

How and when did Old World rat snakes disperse into the New World?

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Received 20 March 2006; revised 21 August 2006; accepted 17 September 2006

Available online 27 September 2006

Abstract

To examine Holarctic snake dispersal, we inferred a phylogenetic tree from four mtDNA genes and one scnDNA gene for most species of the Old World (OW) and New World (NW) colubrid group known as rat snakes. Ancestral area distributions are estimated for various clades using divergence–vicariance analysis and maximum likelihood on trees produced using Bayesian inference. Dates of divergence for the same clades are estimated using penalized likelihood with statistically crosschecked calibration references obtained from the Miocene fossil record. With ancestral areas and associated dates estimated, various hypotheses concerning the age and environment associated with the origin of rat snakes and the dispersal of NW taxa from OW ancestors were tested. Results suggest that the rat snakes originated in tropical Asia in the late Eocene and subsequently dispersed to the Western and Eastern Palearctic by the early Oligocene. These analyses also suggest that the monophyletic NW rat snakes (the Lampropeltini) diverged from OW rat snakes and dispersed through Beringia in the late Oligocene/early Miocene when this land bridge was mostly composed of deciduous and coniferous forests.

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Keywords: Ancestral area; Divergence date; Colubridae; Rat snakes; Divergence–vicariance analysis; Maximum likelihood; Bayesian inference; Penalized likelihood; Beringia

1. Introduction

Investigating faunal exchange between the New (NW) and Old World (OW) continents of the Holarctic Region in the Tertiary relies upon explicit tests of dispersal hypotheses as continental positions were roughly in the same orientation as they are today. During the mid-to-late Tertiary, two major routes for this exchange existed, either trans-Atlantic or trans-Beringian (Pacific). Examination of the frequency of route use by 57 extant nonmarine animal species suggests that trans-Atlantic routes were most common during the early–mid Tertiary (70–20 mya) and trans-Pacific paths of faunal exchange were more common during the late Tertiary (20–3 mya; [Sanmartin et al., 2001](#)). Assessing the direction of these exchanges suggests that

movements either from the OW to the NW or from the NW to the OW were equally common across many animal groups. These trends were summarized mainly for the following groups of animals: crustaceans, insects, arachnids, teleost fishes, birds, and mammals ([Sanmartin et al., 2001](#)).

Although snakes of individual families and subfamilies (Colubrinae, Elapidae, Natricinae, and Viperidae) are found across the Holarctic Region, literature explicitly examining areas of origin of these groups and the dates and routes of dispersal from areas of origin does not exist ([Lawson et al., 2005](#)). This is surprising given that these families and subfamilies dominate the snake fauna of the Holarctic Region ([Pough et al., 2004](#)). In this paper, we examine the area of origin, dates of origin, area of dispersal, and dates of dispersal for one of the most well-known and conspicuous groups of the holarctic snake fauna, the rat snakes. For this group, we infer the phylogeny, assess ancestral areas of origin, and use key internal-node divergence dates to investigate the areas and date of origin of the holarctic rat snakes.

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The rat snakes are currently represented by 19 genera that have a holarctic and oriental distribution. Once considered members of a single genus, *Elaphe*, the rat snakes have recently been divided into a number of OW and NW genera. In the OW are the genera *Elaphe*, *Zamenis*, *Rhinechis*, *Oocatochus*, *Orthriophis*, *Euprepiophis*, *Oreocryptophis*, *Coelognathus*, and *Gonyosoma*. The Western Palearctic smooth snakes, *Coronella*, are considered closely related to OW rat snakes (Dowling and Duellman, 1978; Nagy et al., 2004; Utiger et al., 2002). Alternatively, NW rat snakes are considered part of a monophyletic tribe, the Lampropeltini, which includes the genera *Arizona*, *Bogertophis*, *Cemophora*, *Lampropeltis*, *Pantherophis*, *Pituophis*, *Pseudelaphe*, *Rhinocheilus*, *Senticolis*, and *Stilosoma* (Dessauer et al., 1987; Dowling, 1952; Dowling et al., 1983; Dunn, 1928; George and Dessauer, 1970; Keogh, 1996; Lawson and Dessauer, 1981; Minton, 1976; Pearson, 1966; Rodríguez-Robles and de Jesús-Escobar, 1999; Schwaner and Dessauer, 1982; Utiger et al., 2002) and, as a group, appears to be related to OW rat snakes. Therefore, in this paper we refer to the group that collectively includes the OW rat snakes and the Lampropeltini simply as rat snakes.

The historical processes that have shaped the distribution of these snakes may include extinction, dispersal, and vicariance (Futuyma, 2005). Using a phylogeny of the extant taxa with geographic distributions mapped onto the tree permits the inference of ancestral distributions found within internal nodes of this phylogeny. These ancestral distributions along with an estimation of time, in turn, may help to identify possible dispersal routes or vicariant events that have produced the current distribution of extant species. For taxa with a poorly known fossil record, such as snakes, using phylogenies of extant species may provide the only means to estimate the date and ancestral area of origin and subsequent trans-Holarctic dispersal events.

The study of snake evolution, where the availability and identification of fossils of modern groups prior to the Miocene is limited (Holman, 2000; Rage, 1987), underscores the need for a combined technique of estimating ancestral area and divergence time using extant taxa. Fossil representatives of the largest extant snake family, the Colubridae, prior to the Miocene are poorly known with respect to affinities with modern taxa (Holman, 2000; Rage, 1984, 1987), although fossils do indicate that this family dates back to the late Eocene in Thailand and from the early Oligocene in North America and Europe (Holman, 2000; Rage et al., 1992; Szyndlar, 1994). Evidence from the fossil record suggests that an explosive radiation in snake diversity with clear modern affinities, particularly with respect to Colubridae, occurred in the early Miocene of Europe and North America (Holman, 2000; Rage, 1987). It is presumed that relatives of modern colubrid groups must have lived throughout the Oligocene, but did not fossilize, remain undiscovered, or are improperly identified. Therefore, the fossil record alone cannot be used to determine the area and date of origin of extant colubrid groups, including the holarctic ratsnakes, with probable occur-

rence prior to the Miocene. Thus, combining ancestral area and divergence date estimation using a phylogeny of extant taxa provides the only means of identifying the area and date of origin for modern species of snakes. As stated by Near and Sanderson (2004), the taxa that necessitate molecular divergence date estimation are usually those with a poor fossil record.

Using DNA sequences of four mitochondrial and one single-copy nuclear gene from NW and OW ratsnakes, along with the European and North American Miocene colubrid fossil record (Holman, 2000; Ivanov, 2001, 2002; Rage, 1987) to calibrate rates of molecular evolution, we: (i) infer a phylogeny for the ratsnakes, (ii) assess putative ancestral areas, and (iii) estimate times of divergences at key nodes. Although generally accepted, but poorly demonstrated, we examine the assumption that the Lampropeltini are a monophyletic NW group derived from OW ratsnakes (Dessauer et al., 1987; Dowling, 1952; Dowling et al., 1983; Dunn, 1928; George and Dessauer, 1970; Keogh, 1996; Lawson and Dessauer, 1981; Minton and Salanitro, 1972; Pearson, 1966; Rodríguez-Robles and de Jesús-Escobar, 1999; Schwaner and Dessauer, 1982; Utiger et al., 2002). Provided that the Lampropeltini are monophyletic and derived from OW ratsnakes (Utiger et al., 2002), we infer the ancestral area for these groups and estimate dates of divergence from their most recent common ancestor (MRCA). With this ancestral area and divergence date information, we test two possible dispersal routes connecting the Nearctic and Palearctic: the older pre-Oligocene trans-Atlantic land bridges connecting Europe with Eastern North America (Liebherr, 1991; McKenna, 1983; Tiffney, 1985) and the more recent trans-Beringian land bridge connecting Eastern Asia with Western North America (Lafontaine and Wood, 1988; Mathews, 1979; McKenna, 1983; Nordlander et al., 1996; Tangledler, 1988). Both of these land bridges might have provided suitable habitat for snakes to cross from the OW to the NW throughout the earlier part of the Tertiary (65–45 mya; Fig. 1; Table 1). If dispersal occurred after 45 mya but prior to 14 mya, then movement across the warmer Beringia with associated suitable habitat (Pielou, 1979) appears more probable, given that the trans-Atlantic routes either no longer existed or did not provide appropriately warm habitats after this date (Fig. 1; Table 1; Sanmartin et al., 2001). This approach assumes that ancestral ratsnakes dispersing across either of these northern routes were adapted to the same environmental conditions as extant taxa that live in OW and NW northern extremes (*Pantherophis obsoletus*, *Pituophis melanoleucus*, *Lampropeltis triangulum*, *Coronella austriaca*, *Elaphe dione*, *E. longissima*, and *Oocatochis rufodorsatus*) (Arnold et al., 1978; Conant and Collins, 1991; Schulz, 1996; Staszko and Walls, 1994). Our tests also assume that divergences between OW and NW taxa did not occur prior to the Tertiary, because hypotheses of dispersal between these areas assume that the continents are roughly in the same positions as they were from the Eocene to modern times.

