Integrative and Comparative Biology

Integrative and Comparative Biology, volume 52, number 2, pp. 321–330
doi:10.1093/icb/ics088

Society for Integrative and Comparative Biology

SYMPOSIUM

Preliminary Insights into the Phylogeography of the Yellow-bellied Sea Snake, *Pelamis platurus*

Coleman M. Sheehy III,[1,*] Alejandro Solórzano,[†] Joseph B. Pfaller[‡,§] and Harvey B. Lillywhite[§]

*Amphibian and Reptile Diversity Research Center, Department of Biology, University of Texas at Arlington, Arlington, TX 76010, USA; [†]Serpentario Nacional and Museum of Zoology, University of Costa Rica, San Jose, Costa Rica; [‡]Archie Carr Center for Sea Turtle Research, University of Florida, Gainesville, FL 32611, USA; [§]Department of Biology, University of Florida, Gainesville, FL 32611, USA

From the symposium "New Frontiers from Marine Snakes to Marine Ecosystems" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2012 at Charleston, South Carolina.

[1]E-mail: cmsheehy@uta.edu

Synopsis The yellow-bellied sea snake, *Pelamis platurus* (Elapidae, Hydrophiinae), has the largest distribution of any snake species, and patterns related to its distribution and regional color variation suggest there is population structuring in this species. Here, we use mitochondrial (ND4, Cyt-b) and nuclear (RAG-1) DNA to (1) test whether genetic variation is associated with local variation in color pattern, and (2) assess whether large-scale patterns of genetic variation are correlated with geographic distribution across the Pacific Ocean. We found low levels of genetic variation and shallow population structure that are correlated with local variation in color pattern and with geographic distribution. The low levels of genetic divergence indicate a relatively high rate of gene flow throughout the Pacific region and/or a recent expansion of range, both of which could be attributable to the passive drifting of these snakes on oceanic surface currents. The mtDNA data conform closely to a model of past exponential population growth, and this may have been associated with the species' large eastward and westward expansion of range. The pattern of low nucleotide and high haplotype diversity suggests that this population growth occurred in the relatively recent past. Data from drifting buoys can potentially act as informative models for predicting patterns of drifting in *Pelamis* and for generating additional testable hypotheses relating to its population structure and biogeography. Future studies should employ nuclear microsatellite markers to investigate population structure in this species at a finer scale. The exploitation of oceanic currents as a novel and highly efficient dispersal mechanism has likely facilitated gene flow throughout the Pacific Ocean in this uniquely pelagic species of sea snake, resulting in a distribution spanning over half of the earth's circumference.

Introduction

The colonization of tropical marine habitats has occurred independently in the snake lineages Acrochordidae, Laticaudinae, Homolopsidae, and Hydrophiini. With more than 60 species in 19 genera currently recognized, the Hydrophiini, or true sea snakes, are the most diverse and the most widely distributed of all the lineages of marine snakes (Heatwole 1999; Sanders et al. 2008). Most of the Hydrophiini are distributed from the western Pacific through Indonesia and northern Australia to the Persian Gulf (Heatwole 1999). However, the yellow-bellied sea snake (*Pelamis platurus*) is uniquely pelagic and ranges throughout the tropical and subtropical waters of the Pacific and Indian Oceans (Heatwole 1999). It is the only species of sea snake that has colonized the far eastern Pacific and western Indian Oceans (Kropach 1975). As a result, *Pelamis* has the largest distribution of any snake, marine or terrestrial (Minton 1975). However, despite its exceptionally wide distribution, we thought this species might be heterogeneous throughout the Indo-Pacific region. Museum records and collection data suggest that *Pelamis* is more commonly associated with the relatively shallow waters of continental shelves and island archipelagos, and less common or absent in the deepest waters (Hecht et al. 1974; Heatwole 1999). Consequently, extensive gaps associated with deep-water may restrict gene flow causing regional population structuring within the species (Heatwole 1999).

Advanced Access publication June 1, 2012

Population structure may also be correlated with variation in color pattern in this species. Snakes along much of the Pacific coast of Costa Rica exhibit the species' typical color pattern consisting of a dark dorsum and yellow venter, which may contain a brown stripe, black markings, neither, or both (Tu 1976; Fig. 1A). This variation in color pattern is continuous and occurs throughout the range of the species (Kropach 1975). However, a southern Costa Rican population consists of yellow individuals within Golfo Dulce (Kropach 1975; Solórzano 2011; Bessesen 2012). This yellow population is characterized by lacking black color altogether or having it reduced to small sparse patches of dark pigment on the dorsum or top of the head (Solórzano 2011; Bessesen 2012) (Fig. 1B). Solórzano (2011) and Bessesen (2012) found that all of the *Pelamis* in the far inner Golfo Dulce basin were yellow and hypothesized that the effects of topography on oceanic currents flowing along the coast and within the Gulf may be maintaining this polychromatism by restricting gene flow into and out of the inner Gulf.

Patterns related to distribution and regional variation in color suggests there is structuring of *Pelamis* populations. However, little is known regarding levels of genetic variation across the range of this species. Thus, the goals of this study are two-fold. First, we use mitochondrial (mt) and nuclear (n) DNA to test if genetic diversity is associated with color pattern variation in Costa Rican populations. Second, we assess whether phylogeographic patterns are correlated with geographic distribution across the Pacific Ocean.

