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Assembly of the eastern North American herpetofauna: new evidence from lizards and frogs

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Darwin first recognized the importance of episodic intercontinental dispersal in the establishment of worldwide biotic diversity. Faunal exchange across the Bering Land Bridge is a major example of such dispersal. Here, we demonstrate with mitochondrial DNA evidence that three independent dispersal events from Asia to North America are the source for almost all lizard taxa found in continental eastern North America. Two other dispersal events across Beringia account for observed diversity among North American ranid frogs, one of the most species-rich groups of frogs in eastern North America. The contribution of faunal elements from Asia via dispersal across Beringia is a dominant theme in the historical assembly of the eastern North American herpetofauna.

Keywords: Reptilia; Amphibia; North America; biogeography; phylogeny

1. INTRODUCTION

Episodes of intercontinental dispersal have had a dramatic impact on current large-scale floral and faunal distribution patterns. Intercontinental dispersal occurs primarily when tectonic plates collide (Macey *et al.* 2000; Bossuyt & Milinkovitch 2001), when decreasing sea-levels expose land bridges (Wallace 1876; Darwin 1883), or when climatic changes produce dispersal corridors in otherwise inhospitable areas. A faunal exchange between Africa and Asia was induced by the plate-tectonic closing of the Tethys Sea, and a major exchange between North America and South America occurred following formation of a land bridge by the combined influence of plate tectonics and a decrease in sea-level (Brown & Lomolino 1998).

Climatic fluctuations producing episodic connections between Eurasian and North American faunal and floral elements have been invoked recently to explain disjunct distributions in bacteria (Maekawa *et al.* 2005), flowering plants (Wen 1999), aphids (von Dohlen *et al.* 2002), alligators and cryptobranchid salamanders (Pough *et al.* 2004) and even humans (Brown & Lomolino 1998). Particularly important for herpetofaunal exchanges was a Mid-Miocene connection of eastern Eurasia and eastern North America by a continuous temperate deciduous forest; this connection provided habitats for amphibian and reptilian groups otherwise excluded from the high latitudes at which Eurasia and North America are in close proximity.

Several eastern North American lizard and frog groups may have entered this region from Asia during the Miocene. We present molecular phylogenetic evidence of intercontinental faunal exchanges for ranid frogs and for two genera of scincid lizards, which share disjunct distributions in eastern North America and eastern Asia. The broader phylogenetic contexts of both groups place their origins in the Old World (Bossuyt & Milinkovitch 2001; Brandley *et al.* 2005), indicating that the eastern North American species descend from Eurasian ancestors.

2. MATERIAL AND METHODS

The scincid genera, *Scincella* and *Eumeces*, are placed in separate subfamilies, Lygosominae and Scincinae, respectively. No previous phylogenetic work has considered all Northern Hemisphere genera in this family. In the Lygosominae, we sample *Scincella lateralis* from eastern North America and compare it to three species of *Scincella* and *Sphenomorphus indicus* from eastern Asia. In the Scincinae, we sample each species of *Eumeces* (including *Neoseps reynoldsi*) occurring east of the Mississippi River, several western North American *Eumeces* and two eastern Asian species (*Eumeces capito* and *Eumeces quadrilineatus*). Central American *Eumeces* are not part of the North American radiation (Brandley *et al.* 2005). Based on morphological characters, Griffith *et al.* (2000) suggest that North American scincines form a monophyletic group. We sample all scincine genera occurring in western Asia and most from North Africa (*Chalcides*, *Eurylepis*, *Novoeumeces*, *Ophiomorus* and *Scincus*). To root the phylogenetic hypothesis of the Scincidae, members of two separate families are included, *Eremias grammica* from the Lacertidae and *Platysaurus capensis* from the Cordylidae.