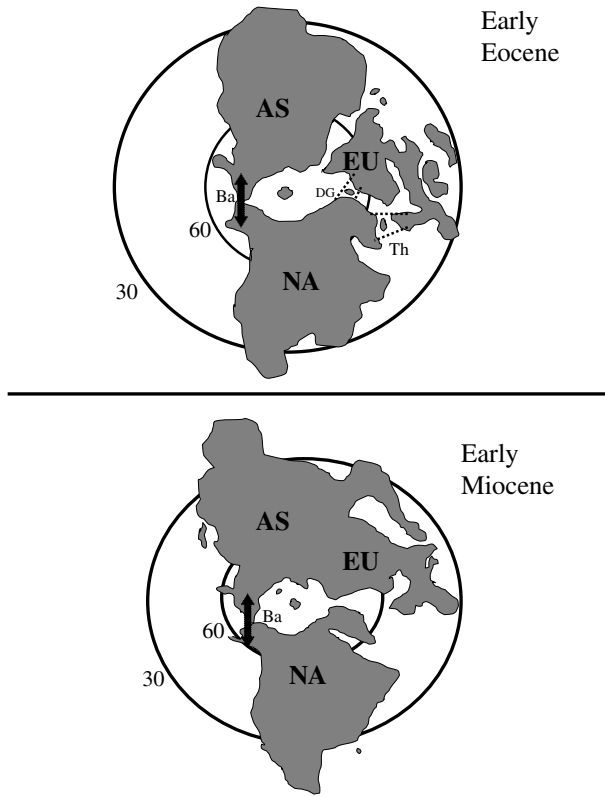


Fig. 1. Maps adapted from Figs. 3 and 4 in Sanmartin et al. (2001) showing the orientation of the Asian (AS), European (EU), and North American (NA) continents and the relative sea levels during the early Eocene (~50 mya) and early Miocene (~20 mya). Holarctic dispersal routes through Beringia (Ba), the Thulean North-Atlantic bridge (Th), and the De Geer North-Atlantic bridge (DG) are also depicted.

We also infer the ancestral area and date of origin for the root of the tree that gave rise to all ratsnakes. With this information, we examine the age and routes of dispersal of ratsnakes from areas of origin throughout the OW. As sug-

gested by Ivanov (2001, 2002), occurrences in the fossil record show that colubrids began appearing and replacing boids in Europe from its eastern reaches or from Asia during the Oligocene.

2. Methods and materials

2.1. Sampling, sequencing, and alignment

This study represents the most comprehensive molecular survey of OW and NW ratsnakes (Table 2). For OW ratsnakes, we used tissue samples from 33 taxa that include all genera: *Coelognathus*, *Elaphe*, *Rhinechis*, *Oocatochus*, *Oreophis*, *Orthriophis*, *Euprepiophis*, and *Gonyosoma*. Recently recognized species were represented by at least one taxon from their species complex. For those newly recognized taxa, our dataset used: *Elaphe quatuorlineata* for *E. sauromates* (Helfenberger, 2001; Lenk et al., 2001), *E. shrenckii* for *E. anomola* (Helfenberger, 2001), and *Zamenis lineata* for *Z. longissima* (in part; Lenk and Wüster, 1999). The study did not include *Euprepiophis perlaceus* due to its extreme rarity and the claim that it may not be a distinct species from *E. mandarina*, which is included in our study (Schulz, 1996). The rare *E. leonardi* is a junior synonym of *E. bella* and we use the latter of those two names (Schulz et al., 2000). A specimen considered to be of the genus *Orthriophis* (possibly the species *O. taeniurus*) secured by J. Slowinski in Myanmar was also included. This specimen is listed under the name of the California Academy of Sciences collection number: *Orthriophis* sp. CAS 221547. In addition to these OW taxa above, other genera considered closely related to OW ratsnakes were included, these were: *Coronella girondica*, *Spalerosophis diadema*, *Ptyas korros*, and *Ptyas mucosus*. For NW ratsnakes (*Pantherophis*, *Pseudelaphe*, *Bogertophis*, and *Senticolis*), we included most genera of the Lampropeltini and multiple species of NW

Table 1
Possible dispersal routes for New World and Old World taxa during the Cenozoic

Connection name	Areas connected	Range (my)	Habitat	Climate	Appropriate for snakes
<i>Trans-Atlantic connection</i>					
Thulean Bridge	S. Europe–British Isles–Queen Elizabeth Islands	65–50	Para-tropical/sub-tropical/polar broad-leafed forest	Warm	Possible prior to 50 mya
DeGreer Bridge	Scandinavia–northern Greenland–Canadian Arctic Archipelago	65–39	Para-tropical to temperate woodland	Cool	No – cold-adapted organisms only
Greenland–Faeroes Bridge	Greenland–Faeroes bridge (island chain connection)	65–24	Para-tropical/sub-tropical to temperate woodland	Cool	No – island chain only
<i>Trans-Beringian land bridge</i>					
Paleocene–Eocene Beringian Bridge	NE Asia to NW North America	65–34	Boreotropical forest	Warm/temperate	Possible
Oligocene Beringian Bridge	NE Asia to NW North America	34–24	Temperate woodland (deciduous/coniferous forest)	Moderate	Possible
Early- to mid-Miocene Beringian Bridge	NE Asia to NW North America	24–14	Temperate woodland (deciduous/coniferous/broad-leafed forest)	Warm/temperate	Possible
Late Miocene to late Pliocene Beringian Bridge	NE Asia to NW North America	14–3.5	Coniferous to taiga	Cool	No – too cold

Name and area connected as well as the range in dates of millions of years before present (my), habitat, climate and likelihood of snakes using these routes are considered.

Table 2 (continued)

Taxon	Museum	Voucher number	GenBank accession numbers are in the following gene order: <i>cytb/ND1/ND2/ND4/c-mos</i>	Origin
<i>Zamenis hohenackeri</i>		No voucher	DQ902137/DQ902191/DQ902250/DQ902320/DQ902098	Turkey
<i>Zamenis lineata</i>		No voucher	DQ902138/DQ902192/DQ902251/DQ902319/DQ902099	Italy
<i>Zamenis persica</i>		No voucher	DQ902117/DQ902178/DQ902225/DQ902297/DQ902075	Unknown
<i>Zamenis situla</i>	CAS	175031	DQ902125/DQ902189/DQ902234/DQ902303/DQ902083	Unknown

ratsnakes (formerly recognized as *Elaphe*). Data from hemipen morphology, dentition, squamation, immunological distance, allozymes, and mtDNA sequences have indicated that the NW ratsnake genera, *Pantherophis*, *Pseudelaphe*, *Bogertophis*, and *Senticolis*, are members of a tribe that includes other genera (*Cemophora*, *Lampropeltis*, *Stilosoma*, *Pituophis*, and *Arizona*) traditionally not considered ratsnakes (Dessauer et al., 1987; Dowling, 1952; Dowling et al., 1983; Dunn, 1928; George and Dessauer, 1970; Keogh, 1996; Lawson and Dessauer, 1981; Minton, 1976; Pearson, 1966; Rodríguez-Robles and de Jesús-Escobar, 1999; Schwaner and Dessauer, 1982; Utiger et al., 2002). Consequently, as potential allies to the NW ratsnakes, we have included *Arizona elegans*, *Stilosoma extenuatum*, *Lampropeltis calligaster*, *Cemophora coccinea*, and *Pituophis melanoleucus* within our ingroup. As a close outgroup to ratsnakes (Colubrinae) we used the NW and OW racer species: *Salvadora mexicana*, *Masticophis flagellum*, *Hemerrhous hippocrepis*, *Coluber constrictor*, *Spalerosophis diadema*, *Hierophis viridiflavus*, and *Macroprotodon cucullatus* (Nagy et al., 2004). Additional more distant outgroup taxa were included as representatives of three other colubrid subfamilies that have never been considered allied to any of the OW or NW ratsnakes (Lawson et al., 2005). For the outgroups, we used one natricine, *Thamnophis sirtalis*, one psammophine, *Malpolon monspessulanus*, and two xenodontines, *Farancia abacura* and *Heterodon simus*.