Methods

Collection of data

Individuals of *Pelamis platurus* with normal color pattern were collected from the Golfo de Papagayo, Guanacaste, Costa Rica ($n = 16$), and from the Taiwan Strait near Hsinchu County, Taiwan ($n = 1$) (Table 1). Yellow *Pelamis* ($n = 11$) were collected from Golfo Dulce, Puntarenas, Costa Rica. Snakes in Costa Rica were usually collected along slicks from a small boat by hand or by using a handheld net (Brischoux and Lillywhite 2011) and subsequently released following collection of tissue. Small samples of tissue were collected from live snakes by

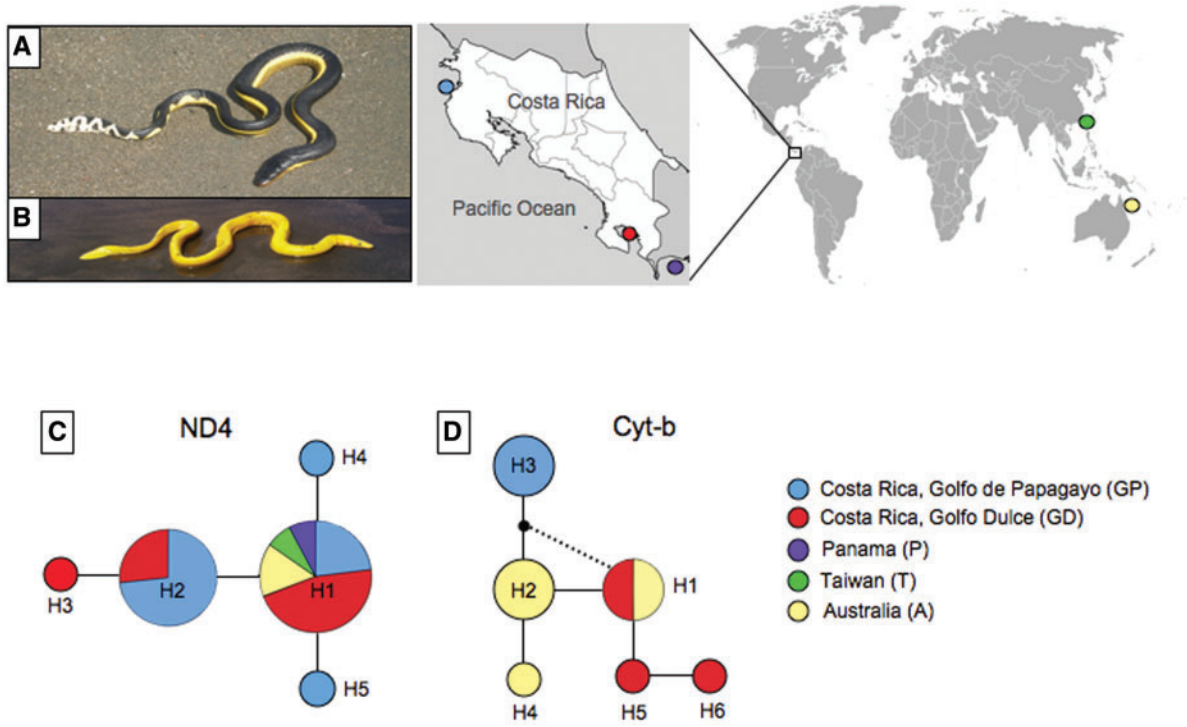

Fig. 1 Variation in color pattern in the sea snake *Pelamis platurus* from (**A**) Golfo de Papagayo and (**B**) Golfo Dulce, Costa Rica. Statistical parsimony (haplotype) networks for (**C**) ND4 and (**D**) Cyt-b. Haplotype colors refer to the respective sampled populations shown on the map. Larger circles and pie slices in haplotype networks contain relatively more individuals. Each line represents a single mutational step, and closed black circles represent hypothetical haplotypes. Haplotype numbers for ND4 (H1–H5) and Cyt-b (H1–H6) refer to Tables 3 and 4, respectively, and to the ND4 gene tree (Fig. 2). The dotted line in the Cyt-b network represents an alternative link between a hypothetical haplotype and haplotype H1.

Table 1 Sample information and GenBank accession numbers for 33 *Pelamis platurus* specimens included in this study

