Molecular Phylogenetics and Evolution 53 (2009) 537–546

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Vicariance and dispersal form a ring distribution in nightsnakes around the Gulf of California

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ARTICLE INFO

Article history:
Received 31 March 2009
Revised 22 May 2009
Accepted 29 May 2009
Available online 6 June 2009

Keywords:
Baja California
Biogeography
Genomics
Hypsiglena
mtDNA
Nuclear DNA
Hierarchical sampling
Dual-peninsular effect

ABSTRACT

Plate tectonics can have profound effects on organismal distribution and is often the driving force in speciation. Through geologic processes, the Baja California Peninsula depicts two faunal patterns: one through southern vicariance with Cape separation, and the other through dispersal onto the northern peninsula, referred to as a 'dual-peninsular effect.' Here we apply a hierarchical sampling strategy that combines population-level sequence data (~800 bp, *nad4* region) with complete mt-genome data (aligned 15,549 bp) and 5 nuclear protein encoding loci (3315 bp), to test whether both patterns have occurred in one group of nightsnakes (*Hypsiglena*). The geologic formation of the peninsula is thought to have occurred in three stages: (1) Cape separation from mainland Mexico; (2) the northern peninsula separated, forming the northern Gulf of California; and (3) the peninsula was united through volcanic activity, while moving northward causing collision with southern California. However, the timing of events is debated. We explore phylogenetic relationships and estimate dates of divergence for nightsnakes using our hierarchical sampling strategy. Our data support both 'southern-vicariance' and 'northern-dispersal' have occurred in nightsnakes, forming a ring distribution around the Gulf of California. Two divergent forms are sympatric on the southern half of the peninsula with no indication of hybridization. Nightsnakes represent the first group to depict the 'dual-peninsular effect' with extensive overlap on the Baja California Peninsula.

1. Introduction

The Baja California Peninsula presents an ideal setting to explore biogeographic systems because of complex geologic history and association with western North American biotic elements. Baja California separated from the west coast of what is now mainland Mexico as the peninsula was shifted on a transform fault from the North American Plate to the Pacific Plate (Atwater, 1970; Stock and Hodges, 1989). The Cape region of Baja California initially separated from mainland Mexico near the area north of Puerto Vallarta, in Jalisco, Mexico (Fig. 1a) before the remaining peninsula (Carreño and Helenes, 2002; McQuarrie and Wernick, 2005), and three approximate dates for this event have been estimated to have occurred: 5.5 mya (Curray and Moore, 1984); or 7.5–8.2 mya (Oskin and Stock, 2003); or 12–14 mya (Ferrari, 1995; Henry and Aranda-Gomez, 2000). Later in time, the northern portion of the peninsula is thought to have separated from mainland Mexico (Fig. 1b), and moved northward and collided with southern California (Fig. 1c). The northern region was eventually connected to the Cape, presumably by a series of rising volcanoes that eventually formed the peninsula. Also during the late Miocene, the Gulf of California extended much farther to the north than present day, with the northern maximum extent estimated to have occurred at 6.3–6.5 mya (McDougall et al., 1999), isolating the northern peninsular ranges from the deserts of western North America. Since then, the head of the Gulf of California has retreated to its current position, leaving a series of periodic lakes in the area immediately to the northwest (Stokes et al., 1997) and the peninsula continues to collide with California (Fig. 1d).

Baja California has attracted the attention of many biologists, mainly because of its unique flora and fauna and their associations with continental forms (Schmidt, 1922; Van Denburgh, 1922; Wiggins, 1960). Initial hypotheses for most faunal associations with the peninsula were based on putative dispersal events from the north onto recently uplifted mountains and other habitable regions (Fig. 2a) followed by more recent climatic changes (Savage, 1960; Wiggins, 1960; Truxal, 1960). Under the acceptance of plate tectonic theory, some hypothesized a vicariant separation from the south for many occupants of the peninsula associated with the geologic separation of the Cape region from mainland Mexico (Fig. 2b; Leviton and Tanner, 1960; Murphy, 1975, 1983; Seib, 1980; Papenfuss, 1982). As the availability of phylogenetic data

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doi:10.1016/j.ympev.2009.05.037

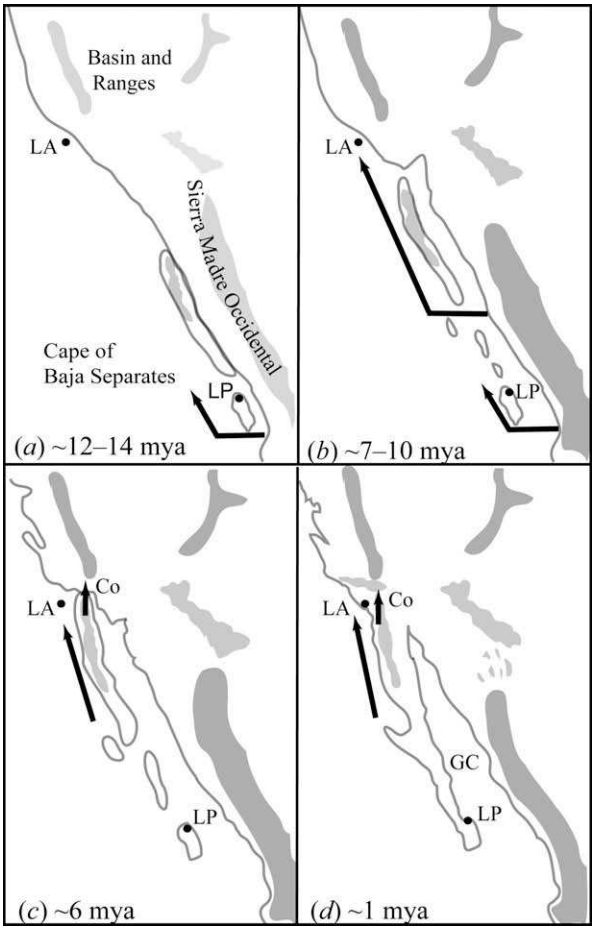

Fig. 1. Geologic history of Baja California. (a) The Cape of Baja California separates from mainland Mexico. (b) Northern section of the peninsula separates from mainland and moves northward. (c) Northern portion of peninsula collides with North America (Co), extent of gulf reaches southern California. (d) Peninsula is united as one landmass forming the Gulf of California (GC). Dates follow our favored geological hypothesis (see text for alternative dates). Shaded regions represent mountain ranges. Reference points: LA = Los Angeles; LP = La Paz.

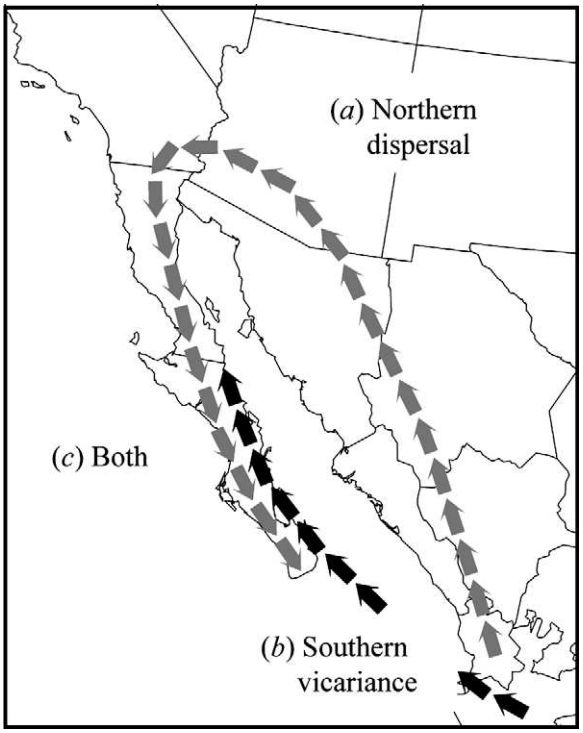

Fig. 2. Biogeographic hypotheses for Baja California. (a) Grey arrows indicate *Northern-dispersal:* dispersal around the gulf and southward onto the peninsula. (b) Black arrows indicate *Southern-vicariance:* separation of the Cape from mainland Mexico. (c) *Ring Hypothesis:* both northern and southern origins for a species complex forming a ring around the Gulf of California.

increased for a variety of taxa, patterns of either vicariance or dispersal were found in different species pairs associated with Baja California and mainland Mexico, depending on whether sister taxa were inferred to be from the south or north, respectively (e.g. Grismer, 1994; Riddle et al., 2000a). Thus, hypotheses for Baja California biota based on dispersal events from the north onto recently uplifted peninsular mountains are referred to as the 'northern-dispersal' hypothesis (Fig. 2a). While those based on vicariant separation from mainland Mexico associated with transform fault separation of the Cape are referred to as the 'southern-vicariance' hypothesis (Fig. 2b). Another alternative difficult to rule out is overwater dispersal (de Queiroz and Lawson, 2008), which could have occurred at any time during or after the formation of the peninsula. In this study, we show a unique 'dual-peninsular effect' (Seib, 1980) has occurred in one group of nightsnakes, where both southern-vicariance and northern-dispersal formed a ring distribution around the Gulf of California (Fig. 2c).