2.2. Acquisition of DNA sequence data

We used the standard method of proteinase K digestion in lysis buffer followed by several rounds of phenol/CHCl₃ extraction (Sambrook and Russell, 2001) to obtain total genomic DNA from samples of shed skin, liver tissue or whole blood. Extracted DNA was precipitated with three volumes of absolute EtOH and the pelleted DNA was then washed with two changes of 80% EtOH before redissolving in TE buffer (Sambrook and Russell, 2001). Stock DNA solution was diluted 1:25 with TE buffer to produce template strength DNA for the polymerase chain reaction (PCR). Using the PCR we amplified the complete gene sequence for the mitochondrial genes *cytochrome b* (*cytb*), *NADH dehydrogenase subunit 1* (*ND1*), and *NADH dehydrogenase subunit 2* (*ND2*), and the partial gene sequence of *NADH dehydrogenase subunit 4* (*ND4*). Additionally, we amplified and sequenced a segment of the single-copy nuclear gene for the oocyte maturation factor (*c-mos*). The PCR product cleanup and cycle sequencing reactions were performed as described in Burbrink et al. (2000) with

sequences being read using either an ABI 310 or 3100 Genetic Analyzer. Primers for the PCR and cycle sequencing reactions were as follows: *cytb* amplification and sequencing L14910 (de Queiroz et al., 2002) and H16064 (Burbrink et al., 2000); *cytb* sequencing only L14919 (Burbrink et al., 2000), L15584 (de Queiroz et al., 2002), H15149 (Kocher et al., 1989), and H15715 (Slowinski and Lawson, 2005); *ND1* amplification and sequencing 16Sb (Palumbi, 1996) and H3518 (de Queiroz et al., 2002), sequencing only L2894 and H3056 (de Queiroz et al., 2002); *ND2* amplification and sequencing L4437 (Kumazawa et al., 1996) and H5877 (de Queiroz et al., 2002), sequencing only L5238 and H5382 (de Queiroz et al., 2002); *ND4* amplification and sequencing *ND4* and tRNA^{leu} (Arévalo et al., 1994); *c-mos* amplification and sequencing S77 and S78 (Slowinski and Lawson, 2005). Using these combinations of primers, both heavy and light strands of each gene were sequenced. All sequences from these five protein-coding genes (*ND1*, *ND2*, *ND4*, *cytb*, and *c-mos*) were first converted to amino acids, which were then aligned using the software DAMBE (Xia, 2000) and then backconverted to the original nucleotide sequences without the potential loss of information at degenerate sites (see Lawson et al., 2004 for technique). Therefore, gaps in the alignment occur in multiples of three, which correspond to the missing amino acids in the alignment. Alignments were also compared to those produced by Clustal X (Thompson et al., 1997). Additionally, the nucleotide sequences were examined by eye using the program Sequencher 4.1.2 (Genecodes, 2000), and an open-reading frame for each given protein-coding gene was determined.

2.3. Phylogenetic analyses

Sequences were combined from the four mitochondrial protein genes (*cytb*, *ND1*, *ND2*, and *ND4*) and one nuclear protein gene (*c-mos*) on all but two taxa of the 41 species of ratsnakes from all genera. Phylogenies from this combined dataset were inferred using Maximum Likelihood (ML) and Bayesian phylogenetic inference (BI). The appropriate model for the ML analysis was selected using Akaike Information Criteria (AIC) in the program Modeltest (version 3.06; Posada and Buckley, 2004; Posada and Crandall, 1998); the starting tree was obtained using the neighbor-joining algorithm. After producing an initial ML estimate using PAUP* (version 4.10b; Swofford, 2003), parameters were re-estimated using this tree, and another ML tree (the best ML tree) was inferred using the updated parameters (cf. Swofford et al., 1996). Support for a tree was obtained

from 1000 nonparametric bootstrap pseudoreplicates (Felsenstein, 1985) under the ML criterion with the preferred model according to AIC using the program PHYML 2.4.4 (Guindon and Gascuel, 2003). Additionally, using the same parameters as the best model chosen using AIC, we estimated support from 100 nonparametric bootstrap pseudoreplicates using PAUP* (version 4.10b; Swofford, 2003). Nonparametric bootstrap values above 75% were considered good support for a bipartition (Hillis and Bull, 1993).

To infer trees and to assess tree support using models incorporating evolutionary information specific to each gene, we performed a mixed-model analysis using BI with MrBayes (version 3.0b4; Huelsenbeck and Ronquist, 2002). Prior to tree inference, two evolutionary models were evaluated for each dataset using Bayes factors on the harmonic mean of the posterior probability (PP) distribution of these models. The first model accounts for differences in evolutionary rates of each of the three codon positions for each gene (thus accounting for three codon positions per gene for all five genes, yielding 15 partitions) using the GTR + Γ + I model with estimated base pair (BP) frequencies for each codon position. For this codon-position-specific model, abbreviated CS (GTR + Γ + I), a single tree was estimated for all 15 codon partitions simultaneously, but all other model parameters were unlinked to insure independent estimation of parameters among partitions. The second method simply applied the GTR + Γ + I model across all positions with no partitioning among genes or codon positions.

For each model, four independent searches were executed to insure convergence of all parameters by comparing the variance across chains within a search to the chain variance among searches using Rubin and Gelman's "r" statistic (Gelman et al., 1995). Searches were considered burned-in when the values for "r" have reached 1.000. All searches consisted of three "heated" and one "cold" Markov chain estimated for 10 million generations, with every 1000th sample being retained, and with default priors applied to all parameters. Parameter stationarity was assumed to have occurred when tree $-\ln L$ values for chains converge on similar generations in all four replicates for each model. Trees sampled before stationarity were discarded. Using Bayes factors to determine the appropriate model for the sequence data requires that the harmonic mean for the model likelihood $f(X|M_i)$ was estimated from the values in the stationarity phase of a Markov chain Monte Carlo run following the methodologies of Newton and Raftery (1994) and Nylander et al. (2004). To compare models, Bayes factors followed the form $2 \log_e B_{10}$, where B_{10} is the ratio of model likelihoods. A Bayes factor value greater than 10 was considered strong evidence favoring the more parameter-rich model (Kass and Raftery, 1995). These BI trees were compared with those obtained using ML and the most credible inferences of relationship were confined to nodes where the posterior probability was greater than 95% and the nonparametric bootstraps were greater than 75%.

2.4. Ancestral area estimation

Both an event-based parsimony and a ML method were used to infer ancestral areas. For the former method, we used divergence–vicariance (DIVA) analysis developed by Ronquist (1997). This event-based method has the advantage of inferring ancestral states based on a three-dimensional (3-D) cost matrix and does not require an explicit hypothesis of area relationships. Given an area distribution and phylogeny for terminal taxa, the DIVA 3-D matrix incurs one cost for dispersal events, a single cost for extinction, and no charge for speciation due to vicariance or allopatric or sympatric speciation. We used the program DIVA 1.1 (Ronquist, 1997) to implement this model given our ML and BI trees and area distributions for ratsnakes (Figs. 2 and 3). All taxa were coded as occurring in a single area or combination of areas as indicated by Schulz (1996), Conant and Collins (1991), Smith and Taylor (1966), Staszko and Walls (1994), and Kohler (2003). The zoogeographic areas include the Western Nearctic (WN), Eastern Nearctic (EN), Western Palearctic (WP), Eastern Palearctic (EP), and the Orient (O) (Futuyma, 2005).

Although Huelsenbeck and Imennov (2002) used a combination of BI and maximum parsimony (MP) to estimate human ancestral areas, a purely stochastic method for ancestral area reconstruction is uncommon. Using the program LaGrange (Ree et al., 2005), we examined the likelihood of ancestral taxa occurring in one or a combination of the five zoogeographic regions coded in the terminal taxa (see above) using ML. This method has the following desirable properties: (i) all possible area reconstructions are considered for a node, each with a probability of occurrence given topological and branch-length information (Huelsenbeck and Bollback, 2001; Huelsenbeck et al., 2003; Pagel et al., 2004), (ii) different rates of dispersal and extinction may be examined to produce the highest likelihood of ancestral area occurrence, and (iii) information using the availability of a dispersal route in a given time may also be modeled (Ree et al., 2005). For all ratsnakes, the probability of an ancestral area using ML was estimated using the topology with branch lengths from the BI 50% majority rule tree. To find the most appropriate rate of dispersal/extinction, we examined several different rates: 0.005/0.005 to 0.009/0.001 in increments of 0.001. Our ML estimation of ancestral areas is compared to those using the DIVA method.

2.5. Divergence date estimation

To estimate ages of divergence it was necessary first to choose appropriate calibration points. Five calibration references were used simultaneously for these trees and were derived from the Miocene fossil record of Europe and North America. The genus *Lampropeltis* is known from the fossil record as early as 15 mya without prior representation from the early Miocene (Holman, 2000). Therefore, the calibration dates for *Lampropeltis* ranged from 15 to

