c]
Cyclura carinata	I	Glaberman et al. 2008[c]
Cyclura cornuta	I	Glaberman et al. 2008[c]
Cyclura rileyi	I	Glaberman et al. 2008[c]
Iguana iguana	I	Glaberman & Caccone 2008[ac]
Family Lacertidae		
Eremias brenchleyi	I	Yuan et al. 2014[ac]
Eremias multiocellata	I	Yuan et al. 2014[ac]
Eremias przewalskii	I	Yuan et al. 2014[ac]
Lacerta agilis	I	Madsen et al. 2000[b]; Olsson et al. 2003[b], 2004[b], 2005a[b], b[b]
Podarcis gaigeae	I	Runemark 2012[d]
Family Polychrotidae		
Anolis sagrei	I	Hung 2013[c]
Family Pythonidae		
Liasis fuscus	I	Wittzell et al. 1999[b]; Madsen & Újvári 2006[b]
Ameiva ameiva	I	Grossberger & Parham 1992[ac]
Family Scincidae		
Ctenotus taeniolatus	I	Murphy et al. 2009[ac]
Egernia stokesii	I	Ansari et al. 2015[ac]
Eulamprus tympanum	I	Murphy et al. 2009[ac]
Lampropholis guichenoti	I	Murphy et al. 2009[ac]
Niveoscincus metallicus	I	Murphy et al. 2009[ac]
Plestiodon chinensis	IIβ	Li et al. 2008[ac]
Pseudemoia entrecasteauxii	I	Murphy et al. 2009[ac]
Saiphos equalis	I	Murphy et al. 2009[ac]
Tiliqua adelaidensis	I	Ansari et al. 2015[ac]
Tiliqua rugosa	I	Ansari et al. 2015[ac]
Family Teiidae		
Ameiva ameiva	I	Grossberger & Parham 1992[ac]
Family Viperidae		
Sistrurus c. catenatus	IIβ	Jaeger et al. 2014[ac]
Vipera berus	I	Madsen et al. 1999[ab]; Madsen et al. 2000[b]
Vipera ursinii rakosiensis	I	Újvári et al. 2002[ab]
Testudines		
Family Cheloniidae		
Caretta caretta	I	Stiebens et al. 2013a[d],b[d]
Family Emydidae		
Malaclemys terrapin	I	McCafferty et al. 2013[ce]
Family Geoemydidae		
Chinemys reevesii	IIβ	Li et al. 2006[ac]; Li et al. 2008[ac]
Family Trionychidae		
Pelodiscus sinensis	I	Xia 1999[ac]; Liu et al. 2006[c]

[a]Study used small sample size ($n < 10$)
[b]Study used restriction fragment length polymorphism (RFLP)
[c]Study used cloning and/or Sanger sequencing
[d]Study used next-generation sequencing
[e]Study used single-strand conformational polymorphism (SSCP)