Ranidae is one of the largest frog families. To investigate the affinities of North American taxa, members of all monophyletic North American species groups are sampled (*R. catesbeiana*, *R. sylvatica*, *R. warszewitschii*, *R. areolata*, *R. pipiens* and all taxa in the *R. boylii* species group; Hillis & Davis 1986; Macey *et al.* 2001). These taxa are compared to a wide range of ranids occurring in Asia and Europe, and rooted with a pipid (*Xenopus laevis*), bufonid (*Bufo andrewsi*) and rhacophorid (*Polypedates leucomystax*).

We sequenced a segment of the mitochondrial genome containing part of *nad1*, *trnI*, *trnQ*, *trnM*, *nad2*, *trnW*, *trnA*, *trnN*, *trnC*, *trnY* and part of *cox1*. Sequencing primers for scincid lizards are from Macey *et al.* (1997) and primers for ranid frogs are from Macey *et al.* (2001).

DNA sequences were aligned manually. Positions encoding proteins were translated to amino acids using MacClade v. 4.03 (Maddison & Maddison 2001) for confirmation of alignment. Alignments of sequences encoding tRNAs were based on secondary

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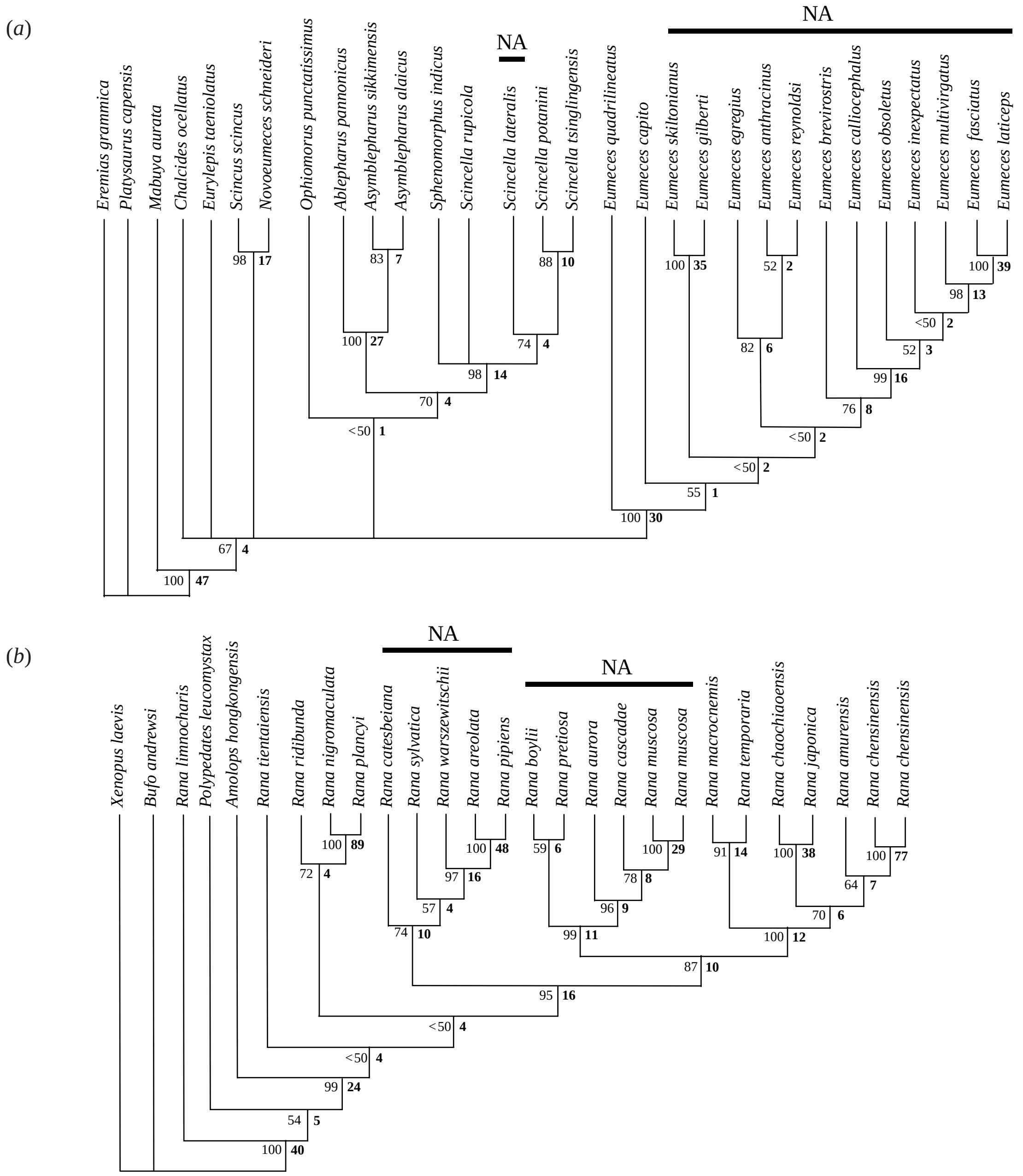