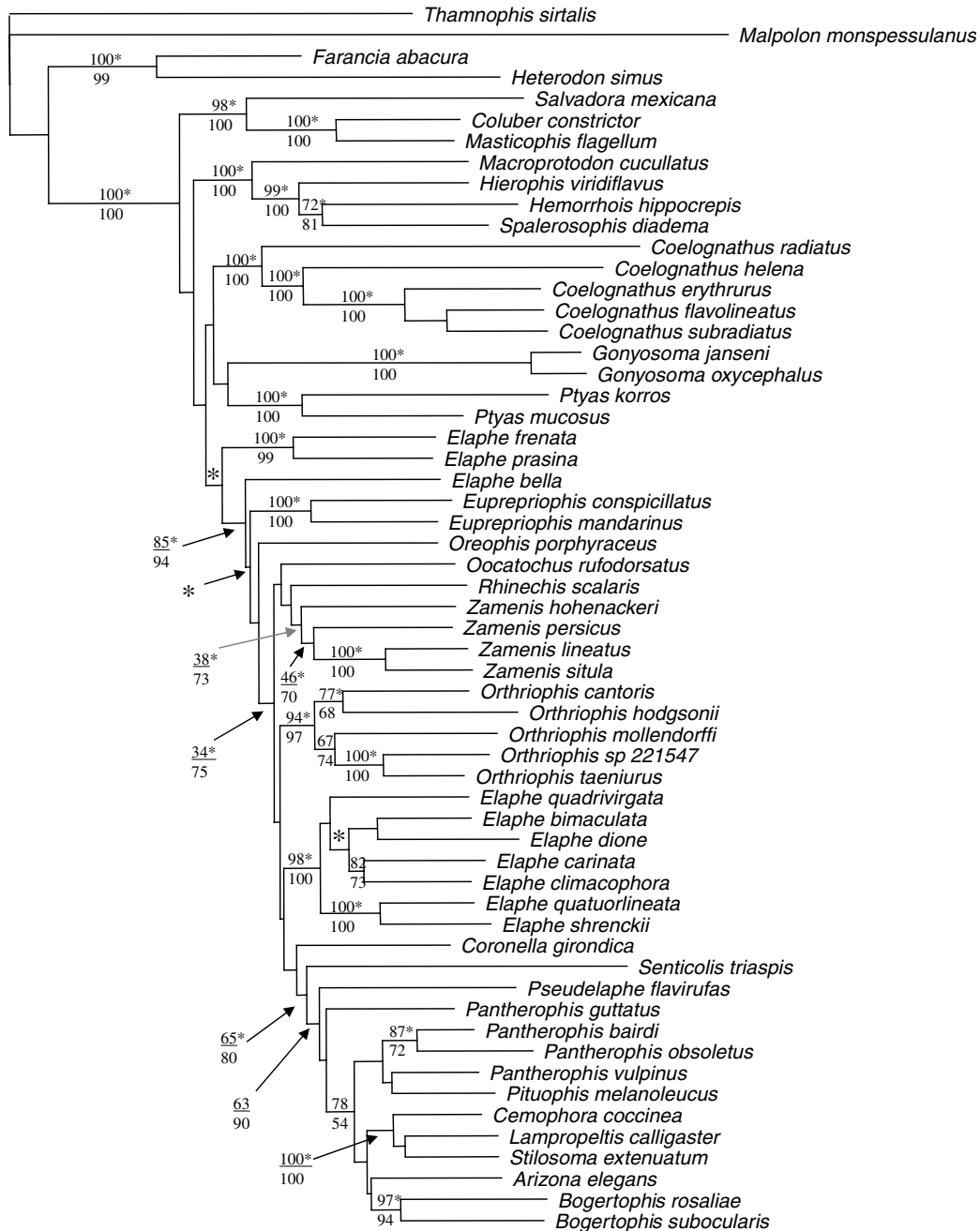


Fig. 2. Inferred phylogeny of the ratsnakes using ML on all genes combined. Asterisks above branches indicate PP support greater than 0.95. Numbers above branches indicate support values from 1000 nonparametric bootstrap pseudoreplicates using the program Phym1 2.4.4 (Guindon and Gascuel, 2003). Numbers below branches indicate support values from 100 nonparametric bootstrap pseudoreplicates using the program PAUP* (version 4.10b; Swofford, 2003).

19mya. The first occurrence of *Pantherophis* (formerly the New World *Elaphe*) at 16mya in the NW was placed as a minimum age estimate at the root of the node joining all *Pantherophis* (Holman, 2000). Although other modern NA colubrids are known from the early Miocene, *Pantherophis* is unknown in the fossil record prior to 20mya, and this date was used as a maximum age estimate for this node. In the OW, fossils considered directly ancestral to *Zamenis situla* and *Z. lineatus* (*E. longissima*) were dated at 6mya (Ivanov, 1997). The first ratsnake (designated as the genus

Elaphe) appeared in Europe 20mya (Ivanov, 2002). For the MRCA of *Z. situla* and *Z. lineatus*, we chose the minimum and maximum range of dates to be from 6 to 20mya. The New World genera *Coluber* and *Masticophis* are known from the early or middle part of the late Miocene, and a minimum date of 11mya was used for their MRCA. A maximum calibration point of 15my was used for the MRCA of *Coluber* and *Masticophis* as neither are known from the middle Miocene, even though other related taxa, *Paracoluber* and *Salvadora*, have been found as far back as

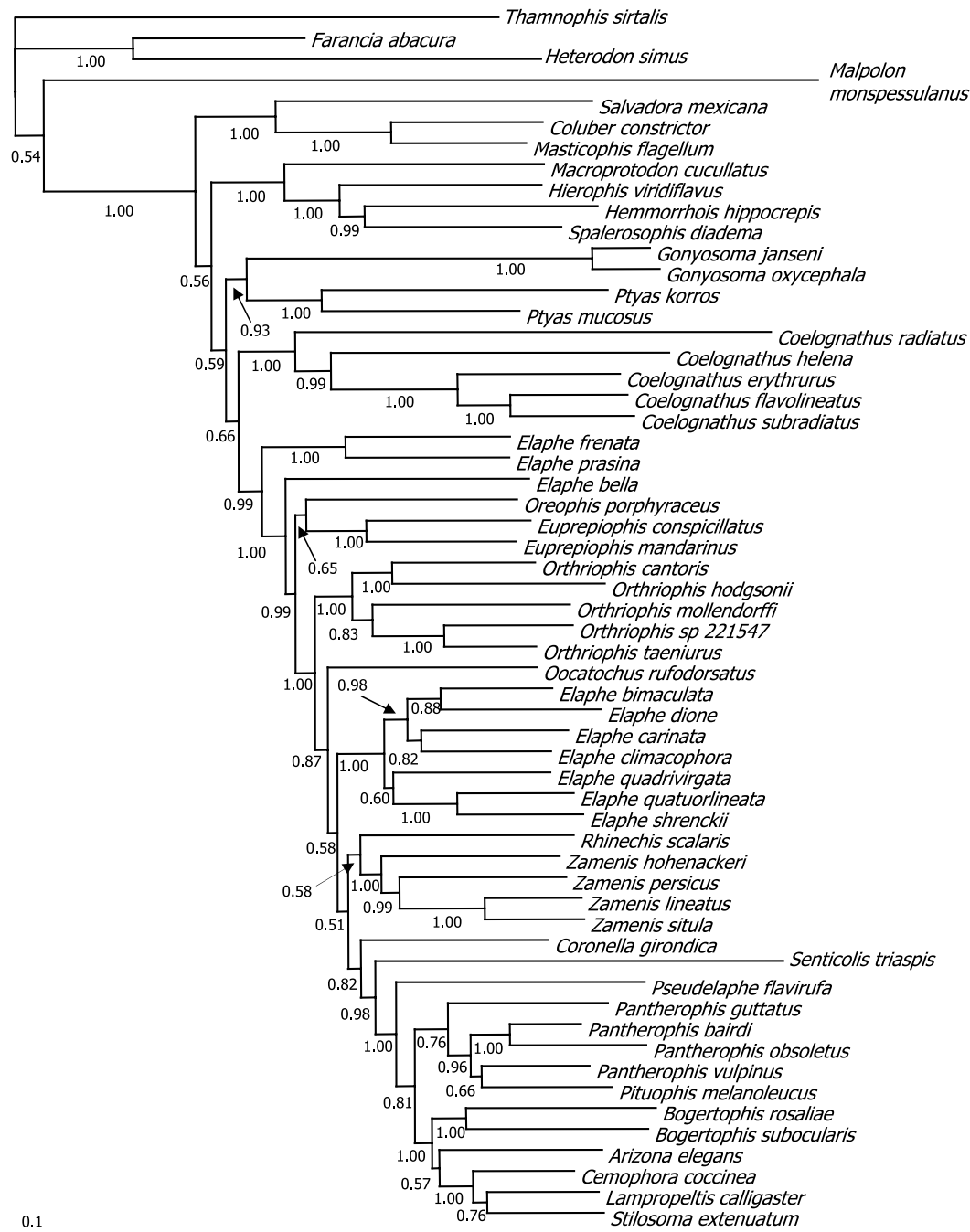


Fig. 3. Inferred phylogeny of the ratsnakes using BI on combined genes partitioned using the CS (GTR + Γ + I) model with PP support indicated below branches.

the early Miocene (Holman, 2000). The sister taxon to *Coluber* and *Masticophis* is *Salvadora* (Nagy et al., 2004). *Salvadora* is known from the early Miocene and as for all extant colubrid genera is completely unknown in the Oligocene. Therefore, the MRCA of *Coluber*, *Masticophis*, and *Salvadora* has been given the minimum and maximum calibration dates of 20 and 24 mya.

Departures of both ML and BI trees from a molecular clock were tested using the likelihood ratio test and Bayes factors, respectively. Trees were constrained to evolve according to a molecular clock for both ML and BI meth-

ods. The $-\ln L$ of the ML tree evolving in a clock-like fashion was compared to the unconstrained tree using the likelihood ratio test, where $P < 0.05$ indicates significant departure from a molecular clock. The harmonic mean estimated from the PP distribution of the clock-constrained model was compared to the unconstrained harmonic mean of PP distribution using Bayes factors (see Section 2.3)

For clock-like trees, absolute ages can be estimated, but when trees are not clock-like, then the semiparametric approach using the penalized likelihood (PL) method with truncated Newton (TN) algorithm of Sanderson (2002) as

implemented in r8S version 1.70 (Sanderson, 2003) may be used. This method has been demonstrated to provide reliable divergence date estimates in simulation studies when global clocks are inappropriate (Linder et al., 2005). Calibration references were placed on the ML and BI trees and date estimates were derived from these trees. For both the BI and ML trees, we determined the appropriate PL parameters. The smoothing parameter was estimated using the cross-validation procedure in r8s version 1.70 (Sanderson, 2003). This parameter is intended to minimize the effects of varying rates in different branches and was chosen among five values differing in a magnitude of 10 and ranging from one to 10,000. In addition, a log penalty function was selected to penalize squared log differences between neighboring branches. The likelihood of solutions for different rates was examined using the checkgradient feature of the program (Sanderson, 2003). To obtain error estimates for divergence date inferences, we used: (1) the BI tree with 1000 nonparametric bootstrap pseudoreplicates of all branch lengths, (2) the BI tree with branch lengths estimated from the PP distribution, and (3) the ML tree with branch lengths estimated from 1000 nonparametric bootstrap pseudoreplicates. All branch-length information using 1000 nonparametric pseudoreplicates was obtained using PAUP* v. 4.10 (Swofford, 2003).