Nightsnakes consist of two genera in the family Colubridae (Dipsadinae) that occur in western North America: *Pseudoleptodeira*, endemic to the Balsas Basin of mainland Mexico, and *Hypsiglena*, which contains eight species in western North America (Mulcahy, 2008). *Hypsiglena tanzeri* occurs in eastern-central Mexico and *H. slevini* (previously *Eridiphas slevini*; Leviton and Tanner, 1960) is endemic to the Baja California Peninsula. The idea that nightsnakes colonized Baja California through each process was previously suggested, with '*E. slevini*' from the south, and '*H. torquata* (*sensu lato*)' from the north (Seib, 1980; Grismer, 1994). Hence, their close relationship was not assumed (see Leviton and Tanner, 1960; Cadle, 1984) and neither hypothesis was actually tested. Mulcahy (2008), used ~800 bp of the NADH dehydrogenase subunit 4 (*nad4*) mtDNA gene region from 178 individuals, and an assessment of morphology and geography to show what was previously considered '*H. torquata*' actually consists of six closely related species, and that '*E. slevini*' was nested among *Hypsiglena*. However, the relationships among species were not well-resolved (Mulcahy, 2008). Here, we test the 'dual-peninsular effect' by using a hierarchical sampling strategy: by collecting complete mt-genome sequence data (~15 aligned kb) from 16 individuals representing the major clades of the phylogeographic data (800 bp *nad4*) from Mulcahy (2008), and we combine the *nad4* + mt-genome data by enforcing a backbone constraint on the phylogeographic data (more individuals and geographic areas but fewer characters) based on the information from the mt-genome data. In addition to the mtDNA data, we collected sequence data from five protein encoding nuclear loci from the individuals for which we collected complete mt-genome data. Most studies to date using complete mt-genome data address higher-level phylogenetic relationships (e.g. Zardoya and Meyer, 2000; Kumazawa, 2007). Instead, we use our mt-genome data, and five nuclear DNA markers, to explore phylogenetic relationships among clades of closely related species, delineated by the phylogeographic data (*nad4* of Mulcahy, 2008). We also estimate the date for our northern divergence (Fig. 2) by calibrating our southern divergence with the geologic separation of the Cape, under two proposed dates to evaluate the discrepancy in these geological dates for the formation of the Baja California Peninsula.

Table 1
Specimen information for tissue samples used in DNA sequence data collection and analyses. All samples were acquired from frozen tissue, with the exception of *H. tanzeri*, which was placed in salt and 95% EtOH. Complete mt-genome sequence data was obtained from 16 individual snakes and a ~5 kb region was collected from the *H. tanzeri* specimen. Terminal numbers for *H. chlorophaea* and *H. ochrorhyncha* used in this study are in first column, haplotypes in second column correspond to numbering system of Mulcahy (2008) and Fig. SM-1 (this study), followed by locality, and voucher information. Nuclear data for five protein encoding loci were taken from each of the same individuals (GenBank: FJ455155–FJ455234).

Species	Haplotype (Mulcahy, 2008)	Locality	Voucher No.	GenBank No.
Hypsiglena torquata	torquata 2	Sinaloa, MX	MZFC 16926	EU728591
H. jani	texana 9	Culberson Co., TX	CAS 228960[a]	EU728592
H. sp. nov.	Cochise 1	Cochise Co., AZ	CAS 228951	EU728580
H. chlorophaea 1	chlorophaea 6	Tucson, AZ	MVZ 237359	EU728593
H. chlorophaea 2	chlorophaea 2	Alamos, Son., MX	ROM 14932	EU728585
H. chlorophaea 3	chlorophaea 4	Ortiz, Son., MX	ROM-JRO 694	EU728577
H. chlorophaea 4	deserticola 5	Salt Lake, UT	MVZ 241611	EU728587
H. ochrorhyncha 1	ochrorhyncha 2	Cape of Baja, MX	MVZ 236396	EU728578
H. ochrorhyncha 2	klauberi 8	Cataviña, BCN, MX	MVZ 236389	EU728589
H. ochrorhyncha 3	klauberi 3	Santa Monica, CA	CAS 229918	EU728582
H. ochrorhyncha 4	nuchalata 5	Sierra Nevada, CA	MVZ 180363	EU728581
H. slevini	slevini 4	Cape of Baja, MX	MVZ 234613	EU728584
H. tanzeri	tanzeri	San Luis Potosi, MX	TCWC A2055	EU728588
Pseudoleptodeira latifasciata	n/a	Guerrero, MX	LSUMZ 39571	EU728579
Sibon nebulatus	n/a	Limon, Costa Rica	MVZ 233298	EU728583
Imantodes cenchoa	n/a	Limon, Costa Rica	MVZ 149878	EU728586
Leptodeira septentrionalis	n/a	Guerrero, MX	MVZ 164943	EU728590

[a] In Mulcahy (2008; p. 1103) for *texana 9:* '228060' should read 228**9**60.

2. Materials and methods

2.1. Geographic sampling

Previous studies have suggested the use of nested sampling for large-scale phylogeographic analyses, with the addition of one or two genes at higher levels (Morando et al., 2003). Analyses of complete mt-genome data show that >7–8 kb of sequence data may be required to find congruence with the entire mt-genome signal (Bonett et al., 2005). Therefore, we collected complete mt-genome data from a subset of individuals representing the major clades recovered by the *nad4* mtDNA data (Mulcahy, 2008) including one specimen from each of the *H. torquata*, *H. jani*, and Cochise clades, and four from each of the Coast and Desert clades of *Hypsiglena*. In addition, we also sampled one specimen each of the Baja California nightsnake (*H. slevini*) and the banded nightsnake (*Pseudoleptodeira latifasciata*). A specimen of the rare Rio Verde nightsnake (*H. tanzeri*) was obtained. This was a poorly preserved specimen (road-kill placed in salt and later 95% EtOH) and we were only able to obtain ~5 kb of mt-sequence data from this individual, spanning the *nad4–cob* region, a large portion of the most informative region for phylogenetic analyses in humans (Non et al., 2007) and nightsnakes (Mulcahy, 2006). All other tissue samples were acquired from frozen liver or muscle tissue. Additionally, complete mt-genomes were sampled from one specimen each of the blunt-headed vinesnake (*Imantodes cenchoa*), the northern cat-eyed snake (*Leptodeira septentrionalis*), and the cloudy snail-eating snake (*Sibon nebulatus*) as close outgroups (Mulcahy, 2007). All specimens for which sequence data were collected for this project are from museum voucher specimens and all sequences were deposited in GenBank (Table 1). The Akamata (*Dinodon semicarinatus*), an Asian colubrid was taken from GenBank (NC001945; Kumazawa et al., 1998) and used to root the tree. In addition to our mtDNA data, we also collected sequence data from five protein encoding nuclear loci (SLC30A1, ZEB2, FSHR, NT3, and DNAH3; see Townsend et al., 2008) from individuals for which we obtained complete mt-genome data. These loci were initially screen for deep-level (i.e. Family) relationships among squamates (Townsend et al., 2008); thus, we explore their use among closely related species.

2.2. Laboratory protocols and alignment

Complete mt-genome data were collected in the Evolutionary Genomics Dept., at the Joint Genome Institute (U.S. Department of Energy) following protocols described elsewhere (Macey et al., 2005) with the following modifications. Initial amplifications were conducted with combinations of specific primers designed from sequence alignments, of the *cob* and *nad4* regions (Mulcahy, 2007), for the same specimens that were used in this study (Table 1) and from primers described in Macey et al. (1997). Additionally, perfectly matching primers were designed based on preliminary data to complete the amplification of some of the mt-genomes (a list of all primers is shown in Table SM-1). Advanced snakes are known to have two identical Control Regions (CRs) in the mt-genome (Kumazawa et al., 1998), therefore we avoided amplifying both CRs in one amplification in order to build sequence contigs for each CR separately. Data for the nuclear protein encoding loci were collected using the primers and methods described in Townsend et al. (2008).