Voucher ID[a]	Locality	Latitude	Longitude	ND4	Cyt-b	RAG-1
ASL 587	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966042**	**X**	**X**
ASL 588	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966041**	**JQ966023**	**X**
ASL 591	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966029**	**JQ966021**	**X**
ASL 592	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966030**	**X**	**JQ966028**
ASL 594	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966031**	**X**	**JQ966027**
ASL 603	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966032**	**X**	**X**
ASL 604	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966040**	**X**	**X**
ASL 605	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966036**	**X**	**X**
ASL 606	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966037**	**X**	**X**
ASL 609	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966039**	**X**	**X**
ASL 689	**Costa Rica, Golfo Dulce (GD)**	**NA**	**NA**	**JQ966035**	**JQ966022**	**X**
CMS 025	**Costa Rica, Golfo de Papagayo (GP)**	**10.59385**	**−85.69992**	**JQ966055**	**X**	**X**
CMS 106	**Costa Rica, Golfo de Papagayo (GP)**	**10.77174**	**−85.67612**	**JQ966056**	**X**	**X**
CMS 108	**Costa Rica, Golfo de Papagayo (GP)**	**10.77174**	**−85.67612**	**JQ966054**	**X**	**X**
CMS 112	**Costa Rica, Golfo de Papagayo (GP)**	**10.76229**	**−85.68238**	**JQ966052**	**X**	**X**
CMS 113	**Costa Rica, Golfo de Papagayo (GP)**	**10.76229**	**−85.68238**	**JQ966047**	**X**	**X**
CMS 114	**Costa Rica, Golfo de Papagayo (GP)**	**10.7588**	**−85.68863**	**JQ966053**	**X**	**X**
CMS 115	**Costa Rica, Golfo de Papagayo (GP)**	**10.75900**	**−85.69073**	**JQ966043**	**X**	**X**
CMS 116	**Costa Rica, Golfo de Papagayo (GP)**	**10.75048**	**−85.69022**	**JQ966049**	**X**	**X**
CMS 127	**Costa Rica, Golfo de Papagayo (GP)**	**10.58292**	**−85.69555**	**JQ966034**	**JQ966024**	**X**
CMS 128	**Costa Rica, Golfo de Papagayo (GP)**	**10.58656**	**−85.70104**	**JQ966033**	**JQ966025**	**X**
CMS 140	**Costa Rica, Golfo de Papagayo (GP)**	**10.60070**	**−85.68008**	**JQ966044**	**X**	**X**
CMS 153	**Costa Rica, Golfo de Papagayo (GP)**	**10.57461**	**−85.75778**	**JQ966048**	**X**	**X**
CMS 161	**Costa Rica, Golfo de Papagayo (GP)**	**10.54615**	**−85.77782**	**JQ966045**	**X**	**X**
CMS 178	**Costa Rica, Golfo de Papagayo (GP)**	**10.62850**	**−85.64478**	**JQ966046**	**X**	**X**
CMS 224	**Costa Rica, Golfo de Papagayo (GP)**	**10.58979**	**−85.70365**	**JQ966050**	**X**	**JQ966026**
CMS 231	**Costa Rica, Golfo de Papagayo (GP)**	**10.59345**	**−85.70107**	**JQ966051**	**X**	**X**
CMS 258	**Taiwan, Taiwan Strait near Hsinchu County (T)**	**NA**	**NA**	**JQ966038**	**X**	**X**
CSIRO - no #	Australia, Gulf of Carpentaria, NT (A)	−14.80	130.00		DQ233975	
NR8918	Australia, Richmond River, NSW (A)	−28.80	153.55	FJ593233	DQ233977	FJ587124
NR8922	Australia, South Ballina, NSW (A)	−28.80	153.55	FJ593234	DQ233978	FJ587125
SAM 55127	Australia, Broken Head, NSW (A)	−28.80	153.55		DQ233976	
UMMZ 209799	Panama, Golfo de Chiriquí (P)	NA	NA	U49299		

Voucher IDs are either museum or field numbers. GPS coordinates are WGS84 datum. Negative latitude coordinates are south of the Equator. Negative longitude coordinates are west of the Prime Meridian. Sequences added specifically in this study are indicated in bold.
[a]Voucher information: ASL = Alejandro Solórzano (private collection, Serpentario Nacional, Costa Rica); CMS = Coleman M. Sheehy (field number, UTA); CSIRO = Commonwealth Scientific and Industrial Research Organisation, Australia; SAM (including NR) = South Australian Museum, Australia; UMMZ = University of Michigan Museum of Zoology, USA.

removing the tip of the tail (usually only the terminal scale) using micro-surgical scissors. Muscle tissue was also collected from dead snakes. Tissue samples were stored in either 95–100% ethanol or tissue lysis buffer in the field. Snake tissues were taken from both sexes (determined by examining the tail for the presence of hemipenes) and from a variety of age classes.

Extraction of DNA, amplification, and sequencing

Genomic DNA was extracted from tissues using a Qiagen DNeasy kit (Qiagen, Valencia, California, USA). Three loci were used: (1) a 620-base pair-fragment of the mitochondrial NADH dehydrogenase subunit 4 (ND4) without tRNAs, (2) a 828-base pair-fragment of the mitochondrial cytochrome-b gene (Cyt-b), and (3) a 955-base pair-fragment of the nuclear

recombination activating gene 1 (RAG-1). Gene fragments were amplified using polymerase chain reactions (PCR) (see Table 2 for primers used). All amplification reactions used GoTaq® Green Master Mix, 2X (Promega Corporation, Madison, Wisconsin, USA). Thermal cycling was performed on a GeneAmp® PCR System 9700 machine (Applied BioSciences, Foster City, California, USA). PCR product was quantified by visualization on 1% agarose gel stained with ethidium bromide. Successfully amplified PCR products were prepared for sequencing by using the ExoSAP-IT kit (United States Biochemical). A BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) was used for sequencing following the manufacturer's protocol and using PCR primers. The sequenced products were precipitated, using an ethanol/sodium acetate method, and rehydrated in HPLC purified formamide (HIDI). The sample was then analyzed on an ABI PRISM 3100xl Genetic Analyzer in the Genomics Core Facility at the University of Texas at Arlington, USA. Resulting DNA sequences were submitted to GenBank (Table 1 for accession numbers).

ND4 sequences of *Pelamis* ($n = 31$) were obtained from sites in Golfo de Papagayo, Costa Rica ($n = 16$), Golfo Dulce, Costa Rica ($n = 11$), and Taiwan ($n = 1$). ND4 sequences from Panama ($n = 1$) and Australia ($n = 2$) were downloaded from GenBank (Table 1). Cyt-b sequences ($n = 9$) were obtained from sites in Golfo de Papagayo, Costa Rica ($n = 2$) and Golfo Dulce, Costa Rica ($n = 3$). Cyt-b sequences from Australia ($n = 4$) were downloaded from GenBank (Table 1). RAG-1 sequences ($n = 5$) were obtained from sites in Golfo de Papagayo, Costa Rica ($n = 1$) and Golfo Dulce, Costa Rica ($n = 2$). RAG-1 sequences from Australia ($n = 2$) were downloaded from GenBank (Table 1). Alignments were constructed using the program Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and edited by eye using MacClade 4.08 (Maddison and Maddison 2005). Base pair differences among sequences were generated in MEGA 5 (Tamura et al. 2011).