Calibration references of fossils may underestimate branch ages if they do not represent the oldest date for a group (Marshall, 1990), may be misidentified and thus incorrectly placed at internal nodes (Doyle and Donoghue, 1993; Lee, 1999; Magallon and Sanderson, 2001), and/or have been incorrectly dated from the matrix in which they were derived (Conroy and Van Tuinen, 2003). Therefore, we examined the appropriateness of our fossil calibration references using a fossil-based model cross-validation to identify the optimal model of molecular evolution in the context of rate smoothing (Near and Sanderson, 2004). This method verifies that the minimum and maximum constraints for a node are within the range of the predicted values for that node. This is done by removing fossil constraints from a node and checking predictions at this same node using other fossil calibration references distributed throughout the tree. Where fixed nodes are required to run this analysis in r8s v 1.7 (Sanderson, 2003), we performed the test using the minimum value for that node.

To examine the distribution of ages of these calibrated nodes, we also removed, in turn, each of the five calibration points to examine the difference in predicted age of that reference using the other four remaining calibrated ages over all 1000 bootstrapped branch lengths for the BI and ML trees and over the PP distribution of branch lengths from the BI tree. If the ranges of fossil dates obtained from the literature are correct, then it is expected that this fossil calibration reference would fit within the distribution of ages estimated for that same node by the other four fossils in the absence of that constraint.

3. Results

3.1. Sequence and alignments

The number of amino acids (aa) for each protein was fairly constant across all ingroup and outgroup taxa. *Cytochrome b* consisted of 372 aa, *ND1* 321 aa, *ND2* 342 aa, *ND4* 232 aa, and *c-mos* 187 aa. An open-reading frame for all alignments was produced with gaps located only in a small area in two of the genes. For the portion of *c-mos* acquired here, the genus *Malpolon* had the sequence GAT (aspartic acid) inserted at aa position 101. Additionally, at the beginning of the *ND1* gene sequence several six or nine base-pair gaps (yielding an open-reading frame) occurred for many taxa and were not restricted to any taxonomic group. Also in this gene, the outgroup, *Thamnophis*, had an insertion of CAA (glutamine) following the first 15 base pairs from the start codon.

3.2. Phylogenetic inference

Similar tree topologies and support values were produced using ML and BI methods. For ML, AIC chose the GTR+ Γ + I model with the following parameters estimated after two iterations correspond to: $r_{AC}=0.224$, $r_{AG}=6.069$, $r_{AT}=0.515$, $r_{CG}=0.285$, $r_{CT}=6.245$, $r_{GT}=1.00$, $\Gamma=0.464$, $I=0.400$. This produced a single tree with a $-\ln L$ of 81085.56 (Fig. 2).

Burn-in using BI for the less parameter-rich model (GTR+ Γ + I) occurred prior to 1×10^6 generations, whereas burn-in for the more parameter-rich model (CS(GTR+ Γ + I)) occurred just prior to 4×10^6 generations. Harmonic means were calculated for the $-\ln L$ of trees obtained from the posterior probability distribution and yielded a value of 80372.18 for the former model and 79839.84 for the latter. Because Bayes factors, at a value of 1064.68, chose the more parameter-rich model (CS(GTR+ Γ + I)), the BI tree and accompanying PP support values were derived from this model (Fig. 3).

In both the ML and BI trees, the Lampropeltini is monophyletic and derived from OW ratsnakes. Although support is not high, both methods suggest that the WP genus *Coronella* is the sister taxon to the Lampropeltini. The sister group to *Coronella* plus the Lampropeltini clade differs between methods. Bayesian inference suggests, with poor support, that *Zamenis* with *Rhinechis* is the sister group to the clade composed of *Coronella* and the Lampropeltini (Fig. 3), whereas ML identifies *Elaphe* (in part) as the sister genus (Fig. 2). Although support is low, *Rhinechis* appears as the sister taxon to the genus *Zamenis* (BI and ML) with the placement of *Oocatochus* as the sister genus to the *Zamenis/Rhinechis* clade (ML). Bayesian inference suggests that *Oocatochus* is the sister taxon to a clade consisting of *Elaphe* (part), *Rhinechis*, *Zamenis*, and the Lampropeltini, with *Orthriophis* as the sister taxon to this clade. As indicated by high PP values, the sister taxon to the large clade defined by the MRCA of *Orthriophis*, *Oocatochus*,

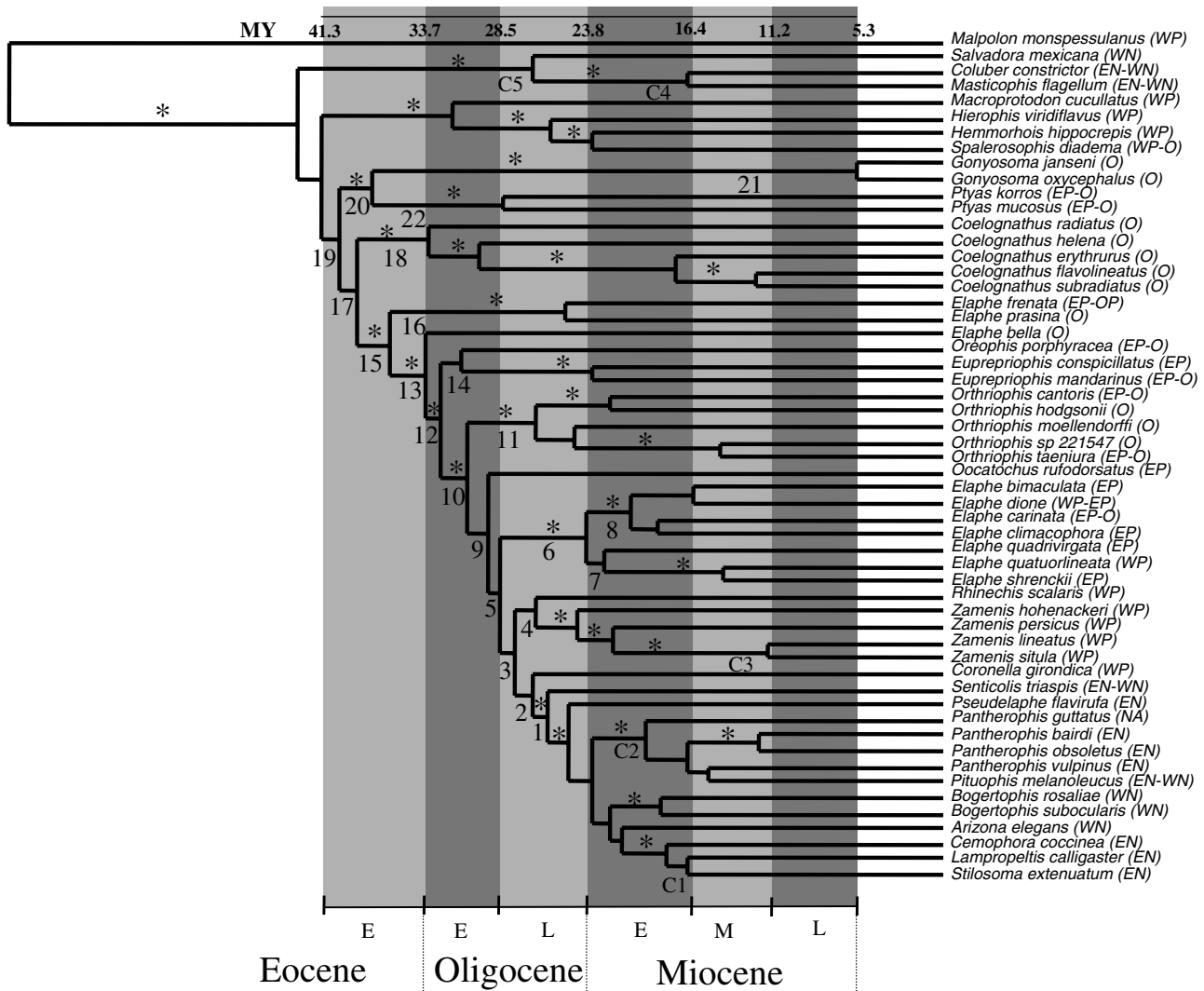


Fig. 4. Chronogram of the phylogeny presented in Fig. 3 generated using PL with dates of divergence indicated. Exact dates and geographic areas for numbered nodes are listed in Table 3. Geographic areas of terminal taxa are abbreviated as follows: WP, Western Palearctic; EP, Eastern Palearctic; O, Oriental; WN, Western Nearctic; EN, Eastern Nearctic. Asterisks above branches indicate branch support greater than 95% posterior probability support.