Complete mt-genomes were assembled and annotated in MacVector® 7.2.2 (Accelrys Inc., 2004). Protein coding genes were inspected and translated into amino acid sequence to verify authenticity and aligned by amino acid sequence. For genes encoding transfer RNA (tRNA), presumptive secondary structure was compared with *Dinodon* (Kumazawa et al., 1998) for length variation in stem and loop regions (Macey and Verma, 1997) and aligned based on inferred stem–loop secondary structure. Only one control region was used in the alignment because of redundancy. The first copy of the control region (CR1), the small (*rrn S*) and large (*rrn L*) ribosomal RNA subunits (12S and 16S *rRNAs*, respectively) were aligned with the ClustalW (v1.4) option in MacVector and inspected by eye. Loop regions of *tRNAs*, *rRNAs*, and CR1 were omitted from the complete dataset, which resulted in the exclusion of 835 positions, for an alignment of 15,549 bp of mt-genome sequence data.

2.3. Phylogenetic analyses

Maximum parsimony analysis was conducted in PAUP* (version 4.0b10; Swofford, 2000) on the complete sequence alignment (15,549 bp) of mt-genome data (excluding loops/gaps in CR1, *rRNAs*, and *tRNAs*), with the heuristic search option using 100 random stepwise additions and TBR branch swapping. Bootstrap support values were measured with 1000 full heuristic replicates, each with 100 random stepwise additions. Decay values (Bremer, 1994) were measured using AutoDecay 5.0 (Eriksson, 2001). Bayesian analyses were conducted in MrBayes 3.1.1 (Huelsenbeck and

Ronquist, 2001). Various partition models were explored elsewhere (Mulcahy, 2006) and here the following partition strategy was used: one for each codon position in each of the protein coding genes with the same substitution model, one for each *tRNA* with the same substitution model, and one each for the *rRNA*s, and CR1. Substitution models were selected using the Akaike Information Criterion (Posada and Buckley, 2004) in MrModeltest 2.1 (Nylander, 2004). This resulted in 30 partitions for the entire dataset (Table SM-2). Four separate Bayesian analyses were run to ensure searches did not become fixed on local optima, each for 10×10^6 generations, using four heated chains (user default values), sampling trees every 1000 generations. Log-likelihood scores were plotted against generations to assess stationarity using Tracer 1.4 (Rambaut and Drummond, 2004). Runs reached stationarity by the first 1×10^6 generations, therefore the first 1500 trees were discarded and a 50% majority-rule consensus tree was taken. Nuclear data were analyzed separately using the same methods for parsimony as the mt-genome data. For Bayesian analyses, the nuclear data was partitioned by codon position for each locus, resulting in 15 partitions. These were run in two separate analyses for 50×10^6 generations, therefore the first 12,500 trees were discarded and a 50% majority-rule consensus tree was taken. Posterior-probabilities of 0.95 or greater were considered significant for Bayesian analyses. A parsimony bootstrap analysis was conducted on the 110 unique haplotypes from Mulcahy (2008) with a backbone topology enforced based on the phylogenetic position of the individuals for which we obtained complete mt-genome sequence data. This analysis was run in PAUP* for 1000 replicates, each with 100 random additions per replicated and TBR branch swapping.

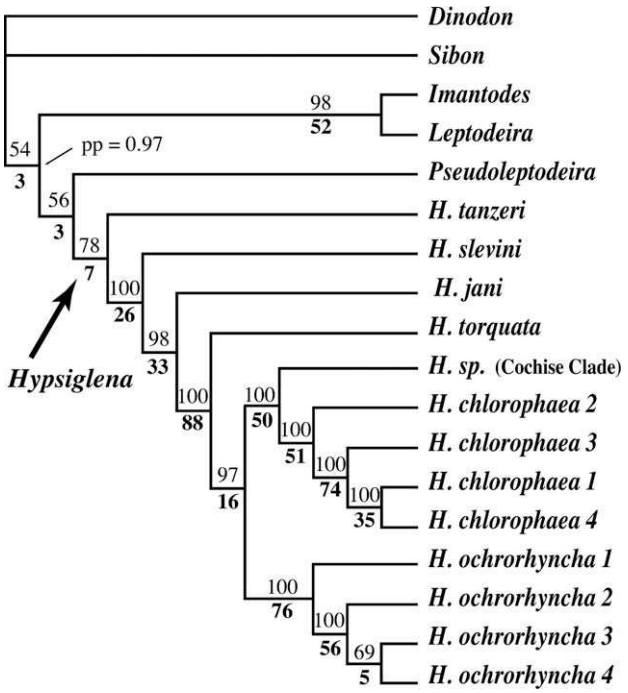

Fig. 3. Phylogeny of nightsnakes based on complete mt-genome data (15,549 aligned bp, 3836 parsimony-informative). The identical topology was recovered using both parsimony (12,814 steps, CI = 0.588, RI = 0.447) and Bayesian (average $-\ln L = 81{,}989$) analyses. Nodal support is shown with parsimony bootstrap values above and decay indices below (boldface), all nodes received Bayesian posterior probabilities of 1.0 except the most basal node (indicated by pp = 0.97).

2.4. Estimating divergence times

In order to estimate dates of divergence for the major clades of nightsnakes, we used the penalized likelihood method (Sanderson, 2002) implemented in the r8s software package (version 1.71; Sanderson, 2006) on the mt-genome data. We used our Bayesian consensus topology (identical to our parsimony topology), with branch lengths obtained under the 30-partition strategy (see Section 2.3) for our input tree in r8s to optimize the rate smoothing factor. Penalized likelihood allows rates to vary among branches, where a penalty is imposed for models with substitution rates that change faster from branch to branch. The smoothing factor determines the penalty enforced, which is optimized through multiple iterations of a cross-validation procedure, testing different smoothing factors (Sanderson, 2002). We used an 'additive' penalty function because we are calibrating a deep node and are interested in estimating more shallow nodes. We created 100 bootstrap replicates of our mt-genome data using 'seqboot' in the Phylip (version 3.68) software package (Felsenstein, 2004), estimated branch lengths for each bootstrap-replicated dataset on the optimal topology recovered in this study using PAUP*, estimated smoothing factors for each tree with branch lengths (using the cross-validation procedure), and estimated dates of divergence for each node of interest from the bootstrap-replicated datasets in r8s. We then estimated the upper and lower confidence intervals following Burbrink and Pyron (2008; using their provided spreadsheet) and report the standard normal and ABCq methods.

There are three hypothesized dates for the timing of the separation of the Baja California Peninsula from mainland Mexico: 5.5 mya (Curray and Moore, 1984); 7.5–8.2 mya (Oskin and Stock, 2003); and 12–14 mya (Ferrari, 1995; Henry and Aranda-Gomez, 2000). The age for the maximum extent of the northern Gulf of California is 6.3–6.5 mya (McDougall et al., 1999). Recent studies support extension of the gulf that occurred from ~6 to 12 mya (Henry and Aranda-Gomez, 2000) and the peninsula was near its current position by ~5.5 mya (Stock and Hodges, 1989). Therefore, we did not use the 5.5 mya (Curray and Moore, 1984) hypothesis of the Cape separation from mainland Mexico, because this postdates the northern maximum extent of the gulf. Thus, we conducted the date estimation in r8s twice, each time with only the node representing the Cape separation (node 3, see Section 3) calibrated, once with the early estimate of 13.0 mya (12–14 mya from Ferrari, 1995) and a later estimate of 7.8 mya (7.5–8.2 mya from Oskin and Stock, 2003). The consecutive, basal phylogenetic placements and mainland geographic distributions of *Pseudoleptodeira* and *H. tanzeri*, coupled with the nested phylogenetic placement of *H. slevini*, and its occurrence on the southern portion of the peninsula, topologically support the *H. slevini* divergence to coincide with the Cape separation from mainland Mexico (see Section 3). Hence, we assume the *H. slevini* divergence to be consistent with the Cape separation, and use dates from the two competing hypothesis of timing for this event to calibrate our tree and estimate dates for remaining divergences.

The Coast to Desert + Cochise clade split (node 6, Fig. 3) is proximal to, and likely associated with the northern extent of the Gulf of California. The date for the maximum gulf extent is well-accepted 6.3–6.5 (McDougall et al., 1999; Oskin and Stock, 2003). By using the more confident *H. slevini* divergence, associated with the Cape separation (node 3), we estimate the northern divergence (Coast to Desert + Cochise; node 6) with the two hypothesized dates for the Cape separation. Therefore, we evaluate the results for the different competing hypotheses for the timing of the separation of the Cape (7.8 vs. 13 mya) by comparing our estimated dates for the Coast to Desert + Cochise divergence (node 6), to the maximum extent of the northern gulf (6.3–6.5 mya), the presumed cause of this divergence. In summary, this approach allows us to evaluate the two competing hypotheses for the timing of the Cape separation, by comparing our estimated date for the northern divergence (Coast to Desert + Cochise; node 6) with the more reliable geological estimate for the northern extent of the Gulf of California.