Figure 1. Phylogenetic relationships among (*a*) scincid lizards and (*b*) ranid frogs. Bars indicate taxa occurring in North America (NA); all other taxa are from Asia, Europe and Africa (see text). Bootstrap values are shown to the left of the branches, and decay indices to the right. The two *R. muscosa* samples represent the two major clades (Macey *et al.* 2001). (*a*) The strict consensus of three equally parsimonious trees with a length of 5788 steps resulting from analysis of 1832 positions (949 informative) from the *nad1–cox1* mtDNA region. (*b*) The single most parsimonious tree with a length of 5320 steps resulting from analysis of 2065 positions (951 informative) from the *nad1–cox1* mtDNA region.

structural models (reviewed in Macey & Verma 1997). Regions with extensive length variation were deemed unalignable and excluded from phylogenetic analyses.

The alignment for scincid lizards is 1933 positions of which 101 were excluded from analyses. Of the remaining 1832 positions, 949 are parsimony informative. The alignment for ranid frogs is 2126 positions of which 61 were excluded from analyses. Of the remaining 2065 positions, 951 are parsimony informative. These sequence alignments are available in electronic supplementary material, appendices A and B.

Phylogenetic trees were inferred by parsimony using PAUP* beta v. 4.0b8 (Swofford 2001) using default settings. Bootstrap resampling (Felsenstein 1985) was applied to assess support for individual nodes using 500 replicates. Decay indices (='branch support' of Bremer 1994) were calculated for all internal branches using searches that retained suboptimal nodes. All searches were heuristic using 100 random additions per replicate or search (see appendix C in electronic supplementary material for maximum-likelihood methods).

Museum voucher numbers and locality data for all newly reported sequences are deposited in GenBank files AY607272–AY607316, DQ005638 and DQ471440. A few of these sequences have been extended from other studies (Macey *et al.* 1997, 1998). Previously reported sequences used here are: *X. laevis*, M10217 (Roe *et al.* 1985); *R. catesbeiana*, *R. sylvatica*, *R. temporaria*, *R. boylii*, *R. pretiosa*, *R. aurora*, *R. cascadae* and *R. muscosa*, AF314016–AF314023 and AF314029, respectively (Macey *et al.* 2001).

3. RESULTS

Phylogenetic relationships among the Scincidae and Ranidae are presented in figure 1 (see appendix C in electronic supplementary material for maximum-likelihood results, which are similar). North American scincines do not form a monophyletic group.