Elaphe (part), *Rhinachis*, *Zamenis*, *Coronella*, and the Lampropeltini is a clade comprising *Euprepiophis* and *Oreophis*. In the BI analysis, *E. bella*, *E. prasina*, and *E. frenata* form the base of the ratsnakes, excluding *Gonyosoma* and *Coelognathus*. According to the ML analyses, *E. bella*, to the exculsion of *E. prasina* and *E. frenata*, shares a well-supported MRCA with the Lampropeltini, *Oocatochus*, *Euprepiophis*, *Oreophis*, *Orthriophis*, *Elaphe*, and *Coronella*. Although not well supported, ML analysis suggests that a clade composed of *E. frenata* and *E. prasina* is the sister group to the large clade containing the MRCA of *E. bella* and the Lampropeltini. Once included in this group of ratsnakes were *Gonyosoma* and *Coelognathus*. Neither genus was supported as belonging to this ratsnake clade.

Gonyosoma and *Ptyas* appear to be sister taxa and together are in turn the sister taxon to either *Coelognathus* (ML) or a group composed of the MRCA of all ratsnakes and *Coelognathus*. Bayesian inference places *Coelognathus* as the sister taxon to the holarctic and oriental ratsnakes.

The two species currently included in the genus *Ptyas* are, by genetic distance remarkably diverged, a finding in keeping with that of Nagy et al. (2004). Incrementally sister to the MRCA of *Gonyosoma*, *Coelognathus*, and all holarctic and oriental ratsnakes (including the Lampropeltini) which have at some time been called ratsnakes are the OW racers (*Hemorrhoids*, *Hierophis*, *Macroprotodon*, and *Spalerosophis*), the NW racers (*Coluber*, *Masticophis*, and *Salvadora*), and then the noncolubrids (*Farancia*, *Heterodon*, *Malpolon*, and *Thamnophis*).

3.3. Ancestral area estimation

Both methods of estimating ancestral area, dispersal–vicariance (DIVA) analysis and ML, inferred similar geographic regions for the same nodes using the BI tree (Fig. 4; Table 3). However, all ancestral area inferences have to be considered in light of any statistical uncertainty about the node being estimated. With respect to the ML method,

Table 3
Ancestral area and divergence dates for nodes numbered in Fig. 3

Node	Node support	DIVA	ML	Age using BI tree		Age using ML tree, BL = BS	Total mean age and CI values
				BL = BS	BL = PP		
1	0.980	WN, WN-EN	WN, O	25.633 (2.439)	24.2675 (4.197)	26.271 (2.770)	25.391 (24.920–25.860)
2	0.820	WP-WN, WP-EN, WP-EN-WN	WP	26.640 (2.671)	25.232 (4.634)	27.272 (2.981)	26.381 (25.863–26.897)
3	0.510	WP	WP	28.118 (2.974)	26.464 (5.149)	NA	27.379 (26.532–28.050)
4	0.580	WP	WP	26.039 (3.061)	24.243 (5.902)	27.967 (4.365)	26.083 (25.423–26.750)
5	0.580	WP, EP	WP-EP	28.620 (3.004)	27.389 (5.495)	NA	28.005 (27.195–28.815)
6	1.000	EP	WP-EP	23.523 (3.073)	21.368 (5.014)	23.333 (3.100)	22.744 (22.170–23.300)
7	0.600	EP	EP	21.989 (3.451)	19.545 (6.034)	NA	20.767 (19.880–21.700)
8	0.980	EP	WP-EP	20.146 (2.121)	18.044 (5.507)	NA	19.095 (18.320–19.875)
9	0.870	EP	WP-EP	29.527 (3.243)	28.234 (5.839)	NA	28.881 (28.020–29.600)
10	1.000	EP-O	EP-O	30.650 (3.451)	29.230 (6.153)	29.974 (3.465)	29.951 (29.277–30.623)
11	1.000	O	O	26.012 (3.618)	24.711 (6.099)	24.278 (3.460)	25.000 (24.330–25.670)
12	1.000	EP, O, O-EP	O	32.922 (3.860)	31.457 (7.048)	33.093 (4.073)	32.491 (31.723–33.270)
13	1.000	O, O-EP	O	33.952 (4.057)	32.792 (7.493)	33.831 (4.147)	33.525 (32.703–34.337)
14	0.650	EP, O, O-EP	EP	31.755 (4.708)	29.473 (8.233)	NA	30.614 (29.400–31.830)
15	0.990	O	O	37.470 (4.880)	35.260 (8.524)	37.075 (4.816)	36.602 (35.667–37.540)
16	1.000	O	O	25.382 (3.828)	24.209 (5.675)	24.922 (3.777)	24.838 (24.190–25.483)
17	0.660	O	O	39.292 (5.401)	37.755 (9.392)	NA	38.524 (37.140–39.910)
18	1.000	O	O	33.819 (5.056)	32.108 (9.171)	33.238 (5.637)	33.055 (32.043–34.073)
19	0.590	O	O	40.185 (5.156)	38.662 (9.450)	39.264 (5.130)	39.357 (38.340–40.400)
20	0.930	O	O	38.002 (6.126)	40.076 (9.573)	36.362 (6.409)	38.147 (37.057–39.237)
21	1.000	O	O	5.812 (1.358)	6.455 (1.395)	5.749 (1.309)	6.005 (5.833–6.180)
22	1.000	EP-O	O	27.862 (4.980)	26.431 (9.831)	26.669 (5.104)	26.987 (25.927–28.047)

The most parsimonious ancestral areas for each node using divergence–vicariance analysis (DIVA) and ML on the BI tree (Figs. 2 and 3) are presented. Zoogeographic regions analyzed for ancestral area inferences are abbreviated as follows: Western Palearctic (WP), Eastern Palearctic (EP), Oriental (O), Western Nearctic (WN), and Eastern Nearctic (EN). Mean age estimates and standard deviations (in parentheses) for the labeled nodes in Fig. 3 are presented for the BI tree (Fig. 2) with branch lengths (BL) estimated using 1000 nonparametric bootstraps (BS) or the posterior probability distribution (PP) from the BI analysis, and the ML tree (Fig. 1) with BL estimated using BS. The mean age of all three trees and BL estimates of age along with mean confidence intervals (CI) are presented.

Table 4

Comparison of calibration date ranges obtained from the fossil record for (1) *Lampropeltis*, (2) *Pantherophis*, (3) the most recent common ancestor (MRCA) of *Zamenis situla* and *Z. lineatus*, (4) the MRCA of *Masticophis* and *Coluber*, and (5) the MRCA of all New World (NW) Colubridae

Calibration reference	Fossil date	Predicted date I (tree = BI, BL = BS)		Predicted date II (tree = BI, BL = PP)		Predicted date III (tree = ML, BL = BS)	
		Mean (SD)	Overlap (%)	Mean (SD)	Overlap (%)	Mean (SD)	Overlap (%)
<i>Lampropeltis</i>	15–19	11.496 (1.941)	3.5	10.429 (3.092)	6.7	11.873 (2.138)	7.6
<i>Pantherophis</i>	16–20	16.876 (1.855)	63.5	16.537 (4.465)	32.9	16.189 (2.458)	47.1
MRCA <i>Zamenis situla</i> and <i>Z. lineatus</i>	6–20	10.955 (1.848)	97.9	10.813 (3.439)	91.5	12.326 (2.954)	97.9
MRCA <i>Masticophis</i> and <i>Coluber</i>	11–15	13.040 (1.290)	87.9	20.988 (4.851)	8.9	24.635 (4.504)	1.5
MRCA NW Colubridae	19–24	30.230 (6.114)	12.1	27.222 (9.001)	18.0	30.538 (7.064)	12.6

The predicted mean and standard deviation for a fossil reference was estimated by removing the dates for the fossil reference and using the remaining four fossils to predict the age of this node. Three methods for predicting this date used either the BI or ML tree (Figs. 1 and 2) and branch lengths (BL) estimated using 1000 nonparametric bootstraps (BS) or the posterior probability distribution (PP). The overlap between the original fossil date and the predicted date was assessed by calculating the percent area occupied by the fossil date within the predicted distribution of dates for that fossil node.

using a dispersal/extinction parameter of 0.009/0.001 produced the highest likelihood estimate for ancestral area inference (–534.596). The monophyletic New World Lampropeltini (Fig. 4, node 1), which is supported by a 98% PP value, originated in the Western Nearctic (WN) or the Western and Eastern Nearctic (WN-EN) according to DIVA analysis. When considering all five areas, ML chooses, with similar likelihood values, an ancestral area of origin for the Lampropeltini as the WN or Oriental (O) areas (Table 3). It is unclear why the Oriental zoogeographic region is given a similar likelihood value for the WN considering that all of the Lampropeltini are found in the NW. Although PP support is not high (82%) for sister-

taxon relationship between *Coronella* and the Lampropeltini, DIVA suggests that either the Western Palearctic (WP) with either the WN, the EN, or both are equally probable ancestral areas for the MRCA of these sister taxa. The ML method suggests a WP distribution for the MRCA of the Lampropeltini and *Coronella*. The next well-supported clade combining the MRCA of the Lampropeltini and OW genera is at node 10 (Fig. 4), which includes *Orthriophis*, *Elaphe* (in part), *Rhinechis*, *Oocatochus*, *Zamenis*, *Coronella*, and the Lampropeltini. The inferred ancestral area for this node, as suggested by DIVA and ML, is the EP-O area. Because of the high support for this node and the possibility of a sister-group relationship with *Coronella*, it appears

that the Lampropeltini must have originated from OW ancestors either in the EP-O or WP regions. The origin of ratsnake taxa that includes the MRCA of the *E. frenatal prasina* clade and the Lampropeltini (node 15, Fig. 4) but excludes *Coelognathus* and *Gonyosoma* probably occurred in the Oriental zoogeographic region as predicted by DIVA and ML. The area of origin for the node that subtends all genera ever considered part of the ratsnake group (node 19, Fig. 4; Schulz, 1996; Staszko and Walls, 1994), which includes the MRCA of *Gonyosoma* and *Coelognathus*, the Lampropeltini, and all other ratsnakes, appears to be the Oriental zoogeographic region as indicated by both DIVA and ML analyses.