3. Results

3.1. mt-genome structure in dipsadine snakes

We characterize 16 complete mt-genomes from 13 nightsnakes and three outgroup genera, and a third of the mt-genome for *H. tanzeri* (GenBank: EU728577–EU728593). The 16 complete mt-genomes range in size from 17,197 bp in *H. slevini* to 23,038 bp in *Leptodeira septentrionalis*, and contain the genes encoding 13 proteins, 2 rRNAs, and 22 tRNAs. We recovered two Control Regions (CR1 and 2) with the unique *trnI* and *trnL1-Q-M* tRNA rearrangements typical of advanced snakes. However, none contained the partial *trnP* found in *Dinodon* (Kumazawa et al., 1998). Extensive length variation was discovered in the CRs between genera, and a rearrangement of the conserved sequence blocks (CSB 3, 1, and 2, relative light-strand order) was found in all mt-genomes recovered in this study as compared with *Dinodon* (Kumazawa et al., 1998). All *Hypsiglena* mt-genomes contain two CRs, each ~1 kb in length and virtually identical with a few exceptions (~1–10 bp). The *Pseudoleptodeira* mt-genome contains two CRs of ~1590 bp (CR1 = 1581 bp and CR2 = 1594 bp), identical except for length variation and one bp at the 5′ end of the shorter sequence. The first ~1 kb were easily aligned with the *Hypsiglena* CRs, the rest of the 3′ end appeared as short, random-repeat segments ranging ~10–200 bp.

The *Imantodes* mt-genome contains two CRs with considerable length variation (CR1 = 2878 bp and CR2 = 4110 bp); the first ~1 kb aligned with the other genera, while the rest was composed of hundreds of random repeats of 10 bp up to 1.4 kb for CR1 and 10 bp to nearly 2 kb in length for CR2. The *Leptodeira* mt-genome contains two CRs of 4758 bp and 2789 bp in length, and an insertion of 342 bp between *nad5* and *nad6*. This region is non-coding but is most similar to the *nad6* region of mtDNA in snakes when 'BLAST searched' in GenBank, suggesting a possible duplication event in the past. The inferred secondary structures of the 22 tRNAs were similar to those of *Dinodon*, with considerable truncation of the T-arms, and length variation in the D- and T-loops similar to other snakes (Kumazawa et al., 1998). The *Sibon* mt-genome contains two ~1 kb CRs easily aligned with the others, but contains a large (5702 bp) region of repeated sequences ranging ~100–400 bp in length, between *trnC* and *trnY*, near the origin for light strand replication (O_L). These findings implicate both heavy and light strand replication mechanisms for the evolution of snake mt-genomes.

3.2. Phylogenetic results of mtDNA

The mt-genome data comprises 15,549 aligned base-pairs (3836 parsimony-informative) and parsimony analyses recovered one tree of 14,936 steps in length (for alignment see Supplementary material). Phylogenetic analyses of the mt-genome dataset under both maximum parsimony and Bayesian analyses recovered a single, well supported topology (Fig. 3). This phylogeny recovered *Pseudoleptodeira* sister to *Hypsiglena*, with the mainland *H. tanzeri* as the most basal *Hypsiglena*, followed by the Baja California endemic *H. slevini*, with the remaining mainland forms monophyletic and *H. ochrorhyncha* as highly nested, which also occurs along the entire Baja California Peninsula (Fig. 4). The monophyly of *Hypsiglena* received weak support under parsimony, largely because of the missing data for *H. tanzeri*, the most basal *Hypsiglena*. When this incomplete taxon (*H. tanzeri*) is removed, the parsimony bootstrap value for the monophyly of *Hypsiglena* is 100%. Bayesian analyses recovered posterior-probabilities = 1.0 for all nodes except one (Fig. 3), including the monophyly of *Hypsiglena*. Our backbone constraint on the phylogeographic data (*nad4* region of 110 haplotypes), provides phylogenetic structure and internal rooting for the clades recovered (Fig. 4c), and the phylogeographic data provide the geographic extent of each clade (Fig. 4d; see Fig. SM-1 for all haplotype labels and bootstrap support values).

3.3. Phylogenetic results of the nuclear data

The nuclear data (GenBank: FJ455155–FJ455234) were less informative than the mt-genome data, though they do recover *H. slevini* as one of the most basal divergence among *Hypsiglena*, and *H. ochrorhyncha* as one of the more nested lineages (Fig. SM-2). Of the 3315 bp of nuclear data, only 60 were parsimony informative. Parsimony analyses recovered nine trees of equal length. Bootstrap analyses supported only the monophyly of *Pseudoleptodeira* and *Hypsiglena* (node 1, Fig. 4a =99%), the monophyly of *Hypsiglena* (node 2, Fig. 4a =83%), and two of the 'Coast Clade' individuals as sister taxa (samples *ochrorhyncha* 3–4; =78%). Bayesian analyses recovered similar results, with the same nodes supported by posterior-probabilities of 1.0, 0.99, and 1.0, respectively; all other nodes were supported by ⩽0.75 (Fig. SM-2). The nuclear data do not strongly conflict with the mtDNA; all nodes well-supported by the nuclear data are in agreement with the mtDNA data.

3.4. Divergence times

Our dating analysis, using the 13.0 mya calibration for the Cape–mainland split, node three (12–14 mya from Ferrari, 1995), resulted in estimated dates of divergence for the Coast to Desert + Cochise split (node 6) as 6.4 mya (ABCq CI 4.61–7.89; Fig. 4b and Table 2) and the *Pseudoleptodeira–Hypsiglena* split (node 1) as 25.1 mya (ABCq CI 19.51–29.03; Fig. 4b and Table 2). Our second analysis, employing the calibration of 7.8 my for the Cape–mainland split, node 3 (7.5–8.2 mya from Oskin and Stock, 2003), resulted in estimated dates of divergence for the Coast to Desert + Cochise split (node 6) as 3.9 mya (ABCq CI 3.52–4.46; Table 2) and the *Pseudoleptodeira–Hypsiglena* split (node 1) as 15.1 mya (ABCq CI 12.22–17.56; Table 2). The most likely explanation for the Coast to Desert + Cochise divergence is the maximum northern extent for the Gulf of California, which is estimated to be 6.3–6.5 mya (McDougall et al., 1999). Because our estimated date for this node using the 13.0 calibration (for node 3) was 6.4 mya, we prefer the 12–14 mya (Ferrari, 1995) estimation for the separation of the Cape of Baja from mainland Mexico, over the 7.5–8.2 mya (Oskin and Stock, 2003) estimation. The latter resulted in an estimated date of divergence between the Coast to Desert + Cochise as 3.9 mya, long after the retreat of the northern extent of the Gulf of California (Buising, 1990; Stokes et al., 1997; McDougall et al., 1999). Hence, we report the estimated dates from the 13.0 calibration analysis on relevant nodes in Fig. 4b, whereas estimated dates for all nodes under both calibration points (7.8 and 13.0 mya) are shown in Table 2.

4. Discussion

4.1. Nightsnake biogeography

Nightsnakes achieved a ring distribution around the Gulf of California through a 'dual-peninsular effect' caused by two major tectonic activities. The 'dual-peninsular effect' was initially proposed to describe how some taxa came to occupy the Baja California Peninsula by southern mid-Miocene vicariance, while others colonized the peninsula by late Miocene–Pliocene dispersal from the north (Seib, 1980). Nightsnakes represent the first group documented to occupy the peninsula through both processes, forming a ring distribution with complete secondary overlap. First, Miocene transform fault movement of the Cape of Baja (Stock and Hodges,