Population genetics

Haplotype diversity (h) and nucleotide (per site) diversity (π) were calculated for 31 ND4 sequences using the computer program DnaSP v5 (Librado and Rozas 2009). Haplotype mismatch distributions were constructed in DnaSP using the R_2 test, which tests for past exponential population growth in datasets with small sample sizes (Ramos-Onsins and Rozas 2002). Haplotype networks were generated using parsimony criteria with 95% plausibility in TCS 1.13 (Clement et al. 2000).

To determine whether the yellow *Pelamis* in Golfo Dulce are genetically different from snakes with normal variation in color pattern in the Golfo de Papagayo, we used DnaSP v5 (Librado and Rozas 2009) to implement four statistical tests for detecting geographic subdivision (i.e., X^2, H_S, K_S^*, and Z^*) (Hudson et al. 1992). Significance for the X^2 test was assessed by comparing the observed values with the critical values of the X^2 distribution, whereas significance for the remaining statistics was assessed by permutation tests with 1000 replicates (Hudson et al. 1992).

Phylogenetic analyses

Phylogenetic analyses were conducted using maximum parsimony (MP), maximum-likelihood (ML), and Bayesian methods using the 31 ND4 sequences. Because the genus *Pelamis* is monotypic, we used three closely-related members of the *Hydrophis* group (Ukuwela et al. 2012) to root the trees. The model test option in MEGA 5 identified the Hasegawa–Kishino–Yano model (Hasegawa et al. 1985) plus gamma (HKY+G) as the best-fit model of molecular evolution for the ND4 dataset based on Akaike Information Criterion. A discrete gamma distribution was used to model differences in evolutionary rate among sites (five categories). The ML analyses (using the HKY+G model) and equally weighted MP analyses were conducted in MEGA 5.

Bayesian analyses were conducted with the computer program MrBayes version 3.0 (Huelsenbeck and Ronquist 2001) using the HKY+G model of

Table 2 Names and sequences of primers used in this study

Region	Name	Sequence: 5′–3′	Source
Cyt-b	S20596F (F)	AACCACTCTTGTTAATCAACTACA	Ingrasci 2011
Cyt-b	S21790R (R)	ACCCATGTTTGGTTTACAAAAACAATGCT	Ingrasci 2011
ND4	ND4 (F)	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. 1994
ND4	LEU (R)	CATTACTTTTACTTGGATTTGCACCA	Arévalo et al. 1994
RAG-1	R13 (F)	TCTGAATGGAAATTCAAGCTGTT	Groth and Barrowclough 1999
RAG-1	R18 (R)	GATGCTGCCTCGGTCGGCCACCTTT	Groth and Barrowclough 1999

molecular evolution with four Markov chains (three heated and one cold) per run. Two independent runs were conducted simultaneously, and we determined stationarity to be reached when the standard deviation of the split frequencies became less than 0.01. We ran the analysis for 1,000,000 generations while sampling trees every 1000 generations. The analysis generated 1000 trees, and stationarity was reached after approximately 150,000 generations (150 sampled trees). Therefore, the initial 25% of the stored trees (the first 250 sampled trees) were discarded as burn-in, and a 50% majority rule consensus tree with estimates of Bayesian support was constructed using the remaining 750 sampled trees. Nodal support was provided by bootstrapping for ML (1000 pseudoreplicates) and MP (2000 pseudoreplicates) analyses, whereas posterior probabilities provided nodal support for Bayesian analyses. Because all three analyses produced similar tree topologies, only the ML tree is shown with support values for all three analyses (Fig. 2).

Results

Population genetics

Analysis of 31 ND4 sequences from five Pacific Ocean localities identified five closely related haplotypes, two of which were relatively common ($n = 13$ and $n = 15$) and three were singletons (Fig. 1C). Analysis of nine Cyt-b sequences identified six closely related haplotypes, three of which were found in two individuals each and three were singletons (Fig. 1D). Analysis of 5 RAG-1 sequences identified two haplotypes, of which one was common and one was a singleton. Because the RAG-1 sequences contained just one uninformative polymorphic site, further results for RAG-1 are not presented. Haplotype diversity was relatively high for both mtDNA gene fragments (ND4: $h = 0.61 \pm 0.05$ SD; Cyt-b: $h = 0.92 \pm 0.07$ SD), whereas nucleotide diversity was relatively low (ND4: % $\pi = 0.11 \pm 0.0002$ SD; Cyt-b: % $\pi = 0.20 \pm 0.0004$ SD). The observed ND4 sequence data conformed closely to the expected values under a model of past exponential population growth in the haplotype mismatch distribution (Fig. 3).

The results of the K_S^* and Z^* tests between Golfo Dulce and Golfo de Papagayo localities in Costa Rica were significant ($P < 0.05$); however, the results of neither the X^2 test nor the H_S test were significant ($P > 0.05$).