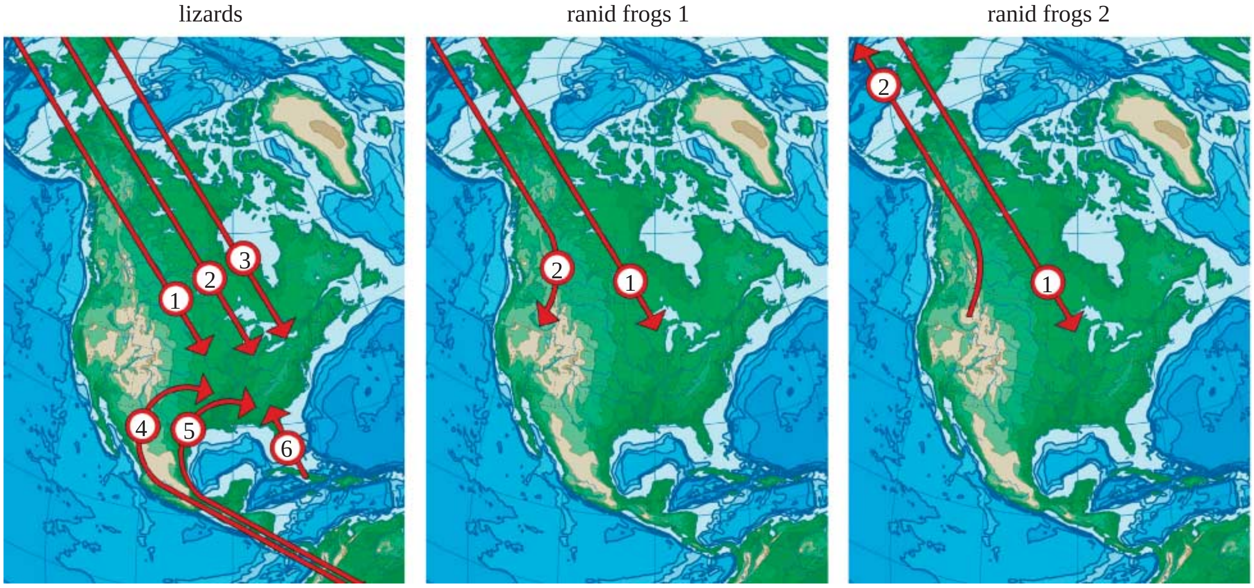

Figure 2. Dispersal of lizards and frogs into continental North America. Eleven of the 14 species of lizards found in eastern North America are derived from three dispersal events from Asia: (1) *Eumeces*, (2) *Scincella* and (3) *Ophisaurus*. Three independent dispersal events from the neotropics explain distributions of three additional taxa: (4) *Sceloporus*, (5) *Cnemidophorus* and (6) a West Indian lineage of *Anolis*. Two dispersal events across Beringia explain the occurrence of ranid frogs in North America. In one hypothesis, both lineages of North American ranids independently dispersed from Asia to (1) eastern and (2) western North America, respectively. Alternatively, there may have been (1) a single dispersal event from Asia to North America, then following divergence of eastern and western North American taxa (2) a subsequent dispersal back to Asia (see text).

Table 1. North American lizard and frog diversity east of the Mississippi River.

lizards	family	origin	no. of species[a]	reference
Ophisaurus	Anguidae	Asian	4	Macey *et al.* (1999)
Eumeces	Scincidae	Asian	6	this study
Scincella	Scincidae	Asian	1	this study and Honda *et al.* (2003)
Anolis	Iguanidae	neotropical	1	Jackman *et al.* (1999)
Sceloporus	Iguanidae	neotropical	1	Wiens & Reeder (1997)
Cnemidophorus	Teiidae	neotropical	1	Reeder *et al.* (2002)
frogs				
Rana	Ranidae	Asian	14	this study
Bufo	Bufonidae	not determined	5	Graybeal (1997)
Acris	Hylidae	neotropical	2	Faivovich *et al.* (2005)
Hyla	Hylidae	neotropical	7[b]	Faivovich *et al.* (2005)
Pseudacris	Hylidae	neotropical	8	Faivovich *et al.* (2005) and Moriarty & Cannatella (2004)
Gastrophryne	Microhylidae	not studied	1	—
Scaphiopus	Pelobatidae	not determined	1	Garcia-Paris *et al.* (2003)

[a] Number of species occurring east of the Mississippi River is after Conant & Collins (1998).
[b] Not including polyploid taxa.