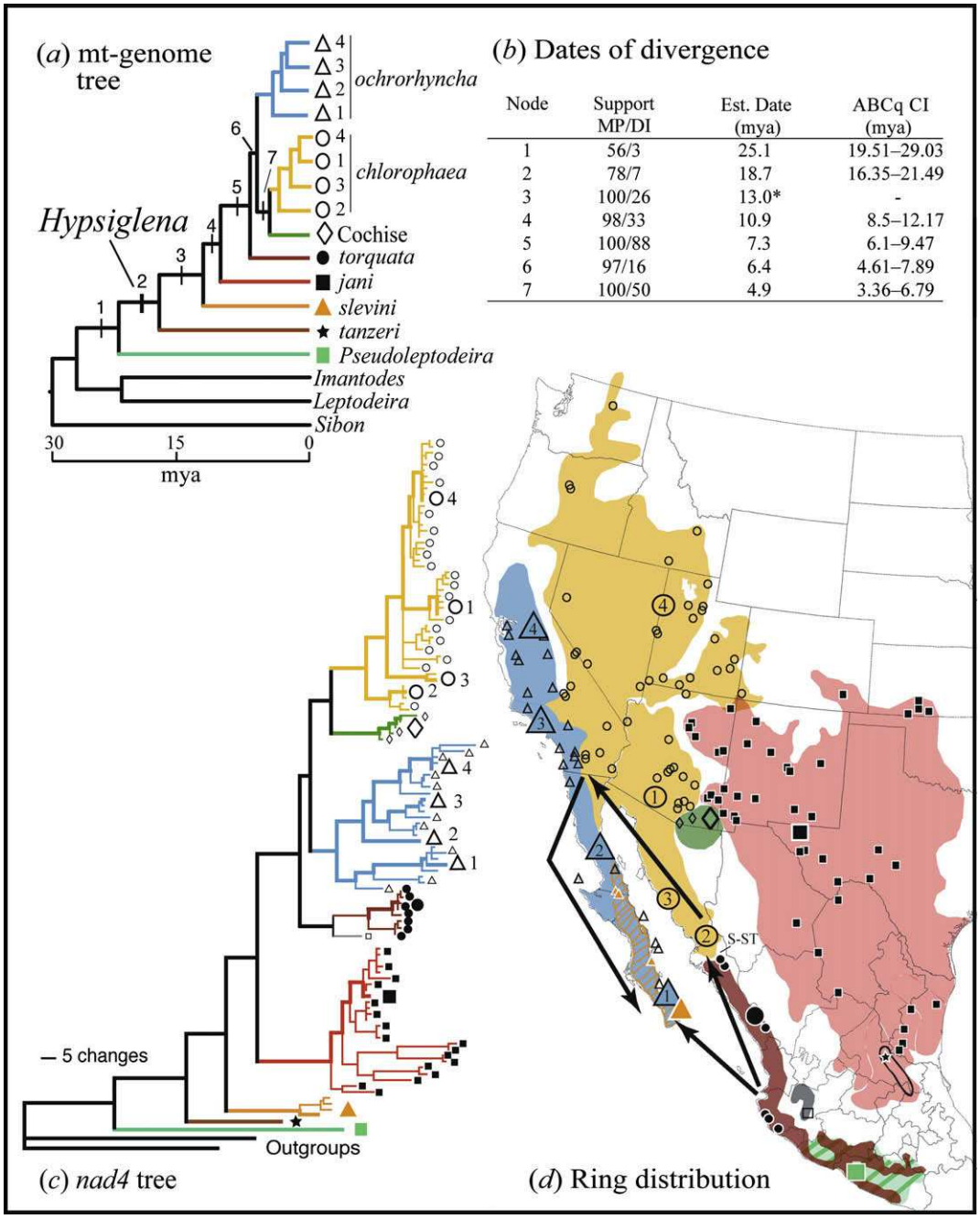

Fig. 4. Hierarchical mtDNA data supporting the ring complex in nightsnakes. (a) Phylogeny based on complete mt-genome data (15,549 bp), numbers following *chlorophaea* and *ochrorhyncha* correspond to samples in Table 1, node numbers 1–7 above branches refer to values in 'b' for nodes above labeled branch. (b) Nodal support and estimated dates of divergence for the major nodes of nightsnakes. Nodes 1–7 received a Bayesian posterior-probability of 1.0, MP = maximum parsimony bootstrap, DI = decay index, ABCq CI = confidence interval, asterisk denotes calibrated node. (c) Phylogeny of the *nad4* mtDNA data recovered with an mt-genome backbone constraint enforced. Bold lines indicate individuals with constrained topology. (d) Map showing the distribution of *Hypsiglena* species around the Gulf of California with *H. slevini* (shown by orange-hatching) and *H. ochrorhyncha* (solid blue) in secondary overlap along the southern half of the peninsula, completing the ring. Black arrows depict routes of dispersal around the Gulf of California. Colors, shapes, sizes, and numbers of terminals in each clade correspond with sampling on the map; S–ST = Sonora–Sinaloa Transition zone, large and small symbols = mt-genome and *nad4* data, respectively. The star represents *H. tanzeri*.

1989; Ferrari, 1995) carried *H. slevini* from mainland Mexico. Second, after late Miocene–Pliocene peninsular collision with North America, mainland *H. ochrorhyncha* were able to invade the peninsula from the north, eventually achieving complete sympatry with *H. slevini* along the southern portion of the peninsula (Fig. 4d).

Currently, there is debate in the geological literature regarding the exact timing of the Cape separation from mainland Mexico: 7.5–8.2 mya (Oskin and Stock, 2003) versus 12–14 mya (Ferrari, 1995; see also Henry and Aranda-Gomez, 2000). We tested these two hypotheses by calibrating our Cape–mainland topological divergence with each of the two competing geological dates (7.8 mya versus 13.0 mya) and evaluated them based on the estimated dates for the northern Coast to Desert + Cochise divergence (node 6; Fig. 4). A ~5.5 mya divergence (Curray and Moore, 1984) was not considered further because this predates the northern extent of the Gulf of California (McDougall et al., 1999), and others suggest the peninsula was near its current position at this time (Stock and Hodges, 1989; Henry and Aranda-Gomez, 2000). Our

Table 2
Estimated dates of divergence for nodes 1–7 (see Fig. 4). Estimated dates of divergence and confidence intervals are shown for analyses with node 3 calibrated at 13.0 mya in columns 2–4 and with node 3 calibrated at 7.8 mya in columns 5–7. Confidence intervals were calculated using the standard normal and ABCq methods. Asterisks indicate calibrated nodes.

Node	Estimated Date (mya)	CI Normal(mya)	CI ABCq(mya)	Estimated Date (mya)	CI Normal(mya)	CI ABCq(mya)
1	25.1	20.23–29.91	19.51–29.03	15.1	12.36–17.78	12.22–17.56
2	18.7	16.1–21.22	16.35–21.49	11.2	9.57–12.82	9.59–12.83
3	13.0*	–	–	7.8*	–	–
4	10.9	9.2–12.74	8.5–12.17	6.6	5.88–7.34	5.47–7.22
5	7.3	5.71–8.87	6.1–9.47	4.3	4.01–4.68	3.89–4.57
6	6.4	4.76–8.03	4.61–7.89	3.9	3.41–4.33	3.52–4.46
7	4.9	3.16–6.57	3.36–6.79	2.9	2.35–3.53	2.19–3.39

data support the earlier 12–14 mya (Ferrari, 1995) date for the Cape separation from mainland Mexico, because this resulted in the northern Coast to Desert + Cochise divergence estimate to be 6.4 mya, within the estimates of the maximum northern extent for the Gulf of California (6.3–6.5 mya; McDougall et al., 1999). Regardless of which timing is more accurate, these relative divergences in *Hypsiglena* mtDNA support our ring hypothesis in nightsnakes, causing the 'dual-peninsular effect.'

The nuclear data were less informative than the mt-genome data; these protein encoding loci were initially selected for Family-level relationship among squamates (Townsend et al., 2008). While these loci seem to be informative for generic-level relationships (Fig. SM-2), they did not perform well at the species-level. The nuclear data do recover *H. slevini* (Cape divergence) as a more basal lineage within *Hypsiglena*, and a monophyletic *H. ochrorhyncha* as the most nested, consistent with the mtDNA data. The nuclear data also recover *H. chlorophaea* as widely polyphyletic; however, this was previously suspected to be a multiple-species complex (Mulcahy, 2008). Most of the variable characters in this data were autapomorphic, and thus not phylogenetically informative. Future studies with these or similar loci on more individuals may prove to be informative using more sensitive methods incorporating coalescence modeling (e.g. Liu et al., 2008).

The backbone constraint enforced on the phylogeographic data (*nad4*, 178 individuals), based on the mt-genome data, provided the overall phylogenetic structure between these lineages, but did not increase support values within each lineage (Fig. SM-1), as suspected by providing the root and internal structure within; particularly in *H. ochrorhyncha* and *H. chlorophaea*, each represented by multiple mt-genomes. We refrained from any area cladogram or other geographic analyses (e.g. Ronquist, 1997) because of our depauperate sampling, particularly in Baja, but note our backbone constraint phylogeny (Fig. SM-1) suggests a south-to-north expansion in *H. slevini*, consistent with Cape expansion to the north. We save alternative geographic analyses for future studies with more complete sampling in these areas.

4.2. Patterns of Baja California biogeography

Several phylogeographic studies have documented patterns of dispersal from the north onto the peninsula, as seen in various groups of lizards (*Aspidoscelis*, Radtkey et al., 1997; *Uta*, Upton and Murphy, 1997; *Callisaurus*, Lindell et al., 2005), gopher snakes (*Pituophis*, Rodríguez-Robles and De Jesus-Escobar, 2000), spiders (*Homalonychus*, Crews and Hedin, 2006), and rodents (*Peromyscus*, Riddle et al., 2000b; *Thomomys*, Alvarez-Castaneda and Patton, 2004; *Ammospermophilus*, Whorely et al., 2004). Fewer studies have documented an earlier Miocene vicariance in the south associated with the initial separation of the peninsula from mainland Mexico, such as in tree lizards (*Urosaurus*, Aguirre et al., 1999), chuckwallas (*Sauromalus*, Petren and Case, 1997), the orange-throat whiptail (*Aspidoscelis hyperythrus*, Radtkey et al., 1997), amphisbaenians (*Bipes*, Macey et al., 2004), and suggested in slender salamanders (*Batrachoseps*, Jockusch and Wake, 2002). A taxon that appears to show both northern-dispersal and southern-vicariance in Baja is nightlizards (*Xantusia*, Sinclair et al., 2004). Yet the absence of *Xantusia* on the west coast of Mexico and their largely disjunct distribution along the peninsula (precluding overlap) warrant further investigation of this intriguing complex. Thus, *Hypsiglena* represents the first group to show both southern-vicariance and northern-dispersal in one complex, that formed a ring around the Gulf of California, with two distinct forms in complete secondary overlap along the southern half of the peninsula with no indication of hybridization (see below).