Base-pair differences

Sequence divergences within and between all localities had a range of 0.0–0.32% for ND4 and 0.0–0.46% for Cyt-b. However, due to their high similarity, we prefer to present differences between sequences in terms of base pairs. For the Costa Rican localities, ND4 sequences differed by 0–1 bases ($n = 16$) within Golfo de Papagayo, 0–1 bases

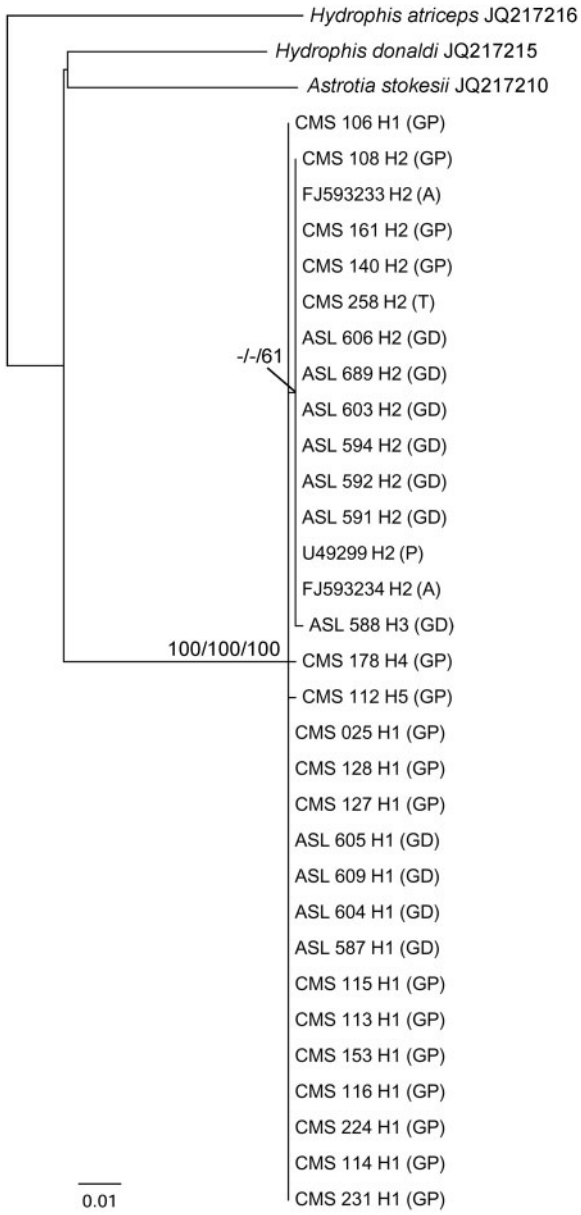

Fig. 2 *Pelamis platurus* gene tree constructed using maximum likelihood to estimate phylogenetic relationships among 31 individuals and five ND4 haplotypes. Units are in substitutions per site. Nodal support is presented as Bayesian posterior probability/maximum-likelihood bootstrap proportions/maximum parsimony bootstrap proportions for nodes with posterior probabilities >0.80 and for bootstrap support >0.50. A dash (-) denotes support below the cutoff value for Bayesian and maximum likelihood. Haplotype numbers (H1–H5) refer to the ND4 haplotype network (Fig. 1B), and to Table 3. Abbreviated localities in parentheses denote regions from which samples were collected and refer to Fig. 1 and Table 1.

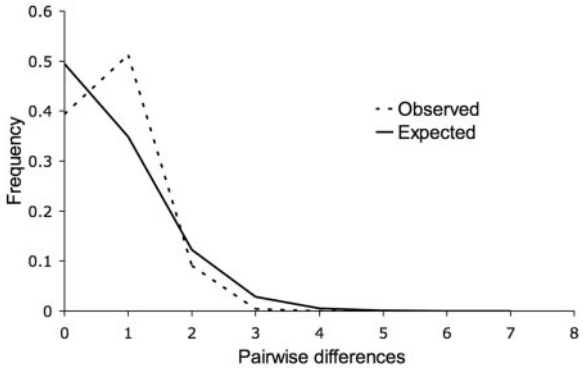

Fig. 3. Mismatch distribution for mtDNA ND4 haplotypes in the sea snake *Pelamis platurus* from the Pacific Ocean. Solid line represents expected values under a model of past exponential population growth. Observed data (dotted line) represent 31 ND4 sequences. Number of pairwise differences (mismatches) is on the *x*-axis. Frequency of mismatches is on the *y*-axis.

Table 3 ND4 haplotype localities and frequencies

Population	N	H1	H2	H3	H4	H5
Costa Rica, Golfo de Papagayo	16	3	11		1	1
Costa Rica, Golfo Dulce	11	6	4	1		
Australia	2	2				
Panama	1	1				
Taiwan	1	1				

Table 4 Cyt-b haplotype localities and frequencies

Population	N	H1	H2	H3	H4	H5	H6
Costa Rica, Golfo de Papagayo	2			2			
Costa Rica, Golfo Dulce	3	1				1	1
Australia	4	1	2		1		

($n = 11$) within Golfo Dulce, and 0–3 bases between the two sites. The Cyt-b sequences differed by 0 bases ($n = 2$) within Golfo de Papagayo, 1–2 bases ($n = 3$) within Golfo Dulce, and 1–3 bases between the two sites. Sequences differed by 0–2 bases (ND4) between Taiwan and the Costa Rican localities (Golfo Dulce and Golfo de Papagayo), and by 0–2 bases (ND4) and by 0–4 bases (Cyt-b) between Australia and the Costa Rican localities. ND4 sequences were identical between snakes from Australia, Taiwan, and Panama. However, ND4 sequences differed by 0–2 bases between Panama and Golfo de Papagayo, and 0–1 bases between Panama and Golfo Dulce.