3.4. Divergence date estimation

A comparison of ML trees produced with and without a clock-like constraint yielded $-\ln L$ values of 81300.82 and 81085.56, respectively. Using a likelihood ratio test with 56 degrees of freedom produced a significant ($P < 0.0001$) χ^2 value of 530.51. Similarly, the PP of trees estimated using BI, with no sites partitioned by codon position and constrained to be clock-like when compared with those free of constraints, produced $\ln L$ harmonic means of -82135.67 and -80372.18 , respectively. Accordingly, Bayes factors selected the model without a clock-like constraint.

Given that both ML and BI demonstrate departures from a molecular clock, we used the PL method with the TN algorithm to estimate dates of divergence. The most appropriate smoothing parameter values are estimated to be from one to 100 as confirmed by using the cross-validation analysis described in Sanderson (2002). The lowest normalized χ^2 error was given by a smoothing parameter of one for either the ML or BI tree (Figs. 2 and 3). All solutions using this smoothing parameter with the PL and the TN algorithm passed the check gradient feature in the program r8s v 1.7 (Sanderson, 2003). Additionally, all calibration references passed the fossil cross-validation implemented in r8s v.1.7 using either the ML or BI tree.

The range of dates obtained from the fossil record for the five calibration references usually occupied greater than 10% of the distribution of the age of the same node predicted in the absence of that fossil calibration, with the predicted normal distribution obtained using one of three methods (ML using 1000 nonparametric bootstrapped branch lengths, BI trees using 1000 nonparametric bootstrapped branch lengths, and the BI tree using branch lengths from the PP distribution; Table 4). For these nodes, the predicted means for *Pantherophis*, the MRCA of *Z. situla* and *Z. lineatus*, and the MRCA of *Masticophis* and *Coluber* (using the BI tree with bootstrapped branch lengths) were within the range of dates obtained from the fossil record. However, the mean predicted dates for certain nodes fell outside of the range obtained from the fossil record. Conversely, for these nodes, the range of dates from the fossil record occupied less than 5% of this predicted distribution (Table 4). This is evident in the earlier estimated

date for the origin of *Lampropeltis* using the BI tree with branch lengths calculated from 1000 nonparametric bootstraps, where the range from the fossil record overlaps in only 3.5% of the right tail of the distribution of the predicted age of that node. Additionally, the predicted age of the MRCA of *Masticophis* and *Coluber* is roughly 10 my earlier than the fossil record age and the area occupied in the left tail of the distribution of this predicted age amounts to only 1.5% of the predicted distribution. However, predicted dates across all nodes vary little when these two references are removed. To examine what effect these different estimates had on inferring divergence dates for all target nodes, we estimated dates using all calibration points and also estimated dates after the removal of the two calibration points that may have originally been erroneously assigned or dated. Estimates for all nodes presented in Fig. 4 using only the remaining three calibration references produced similar divergence times for all target nodes as compared to estimates using all five calibration dates. The range of differences among divergence date estimates using three calibration points or five calibration points was 0.444–5.632 my, with a mean difference of 4.637. Since this narrow range of discrepancies does not change historical interpretations and the fossil cross-validation procedure accepted all five points, we present dates using all calibration values (Fig. 4, Table 3).

All values for divergence date estimates are provided with standard deviations (SD) indicative of our belief that a certain amount of error is associated with these date estimates, but we illustrate them as single dates for clarity (Fig. 4). Additionally, dates of nodes are discussed in terms of combined confidence intervals using both trees and both methods to assess branch-length error estimates (Table 4). Divergence date estimates for the division between the MRCA of the Lampropeltini and *Coronella* are predicted to be 24.9–25.9 mya (Table 3). Using the BI tree, a date of 26.5–28.0 my was estimated for the divergence between the MRCA of the Lampropeltini and *Coronella* and the *Zamenis/Rhinechis* clade. This suggests that the common ancestor of the Lampropeltini and *Coronella* diverged from other OW ratsnakes after 26.5–28.1 mya and that the MRCA of the Lampropeltini would have originated 24.9–25.9 mya. However, the ML tree suggests that the sister group to the MRCA of the Lampropeltini and *Coronella* is the clade occupied by the majority of *Elaphe* (in part) (Fig. 2). Using the ML tree with branch lengths obtained from 1000 nonparametric bootstraps would predict that the Lampropeltini, *Coronella*, and the majority of the *Elaphe* clade would have shared a common ancestor between 28.4 and 28.9 mya and that the MRCA of the Lampropeltini and *Coronella* would have originated between 26.5 and 28.09 mya. If it is assumed that the Lampropeltini are of NW origin and diverged from a *Coronella*-like ancestor from 24.9 to 25.9 mya, then it stands to reason that the MRCA of the Lampropeltini must have used the trans-Beringial route to cross from the OW to the NW. At the time of origin of the Lampropeltini, the trans-Atlantic route was either too far

north (De Geer Bridge), too cold, a chain of islands, or severed completely (Table 1). Choosing the trans-Beringian route necessitates an inferred extinction of the ancestral *Coronella*/lampropeltine taxon in the Eastern Palearctic or in Beringia given that neither the DIVA nor ML predictions of ancestral areas include the EP. Additionally, both well-supported ML and BI nodes (node 10; Table 3) confirm that the Lampropeltini are nested within the OW ratsnakes and this node is dated at 29.3–30.6 mya. It follows that even if *Coronella* is not the sister taxon to the Lampropeltini, the date of this well-supported node eliminates the possibility of a trans-Atlantic route for the dispersal of the MRCA of the Lampropeltini into the NW. This well-supported clade indicates that most OW ratsnakes and their phylogenetically nested NW Lampropeltini originated in the Orient or Eastern Palearctic. Furthermore, the MRCA of all OW ratsnakes, the Lampropeltini with *Coelognathus* and *Gonyosoma* (node 19; Table 3), is predicted to have originated in the early Eocene from 38.3 to 40.4 mya in the Orient as well. This early date still eliminates the possibility that OW ratsnakes (as a progenitor of the lampropeltines) could have invaded the NW using a trans-Atlantic route.

4. Discussion

4.1. Biogeography

The method of combining ancestral area and divergence date estimation has permitted us to make well-supported inferences concerning the time, place of origin, and dispersal among various groups of holarctic ratsnakes and the related NW lampropeltines. Phylogenetic analyses of five genes suggest that the Lampropeltini are monophyletic and related to OW ratsnakes with a possible sister-taxon relationship with the smooth snakes, genus *Coronella* (Figs. 2 and 3). Analyses of ancestral area estimation and the ML and BI phylogenies also indicate that although the MRCA of all extant Lampropeltini share a NW area of origin, the group itself is derived from OW ratsnakes with an ancestral distribution of the WP, EP or O (Table 3). The divergence of the Lampropeltini from OW snakes took place between 24.9 to 25.9 mya (Fig. 4; Table 3). We note that this date for the MRCA of the Lampropeltini is close to the 22 my estimated by Dowling and Maxson (1990) using immunological distance (ID) data with a fixed rate of 1.7 albumin ID units per million years. The most probable ancestral area for the MRCA of the Lampropeltini and their OW sister taxon, *Coronella*, is the WP or combined WP and EN or WN from 25.9 to 26.9 mya. The node subtending the MRCA of the Lampropeltini and *Coronella* (node 3; Fig. 4) indicates a WP ancestral area at 26.5–28.1 mya for all ancestral area estimations. This would necessitate a dispersal event to the Nearctic in either the MRCA of Lampropeltini and *Coronella* (as indicated by DIVA in node 2; Fig. 4) at 25.8–26.9 mya or the Lampropeltini alone (as indicated by ML ancestral area estimation in node 1; Fig. 4)

at 24.9–25.9 mya (Table 3). This WP connection with the NW might imply that the ancestral Lampropeltini would have used one of the North Atlantic routes to cross into the NW (Table 1). However, connections at this time were either severed, only existed as island chains, or would only have provided habitat suitable for cold-adapted organisms. It is possible that the MRCA of the Lampropeltini and *Coronella* crossed Beringia between 24.9 and 25.9 mya, with the subsequent extinction of this ancestor in the EP or Beringia. During the Eocene and part of the Oligocene, Beringia may have been composed of mostly sub-tropical or tropical habitats, and from approximately 16 to 30 mya the Beringian land bridge had become colder and drier giving rise to mixed deciduous and coniferous forest (Wolfe, 1987). Since there are currently several cold-tolerant members among the Lampropeltini (within the *Pantherophis obsoletus* complex and *Lampropeltis triangulum*) ranging into Canada, it is not unreasonable to suggest that the ancestral Lampropeltini would have been able to cross Beringia between 24.9 and 25.9 mya. Several authors have suggested, without explicitly testing any hypothesis, that the Lampropeltini may have crossed into the NW through Beringia at the end of another thermal optimum in the Miocene (16.5–17.2 mya) around 15 mya (Dowling et al., 1983; Utiger et al., 2002; Zubakov and Borzenkova, 1990). However, the earliest fossil evidence of *Pantherophis* and *Lampropeltis* indicates these taxa had already diverged in the Nearctic by this time, 15 and 16 mya, respectively. Since these genera are not connected to the deepest and earliest nodes within the Lampropeltini (*Senticolis* and *Pseudelaphe* represent the two earliest divisions), then a date earlier than 15 my must be inferred for the Beringian crossing.