A limitation in our study is that we were constrained to using the geological Cape separation of Baja as our only calibration source in estimating dates of divergences within *Hypsiglena*. Modern colubrids are poorly represented in the pre-Pleistocene fossil record, and *Hypsiglena* are only known from the Pleistocene (Holman, 1995). So, we assumed *a priori* that separation of *H. slevini* from mainland *Hypsiglena* was consistent with this geological event. We note that since the description of *H. slevini*, experts have assumed its divergence to be consistent with the formation of Baja California (Leviton and Tanner, 1960; Cadle, 1984; Seib, 1980; Grismer, 1994). We estimated dates of divergence to approximate relative separations among lineages of *Hypsiglena*; particularly for the *H. ochrorhyncha* divergence from *H. chlorophaea* and the Cochise Clade (Fig. 4; node 6) at the head of the Gulf of California. We also note that single calibrations are troublesome, particularly for estimating events pre-dating the calibration point (Near and Sanderson, 2004). Therefore, we put less confidence in our *Pseudoleptodeira–Hypsiglena* time estimate.

Hypsiglena are successful overwater dispersers for islands in the Gulf of California, because they occur on many deep water islands (Murphy and Aguirre-Léon, 2002), including Isla Santa Catalina, likely colonized from the mainland (Mulcahy, 2008). They also occur on the Tres Marías islands (McDiarmid et al., 1976), off the coast of western mainland Mexico, and on the Isla Revillagigedo volcanic chain some 200 miles southwest of the Cape of Baja (Tanner, 1944). Though we cannot absolutely rule out overwater dispersal colonization for *Hypsiglena* in Baja California, we can rule out a recent (Plio–Pleistocene) overwater dispersal because of the high levels of sequence divergence [~9–11% uncorrected between *H. slevini* and other *Hypsiglena* from the *nad4* region, commonly used for comparison in other snakes (e.g. Zamudio and Greene, 1997)] and nested tree topology that indicates the *H. slevini* divergence occurred prior to most mainland divergences, with the exception of *H. tanzeri*.

Distinguishing between vicariance and early (Miocene) overwater dispersal would be nearly impossible from sequence divergence data alone because this could have occurred at any point in time during peninsular formation, thus it would not be contingent upon any given amount of sequence divergence. Regardless, the ring distribution would still be achieved; however, we view vicariance more likely than overwater dispersal to a separating landmass because other taxa are thought to show this pattern as well. For

example, Macey et al. (2004) characterized mt-genomes for trans-gulf amphisbaenians (*Bipes*) and estimated a date of divergence for the peninsular endemic *B. biporus* from mainland congeners to be ~55.5 mya, well before the separation of Baja California from mainland Mexico. Yet no one would doubt their occurrence on the peninsula was caused by vicariance—*Bipes* are poor dispersers, unknown from any islands—they simply diverged before peninsular separation. The garter snake *Thamnophis validus* fits a recent overwater dispersal pattern because of the shallow sequence divergence and estimated dates post-date the formation of the peninsula (de Queiroz and Lawson, 2008). By only providing a minimum calibration date for the *H. slevini* divergence, we allowed for the possible recovery of an earlier age estimate, such as that found for *Bipes*. The separated landmass of the Cape region provided new habitat for *H. slevini* to diverge from its mainland relatives. Therefore, we refer to this incident as 'vicariance' because it involved plate tectonics.

4.3. Ring-species or not?

One might at first consider this geographic-genetic ring pattern in nightsnakes to represent a ring species. Defining characteristics of 'ring species' are where populations from an ancestral source disperse around a geographic barrier with the two distinct forms eventually coming into contact, gene flow through populations between the distinct forms without distributional gaps, and gradual morphological variation connecting the distal ends (Irwin et al., 2001). Few cases meet these criteria because most contain gaps within the ring, there is often hybridization at the ends, and genetic discontinuities may exist along the ring (Irwin et al., 2001; Wake, 2006). In *Ensatina* salamanders, distributed around the Central Valley of California, lineages encircling the uninhabited area show intermediate levels of relatedness, and the disparately related forms have come into secondary overlap, with low levels of hybridization (Stebbins, 1949; Moritz et al., 1992; Wake, 1997). Among nightsnakes, there is a gap in distribution across the Gulf of California, the ring has broken up in two places where major habitat differences occur [at the Sonoran–Sinaloan transition zone (Fig. 4d) and the northern extent of the Gulf of California], and some species in the ring are more closely related to other species outside of the ring (e.g. *H. jani*, in the Chihuahuan Desert) then they are to other members of the ring (Fig. 4). Additionally, based on our estimated dates of divergence and phylogenetic relationships, it does not appear that a single species of nightsnake circumscribed the gulf at any one point in time. Therefore, we do not consider nightsnakes to represent a 'ring species.' Instead, nightsnakes appear to have broken up into multiple species while circumscribing the gulf, forming a species complex in the shape of a ring. This system provides a unique opportunity to explore the genetic mechanisms occurring at the interface of speciation where these recently diverged lineages are coming back into secondary contact.

Nightsnakes do show putative evidence for low-levels of hybridization at species contact zones not on the peninsula. The species boundary between *H. torquata* and *H. chlorophaea* occurs at the Sinaloan–Sonoran transition zone (node 5; Fig. 4d). Nightsnakes in this area are morphologically intermediate (Dixon and Dean, 1986) and there is a contact zone in the mtDNA data (Mulcahy, 2008). Elsewhere, *H. ochrorhyncha* and *H. chlorophaea* come into close contact (~30 km) at the northern extent of the Gulf of California (node 6; Fig. 4), in continuously suitable but ecologically different habitats (Mulcahy, 2008). Additionally, individuals comprised of a mix between mtDNA haplotypes and species' distinguishing color patterns occurring on the Colorado Plateau and the Cochise–Chihuahuan boundary, suggest hybridization at these species contact zones (Mulcahy, 2008). The overlapping forms *H. slevini* and *H. ochrorhyncha* in Baja California show no evidence of hybridization, no one has ever suspected or reported hybrid individuals and these lineages show no sign of mixed maternal ancestry (Mulcahy, 2008, this study). Studies of contact zones based on morphology and genetic data can determine the degree of hybridization between lineages (Matocq, 2002), and identify areas to test whether isolating mechanisms can enforce speciation or where the lack of isolating mechanisms may allow lineages to coalesce. Future studies of *Hypsiglena* will focus on fine-scale genetic interactions with more sensitive nuclear markers (e.g. microsatellite DNA loci) at these contact zones to investigate the genetic nature of species boundaries.

Ring complex patterns involving the Baja California Peninsula may be more prevalent than previously understood. Tectonic transform fault separation of the Cape Region and subsequent collision of the northern peninsula with southern California allows for disparate ends of a ring to achieve contact. A revaluation of biotic groups with a 'dual-peninsular effect' in mind could potentially expose ring patterns in numerous other systems inhabiting the peninsula. For instance, a review study revealed that two out of 12 taxa (amphibians, reptiles, and small mammals) examined with mtDNA data show a mainland–Cape vicariance event and four show northern-dispersal, though sampling and data were insufficient to support conclusions in other taxa and none were interpreted as ring species (Riddle et al., 2000a). Further examples of the formation of ring distributions—as shown here for nightsnakes—may be detected in other Baja California taxon groups by performing hierarchical sampling and considering the 'dual-peninsular effect' to create a ring.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

Funding by US DOE, JGI, and University of California, LLNL under Contract No. W-7405-Eng-48, LBNL No. DE-AC03-76SF00098 and LANL No. W-7405-ENG-36, the Departments of Biology at Utah State University and Brigham Young University, and by NSF DDIG (DEB-0206063 awarded to J.R. Mendelson III for DGM) and NSF EF-0334966 (awarded to J.W. Sites Jr.). J.L. Boore and staff at the JGI, and M.M. Pérez-Losada, P. Unmack, and N. Sheffield at BYU, and R.A. Pyron at CUNY provided assistance with logistics. J.V. Vindum at the California Academy of Sciences, R.W. Murphy at the Royal Ontario Museum, D. Dittmann at Louisiana State University, and J.A. McGuire at the Museum of Vertebrate Zoology provided genetic resources and rapid accession of specimens. E.D. Brodie Jr., L. Chan, J.R. Mendelson III, T.J. Papenfuss, J.F. Parham, A. de Queiroz, J.W. Sites Jr., D.B. Wake, and B.L. Williams made valuable comments on the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.05.037.