Haplotype distribution

One of the five ND4 haplotypes was shared among localities in Costa Rica, Panama, Australia, and Taiwan, and one was shared only between Golfo de Papagayo and Golfo Dulce, Costa Rica (Fig. 1C and Table 3). Two of the three singleton ND4 haplotypes were located in Golfo de Papagayo, and one was found in Golfo Dulce (Fig. 1C). Three Cyt-b haplotypes were located each in Australia and Golfo Dulce, with one being shared (Fig. 1D and Table 4). The remaining Cyt-b haplotype was found only in Golfo de Papagayo.

Phylogenetic analyses

Maximum likelihood, maximum parsimony, and Bayesian methods all recovered strong support for a monophyletic *Pelamis platurus* (Fig. 2). One clade containing H2 and H3 ND4 haplotypes was weakly supported (61% MP bootstrap); however, that clade contained snakes from all populations sampled. Of the *Pelamis* collected from Costa Rica, 6 of the 10 snakes (60%) within the H2/H3 haplotype clade were from Golfo Dulce, whereas outside of that clade 12 of the 17 snakes (71%) were from Golfo de Papagayo.

Discussion

We identified shallow population structure in the yellow-bellied sea snake, *Pelamis platurus*, between Golfo de Papagayo and Golfo Dulce, Costa Rica. Each Costa Rican location had unique ND4 haplotypes, although they also shared haplotypes (but at different frequencies). We found no shared Cyt-b haplotypes between the two Costa Rican localities. Two of the tests for geographic subdivision (K_S^* and Z^*) detected significant differences between snakes in Golfo de Papagayo and yellow snakes in Golfo Dulce. However, two other tests (X^2 and H_S) did not find significant differences between the two populations. Given that the test based on X^2 is usually the most powerful of the four in cases involving nonrecombining genes (Hudson et al. 1992), it is surprising that it failed to detect significant differences when other tests did. This suggests that any genetic differences between the two Costa Rican populations are likely to be very small. The phylogenetic analyses also only weakly supported differences between the two populations. However, genetic differences between these populations may not be surprising, given that the yellow snakes in Golfo Dulce and the normal colored snakes outside of the Gulf appear to be allopatric due at least in part to a shallow inner basin sill that impedes the free exchange of water between the inner Gulf and the outer coastal areas (Svendsen et al. 2006; Bessesen

2012). Although the population of yellow *Pelamis* at Golfo Dulce appears to exhibit geospatial, chromatic, and behavioral differences from the rest of the Costa Rican population (Bessesen 2012), we refrain from recommending any taxonomic changes at this time due to the extremely shallow genetic divergence that we identified between the two populations. Insight into whether the Golfo Dulce population is in a stage of incipient speciation might be acquired using finer-scale molecular markers in conjunction with morphological studies.

Genetic variation was small, but strongly correlated with geographic distribution across the Pacific Ocean. The mtDNA haplotypes show a clear signal of genetic structure. Only one of the six Cyt-b haplotypes is shared between Australia and Golfo Dulce; all the other Cyt-b haplotypes are specific for location. There were also four ND4 haplotypes unique to the eastern Pacific, although one common ND4 haplotype was shared among all locations. However, the mtDNA variation between western and eastern Pacific Ocean localities (0.0–0.32% [2 bases] for ND4) was only slightly higher than the within-variation (0.0–0.16% [1 base] for ND4) at both Costa Rican localities. These patterns of haplotype distribution and genetic variation indicate there is shallow genetic structure across the Pacific Ocean.

The pattern of relatively low nucleotide and high haplotype diversity throughout the Pacific Ocean suggests that the Pacific population of *Pelamis* has experienced exponential growth in the relatively recent past (Lukoschek et al. 2007; Sanders et al. 2008, 2010). Similarly, the mtDNA data conform closely to a model of past exponential population growth (Fig. 3), and this may have been associated with the species' large eastward and westward expansion in range. Multiple lines of evidence suggest that the center of radiation for hydrophiine sea snakes occurred in the Australo-Papuan region from a terrestrial elapid ancestor (Keogh 1998; Lukoschek and Keogh 2006). The absence of *Pelamis* in the Caribbean suggests that this species radiated eastward across the Pacific to the western coast of the Americas within the past 2–4 million years after the final closure of the Isthmus of Panama (Kropach 1975; Coates et al. 1992; Schmidt 2007). Recent phylogenetic studies focusing on Australasian elapids in general (Sanders and Lee 2008) and on the *Hydrophis* lineage of hydrophiine sea snakes specifically (Lukoschek and Keogh 2006; Sanders et al. 2008) revealed rapidly-diverged adaptive radiations in these lineages that are consistent with the idea that *Pelamis* has undergone a recent expansion of range.

The shallow population structure and low genetic divergence identified in *Pelamis* suggests that a relatively high rate of gene flow is occurring throughout the Pacific region. Alternatively, this pattern could also be the result of recent range expansion but without ongoing gene flow, as demonstrated for another sea snake, *Aipysurus laevis*, around northern Australia (Lukoschek et al. 2007). Although a recent range expansion likely occurred in *Pelamis*, its unique drifting ecology makes it likely that ongoing gene flow is occurring. *Pelamis* drifts passively with ocean surface currents and can commonly be found in slicks where currents converge (Dunson and Ehlert 1971; Kropach 1971; Lillywhite et al. 2010). In the Golfo de Papagayo, we have found *Pelamis* of both sexes and all age classes in slicks at a maximum rate of 120 snakes seen per hour. The association with slicks provides these snakes with several advantages such as efficiently locating food and mates (Kropach 1975), although the methods by which they arrive at slicks are still debated (Lillywhite et al. 2010; Brischoux and Lillywhite 2011). Thus, oceanic currents are potentially able to transport snakes throughout the Pacific Ocean basin, thereby facilitating gene flow throughout the region. Furthermore, their drifting behavior is likely what originally enabled *Pelamis* to expand their range across the Indo-Pacific region.