The lygosomine *Scincella lateralis* groups with East Asian *Scincella* and *Sphenomorphus* with strong support (bootstrap 98%, decay index 14) to the exclusion of West Asian *Ablepharus* and *Asymblepharus*, which form a monophyletic group (bootstrap 100%, decay index 27). North American members of the scincine genus *Eumeces* appear monophyletic with weak support (bootstrap less than 50, decay index 2), yet they are strongly grouped with East Asian *Eumeces* (*E. capito* and *E. quadrilineatus*, bootstrap 100, decay index 30).

North American ranid frogs also do not form a monophyletic group. Eastern North American ranids (*R. catesbeiana*, *R. sylvatica*, *R. areolata* and *R. pipiens*) and *R. warszewitschii* from Costa Rica together form a clade (bootstrap 74, decay index 10). Western North American ranids form a separate monophyletic group (bootstrap 99, decay index 11), the *R. boylii* species group (Macey *et al.* 2001). Among Eurasian *R. temporaria* and *R. japonicus* species groups form a clade (bootstrap 100, decay index 12) that is the sister taxon to the western North American *R. boylii* species group (bootstrap 87, decay index 10). All other ranids sampled are phylogenetically outside a group comprising eastern North America, western North America and the *R. temporaria*–*R. japonicus* clade.

4. DISCUSSION

Our results identify several intercontinental dispersals that contributed to the current distributions of the lizards and frogs examined here. Among lizards, North American *Eumeces* group with East Asian *Eumeces* to the exclusion of all other Northern Hemisphere scincid lizards, indicating a dispersal event across Beringia from Asia (figure 2). The North American species of *Scincella* is nested within Asian taxa indicating an additional dispersal event from Asia to North America.

Among ranid frogs, two clades of North American species are identified: one corresponding to the *R. boylii* group of western North America and another consisting of eastern North American taxa plus the single Neotropical species *R. warszewitschii*. The North American *Rana* do not form a monophyletic group. The *R. boylii* species group is the sister taxon to the *R. temporaria*–*R. japonicus* clade, and two equally parsimonious interpretations can explain dispersals across Beringia. One hypothesis is that separate dispersals across Beringia from Asia established the eastern and western North American clades, with the eastern North American dispersal preceding the one that established the *R. boylii* group in western North America. Alternatively, a single dispersal event from Asia to North America may have occurred followed by vicariant separation of eastern and western North American forms and a subsequent dispersal from western North America back to Asia to establish the *R. temporaria*–*R. japonicus* clade.

There are 14 native continental species of lizards in eastern North America (east of the Mississippi River; Conant & Collins 1998; table 1). Here, we have determined that ancestral lineages of the six *Eumeces* species and of the single *Scincella* species are both of Asian origin. Macey *et al.* (1999) showed that the ancestral lineage of four eastern North American *Ophisaurus* species is of Asian origin. The three remaining lizard taxa occurring in continental eastern North America, from separate taxonomic groups, are derived from the neotropics: *Anolis* (West Indies; Jackman *et al.* 1999), *Cnemidophorus* (Reeder *et al.* 2002) and *Sceloporus* (Wiens & Reeder 1997). Hence, 11 of the 14 eastern North American lizard species descend from ancestral lineages representing three dispersal events across Beringia. The origins of the 38 frog taxa in eastern North America (Conant & Collins 1998) are less well understood than lizards. All 14 *Rana* species appear to descend from a single dispersal event from Asia because our sampling includes members of all eastern North American species groups. The 17 hylid frogs occurring east of the Mississippi River are descended from a neotropical ancestor, and the North American taxa gave rise to the Eurasian species through dispersal across Beringia (Faivovich *et al.* 2005). Phylogenetic data are not yet available to evaluate geographic origins of the other frog groups in eastern North America.