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Table SM-1. Primers used in this study. Primer combinations for long PCR are shown for each taxon, followed by primer name and sequence.

Voucher	Sample	Primer Combination	Primer Name	Sequence 5'----------3'
MVZ 236396	*H. ochrorhyncha* 1	16Sf.2---Hyp3-ND4r.2	**Forward:**	
"	"	Hyp3-ND4f.1---16Sr.10	16Sf.2 (L3002)*	TACGACCTCGATGTTGGATCAGG
"	"	HypCO3f---HypCBr	Hyp12Sf	AAACTAAGATTAGATACCTTACTA
"	"	Hyp12Sf---HypMetr	Hyp3-ND4f.1	GCATACTCATCAATCAGCCACAT
MVZ 236389	*H. ochrorhyncha* 2	Hyp3-ND4f.1---16Sr.10	HypCO3f	GCAATCTGATACTGACATTTYGT
"	"	16Sf.2---Hyp3-ND4r.2	HypsigLeu2f.1	TGGTGCAAATCCAAGTGGTA
"	"	Hyp12Sf---HypMetr	ImanCBf.1	CGAGACGTACCCTACGGATGAAT
"	"	HypCO3f---HypCBr	ImanLeu1f	GCCATGCAAAAGGCTTAAAACC
CAS 229918	*H. ochrorhyncha* 3	16Sf.2---Hyp3-ND4r.2	ImanNad5f.1	ATCGCATTGGAGATATTGGCCTC
"	"	Hyp3ND4f.1---16Sr.10	ImanND4f.1	GCATATTCATCTATTAGTCACAT
"	"	HypCO3f---HypCBr	LeptND4f.1	GCAGCTATCGGACTTAGTCTTGT
"	"	Hyp12Sf---LeptCO1r	LeptoNad5f2	GCTGCAGCATCAACTCACTTAGA
MVZ 180363	*H. ochrorhyncha* 4	Hyp3-ND4f.1---16Sr.10	LeptoNad6f1	CCGAGATCAGCCAAGAATAACCA
"	"	16Sf.2---Hyp3-ND4r.2	Metf.6 (L4437)*	AAGCTTTCGGGCCCATACC
"	"	Hyp12Sf---HypMetr	PlepCO3f	GCAATCTGATATTGACACTTYGT
"	"	HypCO3f---HypCBr	PSLD-ND4f.1	GCATACTCATCTATCAGTCACAT
MVZ 237359	*H. chlorophaea* 1	Hyp3ND4f.1---16Sr.10	Sibon-ND4f.1	GCATACTCCTCTATCAGCCACAT
"	"	16Sf.2---Hyp3-ND4r.2		
"	"	Hyp12Sf---HypMetr	**Reverse:**	
"	"	HypCO3f---HypCBr	16Sr.10 (H2979)	CCTGATCCAACATCGAGGTCGTA
ROM 14932	*H. chlorophaea* 2	Hyp3-ND4f.1---16Sr.10	CO1r.1 (H5934)*	AGRGTGCCAATCTCTTTGTGRTT
"	"	16Sf.2---Hyp3-ND4r.2	Hyp3-ND4r.2	AGTGTTATAAGGAGGTGTTCTCG
"	"	HypCO3f---HypCBr	HypCBr	AAGTGAAGTGCAAAGAATCGTGT
"	"	Hyp12Sf---HypMetr	HypCO1rev	AAGAGTCAACGRGTRATGAACAC
ROM-JRO 694	*H. chlorophaea* 3	Hyp3-ND4f.1---16Sr.10	HypJ-ND4r.1	AGTGCTATAAGAAGATGTTCTCG
"	"	16Sf.2---Hyp3-ND4r.2	HypMetr	GGTATGGGCCCGATAGCTT
"	"	Hyp12Sf---HypMetr	HypProR.1	GCTGGTTTTGGGGGCYAGAGA
"	"	HypCO3f---HypCBr	HypsigNad6r.1	TTATTCTTAGTTGATCTCGGC
MVZ 241611	*H. chlorophaea* 4	16Sf.2---Hyp3-ND4r.2	ImanCBr.2	AAATGTAATGCGAAGAATCGTGT
"	"	Hyp3-ND4f.1---16Sr.10	ImanContr.1	TGTCTGCAAGCATGGAACGTCTG
"	"	HypCO3f---HypCBr	ImanND4r.2	AATGTTATAAGTAGATGTTCTCG
"	"	Hyp12Sf---HypMetr	LeptCBr.2	AAGAATCGTGTAAGGGTTGGGTC

	H. sp. nov.	
CAS 228951	Cochise Clade	16Sf.2---Hyp3-ND4r.2
"	"	Hyp3ND4f.1---16Sr.10
"	"	HypCO3f---HypCBr
"	"	Hyp12Sf---LeptCO1r
CAS 228960	*H. jani*	Hyp3-ND4f.1---16Sr.10
"	"	16Sf.2---HypJ-ND4r.1
"	"	Hyp12Sf---HypMetr
"	"	HypCO3f---HypCBr
MZFC 16926	*H. torquata*	16Sf.2---Hyp3-ND4r.2
"	"	Hyp3-ND4f.1---16Sr.10
"	"	HypCO3f---HypCBr
"	"	Hyp12Sf---HypMetr
MVZ 234613	*H. slevini*	16Sf.2---Hyp3-ND4r.2
"	"	Erid-ND4f.1---16Sr10
"	"	Hyp12Sf---HypMetr
"	"	HypCO3f---HypCBr
		HypsigNad4f.1---
TCWC A2055	*H. tanzeri*	HypsigLeu2f.1
"	"	LeptoNad5f2---HypsigNad6r.1
"	"	HypsigLeu2f.1---HypProR.1
"	"	LeptoNad6f1----HypProR.1
LSUMZ 39571	*P. latifasciata*	16Sf.2---PSLD-ND4r.2
"	"	PSLD-ND4f.1---16Sr.10
"	"	Hyp12Sf---HypMetr
"	"	PlepCO3f---PSLD-CBr.2
MVZ 233298	*Sibon nebulatus*	Sibe-ND4f.1---16Sr.10
"	"	Metf.6---SibonND5rev
"	"	16Sf---CO1r.1
"	"	Hyp12Sf---HypCO1rev
MVZ 164943	*L. septentrionalis*	ImanCBf.1---16Sr.10
"	"	LeptND4f.1---LeptCBr
"	"	ImanLeu1f---LeptND4r.2
"	"	LeptoND4f.1---LeptoPro r.1
"	"	Hyp12Sf---LeptCO1r
MVZ 149878	*Imantodes cechoa*	16sf.2---Co1r.1

LeptCO1r	GCTGTAAAGTAGGCTCGACTGTC
LeptND4r.2	AGTGTTATGAGAATATGTTCTCG
LeptoPro r.1	GGGCCAGGAGGGCTGTGTTTTCA
PSLD-CBr.2	AAGAATCGTGTAAGAGTTGGATC
PSLD-ND4r.2	AGTGTGATAAGGAGATGTTCTCG
SibonND5rev	CATGTGATGAACAGGGTGATTGG

"	"	ImanND4f.1---LeptCBr.2
"	"	ImanCBf.1---16Sr.10
"	"	ImanLeu1f---ImanND4r.2
"	"	HypCO3f---ImanCBr.2
"	"	ImanNad5f.1---ImanContr.1

*from Macey et al., 1997.

Table SM-2. Partitions used in Bayesian analyses.