The ND4 haplotype and nucleotide diversity we recovered in *Pelamis* are comparable to those identified in *Aipysurus laevis*, which has a geographic distribution more typical of most other hydrophiine sea snakes (Lukoschek et al. 2007). This is surprising given that *Aipysurus laevis* is restricted in distribution to coral reef habitats along the northern continental shelf of Australia and New Guinea (Cogger 1975). The similarities between the two species suggests that they have undergone similar recent histories of range expansion but that, because of their different ecologies, the expansion of range for *A. laevis* occurred on reef habitats around Australia whereas *Pelamis* expanded its range throughout the Indo-Pacific region over a similar time period.

The immense distribution coupled with the drifting behavior of *Pelamis* renders tracking studies of individuals extremely difficult. Kropach (1975) attempted the only mark-recapture study of this species in the Gulf of Panama, but recovery of marked individuals was low (1 out of 961 recovered in the Gulf). Because *Pelamis* passively drifts on surface currents and occurs over such an extensive geographic range, considerable insight can be gleaned from observing the paths of randomly drifting buoys, or drifters. Long-term data on the paths of drifters along surface currents throughout the Indo-Pacific region (Fig. 4) suggest three hypotheses regarding distribution and potential patterns of gene

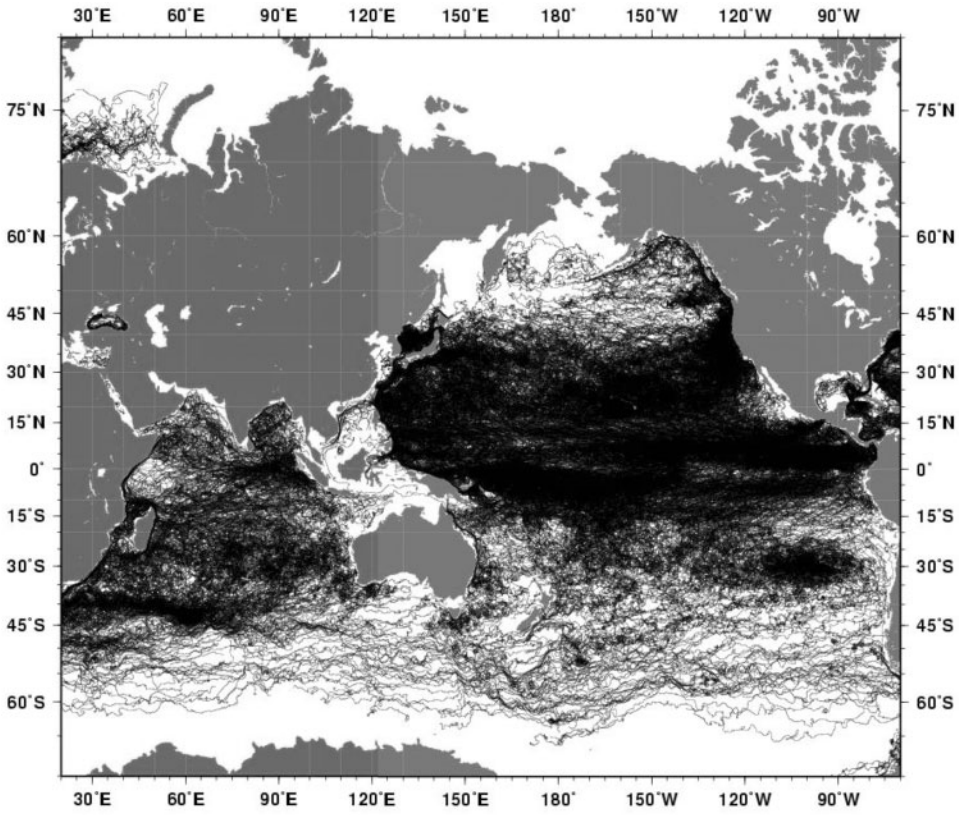

Fig. 4. Spaghetti plot showing 25 years (1978–2003) of data from randomly drifting surface buoys throughout the Indo-Pacific region. The black lines represent the paths of drifting buoys, which numbered several hundred each year. Modified with permission from AJ Mariano (http://oceancurrents.rsmas.miami.edu/basin-maps.html).

flow in *Pelamis*. First, the dense paths of buoys connecting the eastern and western Pacific Ocean suggest that oceanic surface currents are able to transport drifting snakes between the two sides. Second, the distribution of buoy's paths in the Pacific basin appears to be homogeneous and does not contain any gaps. Therefore, if *Pelamis* is drifting passively, any gaps in the distribution should be caused by other factors. The low genetic variation we identified suggests that at least some gene flow is occurring between the eastern and western parts of the Pacific basin, which would likely not occur if large distributional gaps were present. Perhaps collecting biases or nonspecific locality data for specimens are responsible for the idea that distributional gaps exist. Alternatively, the availability of prey over deep water might be lower than over continental shelves (Helfman et al. 2009), which may affect survival of *Pelamis* in these areas causing relatively small or temporary gaps. Third, there appears to be a gap in the density of buoy's paths within the Indonesian region separating the Pacific and Indian Oceans. This suggests that higher levels of genetic population structure might be found between, rather than within, the Indian and Pacific Oceans as a result of restricted gene flow between the two basins. Tissue samples from the Indian Ocean are needed to test this hypothesis.