Because our analyses indicate some uncertainty in the sister-group relationship between *Coronella* and the Lampropeltini (Figs. 2 and 3), other hypotheses that consider the dispersal of the MRCA of the Lampropeltini into the NW may be considered. High support indicated in both the ML and BI trees (Figs. 2 and 3) suggests that the Lampropeltini are definitely nested within the OW ratsnakes by node 10 (Fig. 4) with a combined EP-O area for their MRCA. This node includes the Lampropeltini and the OW ratsnake genera *Orthriophis*, *Oocatochus*, *Elaphe*, *Rhinechis*, *Zamenis*, and *Coronella*. It follows that even if the Lampropeltini diverged at this earlier node from OW ratsnakes between 29.3 and 30.6 mya in the EP-O area, the time constraint would still force them to have dispersed through Beringia to cross into the NW. Additionally, the ancestor of the Lampropeltini in the EP-O would have a direct route of dispersal from northeast Asia through Beringia in suitable habitat. These results are in agreement with dispersal routes of other Asian squamates into North America through Beringia. According to Macey et al. (2006), three independent dispersal events of lizards from Asia to North America through Beringia have resulted in the majority of diversity of lizards in the Nearctic.

Our divergence date estimates suggest that lineage diversification and dispersal of ratsnakes began in the late

Eocene and continued through the Oligocene, despite lack of evidence from the fossil record prior to the Miocene. Ratsnakes (node 15; Fig. 4) as defined here include the Lampropeltini and exclude *Gonyosoma* and *Coelognathus*, and appear to have originated in the Oriental zoogeographic region in the late Eocene (from 35.7 to 37.5 mya). If *Gonyosoma*, *Coelognathus*, and *Ptyas* are included within this group (node 19; Fig. 4) of ratsnakes, the area and date of origin for the entire assemblage would still be the Oriental region and must have occurred in the middle to late Eocene (from 38.3 to 40.4 mya). Our analyses with respect to ratsnake origins are in agreement with Rage (1987) and Rage et al. (1992), who suggested that Asia was the center of origin for Colubridae and Viperidae because the oldest colubrid fossil is from the late Eocene of Thailand. Additionally, the simultaneous appearance of these groups in North America and Europe might indicate that members of these groups migrated to both areas from Asia at roughly the same time (Rage, 1987; Rage et al., 1992), indicating that similar environmental conditions permitted dispersal in these unrelated snake groups. Fossil colubrids with modern affinities are uncommon throughout Asia. Therefore, it is likely that ratsnakes evolved in the Oriental region in the late Eocene, but fossil evidence has not been recovered or has not been identified for this group of snakes. This is in contrast to the Eocene snake fauna of North America and Europe, where fossils attributed to the older and noncolubroid families Scolecophidia, Aniliidae, Boidae, Paleopheidae, and Acrochordidae are well known (Holman, 2000; Rage, 1987). The Oriental Zoogeographic area and late-Eocene date for the origin of ratsnakes would have coincided with rapid global cooling following the maximal global temperatures of the middle Eocene (Burchardt, 1978; Hubbard and Boulter, 1983; Janis, 1993; Prothero, 1994; Shackleton, 1986; Wolfe, 1987). This late-Eocene time frame also coincides with the extinction of many mammals following elevated levels of diversification in the middle Eocene (Stucky, 1990; Sudre and Legendre, 1992). It has also been suggested that the severity of global cooling events of the late Eocene was not as drastic in southeastern Asia as elsewhere and that floral habitats remained tropical and the climate continued to be warm and humid (Leopold et al., 1992; Morley, 1999; Tsubamoto et al., 2004).

Ratsnakes that originated in the Orient (node 15; Fig. 4) at 35.7–37.5 mya (Table 3) may have dispersed to the EP by 32.7–34.3 mya (node 13; Fig. 4) according to DIVA or at the latest by 29 mya according to ML estimates of ancestral areas (node 10 or 14; Fig. 4; Table 3). At this time, the Earth had shifted from the “greenhouse” of the early Eocene to the “ice house” of the late Eocene (Berggren and Prothero, 1992; Prothero, 1994) resulting in the formation of temperate-woodland habitats in most of the EP and O regions (Wolfe, 1985; Janis, 1993; Leopold et al., 1992). Ancestral area inferences and divergence date estimates indicate that ratsnakes may have also dispersed into the WP (node 5 by DIVA or node 9 by ML; Fig. 4) in the Oligocene from 26.5 to 29.6 mya (Table 3). This dispersal of

ratsnakes from the EP to the WP coincides with drying of the Turgai Strait, an epicontinental seaway that separated the EP and WP regions (Cox, 1974; Tangelder, 1988; Tiffney, 1985). This date of dispersal for ratsnakes is also concordant with other biotic exchanges between WP and EP areas documented for this time (Sanmartin et al., 2001). Moreover, fossil evidence indicates that members of the family Colubridae, which includes the ratsnakes, began replacing the family Boidae in Europe by traveling through the Mazury-Mazowsze continental bridge in Poland from the Eastern Palearctic in the early Oligocene (Ivanov, 2001, 2002). At this time, both the WP and EP consisted of temperate woodlands composed of either broad-leaved deciduous trees in the southern regions or mixed coniferous trees in the North (Leopold et al., 1992; Janis, 1993; Wen, 1999; Wolfe, 1985).

4.2. Taxonomic conclusions

Our analyses support some of the recent changes to ratsnake taxonomy in that the species comprising the following genera each form monophyletic groups: *Zamenis*, *Orthriophis*, *Euprepiophis*, and *Coelognathus* (Helfenberger, 2001; Utiger et al., 2002). The monotypic genera *Oreophis*, *Pseudelaphe*, *Senticolis*, *Oocatochus*, and *Rhinechis* cannot be evaluated using monophyly as a criterion for the recognition of a genus. As currently recognized, the OW genus *Elaphe* is paraphyletic given that *E. prasina*, *E. frenata*, and *E. bella* are not the closest relatives of the clade comprising *E. dione*, *E. carinata*, *E. climacophora*, *E. quatuorlineata*, and *E. shrenckii*. *E. prasina* and *E. frenata* appear to be sister taxa and, along with *E. bella* represent early divergences from the clade that includes *Elaphe* sensu Utiger et al. (2002). In order to retain this genus for the monophyletic group of seven species of *Elaphe* that includes the type for the genus (*E. sauromates*) (Pallas, 1814) two new genera are needed: one for the clade formed by *E. prasina* and *E. frenata* and one for *E. bella*. The oldest available genus that should be applied to the monophyletic group of *E. frenata* and *E. prasina* is *Rhadinophis* (Schulz, 1996; Williams and Wallach, 1989). The other taxon, *E. bella*, has no available genus and retaining *Elaphe* for that taxon makes the genus paraphyletic. Given that *E. bella* does not have priority for the genus *Elaphe*, we suggest the name *Maculophis*, which, when translated, means spotted (maculos) snake (ophis) and is in reference to the blotched dorsum. Thus, this species would now be *Maculophis bellus*. We are aware, however, that this does create the unfortunate situation of proposing a monotypic genus.

In the NW, the recently elevated *Pantherophis* (for some of the former NW *Elaphe*, see Utiger et al., 2002) is paraphyletic with respect to *Pituophis*. Since *Pituophis* (Holbrook, 1842) predates *Pantherophis* (Fitzinger, 1843), we suggest that all species in the clade formed by the MRCA of *Pantherophis guttatus* to *Pituophis melanoleucus* (node C2, Fig. 4) be designated as *Pituophis*.

Acknowledgments

We are grateful to the following persons who provided us with tissue samples or allowed us to draw blood from their valuable living specimens; T. Brown, S.D. Busack, J. Campbell, J. Decker, P.G. Frank, N. Helfenberger, U. Joger, R. Knight, S. Landry, H. de Lisle, R.W. Murphy, J.P. O'Brien, K.-D. Schulz, J.B. Slowinski, and R.A. Thomas. E. Visser and C. Elliger helped with laboratory procedures and gave generously their time. We are indebted to the College of Staten Island/CUNY Typhon Computer Cluster users group, especially P. Ivanushkin, A. Kuklov, and M. Lewental. Financial support for this study was provided by the California Academy of Sciences Research Fund.

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