Eighty per cent of lizard species and at least a third of frog taxa occurring east of the Mississippi River are derived from four independent dispersal events across Beringia. The contribution of Asian faunal elements to North America is a dominant theme in the assembly of the eastern North American fauna.

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Bossuyt, F. & Milinkovitch, M. C. 2001 Amphibians as indicators of Early Tertiary 'out-of-India' dispersal of vertebrates. *Science* **292**, 93–95. (doi:10.1126/science.1058875)

Brandley, M. C., Schmitz, A. & Reeder, T. W. 2005 Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Syst. Biol.* **54**, 373–390. (doi:10.1080/10635150590946808)

Bremer, K. 1994 Branch support and tree stability. *Cladistics* **10**, 295–304. (doi:10.1111/j.1096-0031.1994.tb00179.x)

Brown, J. H. & Lomolino, M. V. 1998 *Biogeography*, 2nd edn, p. 691. Sunderland, MA: Sinauer Associates, Inc.

Conant, R. & Collins, J. T. 1998 *A field guide to reptiles & amphibians of eastern and central North America*, 3rd edn expanded, p. 616. New York, NY: Houghton Mifflin.

Darwin, C. 1883 *The origin of species*, 6th edn, p. 458. New York, NY: Appleton.

Faivovich, J., Haddad, C. F. B., Garcia, P. C. A., Frost, D. R., Campbell, J. A. & Wheeler, W. C. 2005 Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bull. Am. Mus. Nat. Hist.* **294**, 1–240. (doi:10.1206/0003-0090(2005)294[0001:SROTFF]2.0.CO;2)

Felsenstein, J. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Garcia-Paris, M., Buchholz, D. R. & Parra-Olea, G. 2003 Phylogenetic relationships of Pelobatoidea re-examined using mtDNA. *Mol. Phylogenet. Evol.* **28**, 12–23.

Graybeal, A. 1997 Phylogenetic relationships of bufonid frogs and tests of alternate macroevolutionary hypotheses characterizing their radiation. *Zool. J. Linn. Soc.* **119**, 297–338. (doi:10.1006/zjls.1996.0068)

Griffith, H., Ngo, A. & Murphy, R. W. 2000 A cladistic evaluation of the cosmopolitan genus *Eumeces* Wiegmann (Reptilia, Squamata, Scincidae). *Russ. J. Herpetol.* **7**, 1–16.

Hillis, D. M. & Davis, S. K. 1986 Evolution of ribosomal DNA: fifty million years of recorded history in the frog genus *Rana*. *Evolution* **40**, 1275–1288.

Honda, M., Ota, H., Kohler, G., Ineich, I., Chirio, L., Chen, S.-L. & Hikida, T. 2003 Phylogeny of the lizard subfamily Lygosominae (Reptilia: Scincidae), with special reference to the origin of the New World taxa. *Genes Genet. Syst.* **78**, 71–80. (doi:10.1266/ggs.78.71)

Jackman, T. J., Larson, A., de Queiroz, K. & Losos, J. B. 1999 Phylogenetic relationships and tempo of early diversification in *Anolis* lizards. *Syst. Biol.* **48**, 254–285. (doi:10.1080/106351599260283)

Macey, J. R. & Verma, A. 1997 Homology in phylogenetic analysis: alignment of transfer RNA genes and the phylogenetic position of snakes. *Mol. Phylogenet. Evol.* **4**, 272–279. (doi:10.1006/mpev.1997.0379)

Macey, J. R., Larson, A., Ananjeva, N. B., Fang, Z. & Papenfuss, T. J. 1997 Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Mol. Biol. Evol.* **14**, 91–104.