Gene Region	Codon Position	Gene Type	AIC Model	BI Partition
nad4pos1	1	protein coding	GTR+G	1
cox2pos1	1	protein coding	GTR+G	1
partATP6pos1	1	protein coding	GTR+G	1
nad4Lpos1	1	protein coding	GTR+G	1
nad1pos1	1	protein coding	GTR+I	2
nad3pos1	1	protein coding	GTR+I+G	3
nad5pos1	1	protein coding	GTR+I+G	3
cobpos1	1	protein coding	GTR+I+G	3
nad2pos1	1	protein coding	GTR+I+G	3
cox1pos1	1	protein coding	GTR+I+G	3
ATP8pos1	1	protein coding	HKY	4
partnad6pos1	1	protein coding	HKY+G	5
cox3pos1	1	protein coding	SYM+I+G	6
cox1pos2	2	protein coding	F81	7
partATP6pos2	2	protein coding	GTR+G	8
partnad6pos2	2	protein coding	GTR+G	8
nad2pos2	2	protein coding	GTR+G	8
nad1pos2	2	protein coding	GTR+I	9
nad5pos2	2	protein coding	GTR+I+G	10
nad4Lpos2	2	protein coding	HKY	11
cox2pos2	2	protein coding	HKY+G	12
cox3pos2	2	protein coding	HKY+I	13
ATP8pos2	2	protein coding	HKY+I	13
nad3pos2	2	protein coding	HKY+I	13
cobpos2	2	protein coding	HKY+I	13
nad4pos2	2	protein coding	HKY+I+G	14
nad3pos3	3	protein coding	GTR	15
partnad6pos3	3	protein coding	GTR+G	16
nad1pos3	3	protein coding	GTR+G	16
nad2pos3	3	protein coding	GTR+G	16
partATP6pos3	3	protein coding	GTR+G	16
nad4Lpos3	3	protein coding	GTR+I+G	17
nad4pos3	3	protein coding	GTR+I+G	17
nad5pos3	3	protein coding	GTR+I+G	17
cobpos3	3	protein coding	GTR+I+G	17
cox1pos3	3	protein coding	GTR+I+G	17
cox2pos3	3	protein coding	GTR+I+G	17
cox3pos3	3	protein coding	GTR+I+G	17
ATP8pos3	3	protein coding	HKY+I	18

rrnL_16S	n/a	ribosomal	GTR+I+G	19
rrnS_12S	n/a	ribosomal	GTR+I+G	19
ControlRegion	n/a	Origin of Replication	GTR+I+G	20
tRNAVal	n/a	tRNA	GTR+I	21
tRNAGlu	n/a	tRNA	GTR+G	22
tRNAArg	n/a	tRNA	GTR+G	22
tRNAGln	n/a	tRNA	HKY	23
partRNASer2	n/a	tRNA	HKY	23
tRNATyr	n/a	tRNA	HKY+G	24
tRNAHis	n/a	tRNA	HKY+G	24
tRNAAla	n/a	tRNA	HKY+G	24
tRNAGly	n/a	tRNA	HKY+G	24
tRNAPhe	n/a	tRNA	HKY+G	24
tRNAMet	n/a	tRNA	HKY+G	24
tRNALeu1	n/a	tRNA	HKY+G	24
tRNAPro	n/a	tRNA	HKY+I	25
tRNATrp	n/a	tRNA	HKY+I	25
tRNACys	n/a	tRNA	K80+G	26
tRNALys	n/a	tRNA	K80+I	27
tRNALeu2	n/a	tRNA	K80+I	27
tRNAAsn	n/a	tRNA	K80+I	27
tRNAAsp	n/a	tRNA	SYM	28
tRNASer1	n/a	tRNA	SYM+G	29
tRNAThr	n/a	tRNA	SYM+G	29
tRNAIle	n/a	tRNA	SYM+I	30

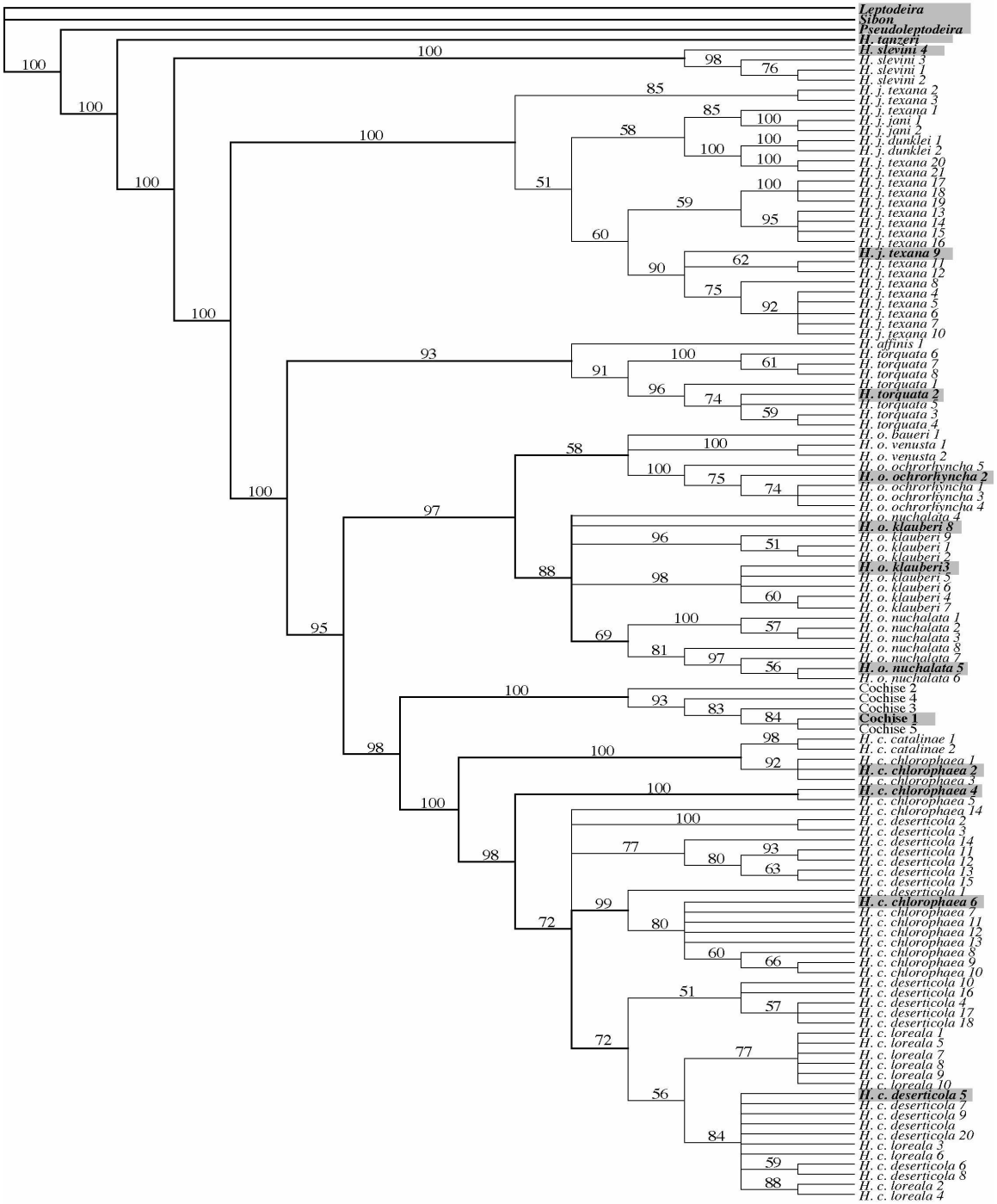

Fig. SM-1. Parsimony bootstrap consensus tree (based on 1000 replicates, w/100 random additions/rep.) of 110 unique *Hypsiglena* haplotypes with a backbone topology enforced for the positions of samples from the mt-genome topology (thick lines and shaded terminals). Haplotypes correspond to Table 1 (this study) and Table 2 (and populations from Table 1 and Fig. 1) of Mulcahy (2008).

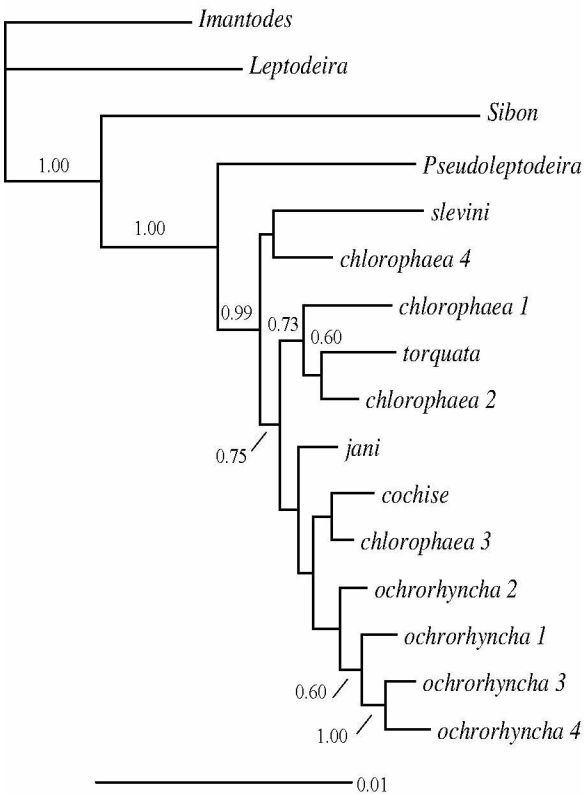

Fig. SM-2. Bayesian phylogram of the combined nuclear loci data. Support values indicate posterior-probabilities, terminals correspond to voucher specimens from Table 1.