Macey, J. R., Schulte II, J. A., Larson, A., Fang, Z., Wang, Y., Tuniyev, B. S. & Papenfuss, T. J. 1998 Phylogenetic relationships of toads in the *Bufo bufo* species group from the eastern escarpment of the Tibetan Plateau: a case of vicariance and dispersal. *Mol. Phylogenet. Evol.* **9**, 80–87. (doi:10.1006/mpev.1997.0440)

Macey, J. R., Schulte II, J. A., Larson, A., Tuniyev, B. S., Orlov, N. & Papenfuss, T. J. 1999 Molecular phylogenetics, tRNA evolution and historical biogeography in anguid lizards and related taxonomic families. *Mol. Phylogenet. Evol.* **12**, 250–272. (doi:10.1006/mpev.1999.0615)

Macey, J. R., Schulte II, J. A., Larson, A., Ananjeva, N. B., Wang, Y., Pethiyagoda, R., Rastegar-Pouyani, N. & Papenfuss, T. J. 2000 Evaluating trans-Tethys migration: an example using acrodont lizard phylogenetics. *Syst. Biol.* **49**, 233–256. (doi:10.1080/10635159950173834)

Macey, J. R., Strasburg, J. L., Brisson, J. A., Vredenburg, V. T., Jennings, M. & Larson, A. 2001 Molecular phylogenetics of western North American frogs of the *Rana boylii* species group. *Mol. Phylogenet. Evol.* **19**, 131–143. (doi:10.1006/mpev.2000.0908)

Maddison, W. P. & Maddison, D. R. 2001 *MacClade, analysis of phylogeny and character evolution, version 4.03*. Sunderland, MA: Sinauer.

Maekawa, K., Park, Y. C. & Lo, N. 2005 Phylogeny of endosymbiont bacteria harbored by the woodroach *Cryptocercus* spp. (Cryptocercidae: Blattaria): molecular clock evidence for a Late Cretaceous–Early Tertiary split of Asian and American lineages. *Mol. Phylogenet. Evol.* **36**, 728–733. (doi:10.1016/j.ympev.2005.03.033)

Moriarty, E. C. & Cannatella, D. C. 2004 Phylogenetic relationships of the North American chorus frogs (*Pseudacris*: Hylidae). *Mol. Phylogenet. Evol.* **30**, 409–420. (doi:10.1016/S1055-7903(03)00186-6)

Pough, H. F., Andrews, R. M., Cadle, J. E., Crump, M. L., Savitzky, A. H. & Wells, K. D. 2004 *Herpetology*, 3rd edn, p. 726. Upper Saddle River, NJ: Pearson Education, Inc.

Reeder, T. W., Cole, C.J. & Dessauer, H.C. 2002 Phylogenetic relationships of Whiptail Lizards of the genus *Cnemidophorus* (Squamata: Teiidae): a test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. *Am. Mus. Novitates* **3365**, p. 61.

Roe, B. A., Ma, D. P., Wilson, R. K. & Wong, J. F. 1985 The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *J. Biol. Chem.* **15**, 9759–9774.

Swofford, D. L. 2001 *Paup* phylogenetic analysis using parsimony (*and other methods), Beta Version 4.0b8*. Sunderland, MA: Sinauer.

von Dohlen, C. D., Kurosu, U. & Aoki, S. 2002 Phylogenetics and evolution of the eastern Asian–eastern North American disjunct aphid tribe, Hormaphidini (Hemiptera: Aphididae). *Mol. Phylogenet. Evol.* **23**, 257–267. (doi:10.1016/S1055-7903(02)00025-8)

Wallace, A. R. 1876 *The geographical distribution of animals*, 2 vols. London, UK: Macmiillan.

Wen, J. 1999 Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Annu. Rev. Ecol. Syst.* **30**, 421–455. (doi:10.1146/annurev.ecolsys.30.1.421)

Wiens, J. J. & Reeder, T. W. 1997 Phylogeny of the spiny lizards (*Sceloporus*) based on molecular and morphological evidence. *Herpetol. Monogr.* **11**, 1–101.