The matrilineal genes ND4 and Cyt-b are both involved with cellular respiration and may be under selection. Thus, the low genetic variation revealed in this study could potentially be due to the past occurrence of one or more selective sweeps. However, selective sweeps may affect one or a few genes, and their potential involvement can often be confirmed or rejected by the inclusion of additional independent markers (Avise 2004). The selective-sweep scenario is unlikely in the *Pelamis* mtDNA markers we used given that a similar pattern of low diversity is observed independently in the nuclear gene RAG-1. Furthermore, oceanic currents provide an excellent mechanism for maintaining high levels of gene flow in this species. An additional consideration is the ability to discriminate between current and recent gene flow between *Pelamis* populations given its recent exponential population growth and expansion of range. Faster-evolving nuclear markers (i.e., microsatellites) will likely be needed to differentiate between these two scenarios.

In summary, experimental and observational studies are improving our understanding of the historical and contemporary population biology of the yellow-bellied sea snake, *Pelamis platurus*, throughout its Indo-Pacific distribution. Although this study used relatively slowly evolving molecular markers, the observed patterns of variation are consistent with a scenario of relatively high levels of gene flow occurring among snakes in the Pacific Ocean. This putative gene flow may be facilitated by the propensity for *Pelamis* to passively drift on ocean currents. Patterns of haplotype and nucleotide diversity suggest that *Pelamis* likely experienced exponential population growth in the relatively recent past, which may have accompanied an expansion of range into the eastern Pacific and western Indian Oceans. The low levels of genetic variation revealed by the mtDNA suggest that future studies should employ faster-evolving nuclear markers to investigate the shallow population structure we found at a finer scale. *Pelamis platurus* is unique among snakes both in its tendency to drift on currents and in its immense geographic range. For perspective, the maximum straight-line distances between snake localities included in this study (15,000–16,000 km) represent ~40% of the earth's circumference, and distances between the western Indian and eastern Pacific Oceans would be far greater still. Thus, a scenario can be hypothesized in which the evolution of floating behavior likely allowed *Pelamis* to exploit oceanic currents as a novel and highly efficient dispersal mechanism that subsequently facilitated gene flow throughout the Indo-Pacific region.

Acknowledgments

We thank Adán Barrera, Françios Brischoux, Harold Heatwole, and Jamie Lillywhite for assistance with fieldwork, and Serge Boucher for accommodations in Costa Rica. Ming-Chung Tu kindly provided *Pelamis* tissues from Taiwan. We thank Arthur Mariano for providing data on drifting buoys. We are grateful to Mahmood Sasa and Carl Franklin for assisting with obtaining permits, and to Eric Smith, Claudia Marquez and the Genomics Core Facility at the University of Texas at Arlington for providing logistical support pertaining to molecular work and sequencing. We thank Thomas Eimermacher and Andrea Martinez for providing valuable discussion and feedback. Christian Cox, Jeffrey Streicher, Harold Heatwole and two anonymous reviewers kindly provided reviews that greatly improved this manuscript. This study fully complied with approved University of Florida, Institutional Animal Care and Use Committee (IACUC) protocol 200902798.

Funding

This work was supported by the National Science Foundation [IOS-0926802 to H.B.L.] and by the Instituto Bioclon [to E.N. Smith]. The Symposium was supported by the National Science Foundation [IOS-1132369 to H.B.L.]; the Society for Integrative and Comparative Biology (SICB) Divisions of Animal Behavior, Comparative Physiology and Biochemistry, Neurobiology, Phylogenetics and Comparative Biology, and Vertebrate Morphology; University of Florida; Sable Systems International; Vida Preciosa International, Inc. (Dave and Tracy Barker); and the Gourmet Rodent, Inc. The Department of Biology at the University of Texas at Arlington provided travel funds to CMS to attend the Symposium.

References

Arévalo ES, Davis SK, Sites JW Jr. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst Biol 43:387–418.

Avise JC. 2004. Molecular markers, natural history, and evolution. 2nd ed. Sunderland (MA): Sinauer Associates, Inc. Publishers.

Bessesen BL. 2012. Geospatial and behavioral observations of a uniquely xanthic colony of pelagic sea snakes, *Pelamis platurus*, residing in Golfo Dulce, Costa Rica. Herp Rev 43:22–26.

Brischoux F, Lillywhite HB. 2011. Light- and flotsam-dependent 'float-and-wait' foraging by pelagic sea snakes (*Pelamis platurus*). Mar Biol 158:2343–47.

Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–60.

Coates AG, Jackson JBC, Collins LS, Cronin TM, Dowsett HJ, Bybell LM, Jung P, Obando JA. 1992. Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. Geol Soc Am Bull 104:814–28.

Cogger HG. 1975. Sea snakes of Australia and New Guinea. In: Dunson WA, editor. The biology of sea snakes. Baltimore (MD): University Park Press. p. 59–139.

Dunson WA, Ehlert GW. 1971. Effects of temperature, salinity, and surface water flow on distribution of the sea-snake *Pelamis*. Limnol Oceanogr 16:845–53.

Groth JG, Barrowclough GF. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. Mol Phylogenet Evol 12:115–23.

Hasegawa M, Kishino H, Yano T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–74.

Heatwole H. 1999. Sea snakes. 2nd ed. Sydney: University of New South Wales Press.

Hecht MK, Kropach C, Hecht BM. 1974. Distribution of the yellow-bellied sea snake, *Pelamis platurus*, and its significance in relation to the fossil record. Herpetologica 30:387–96.

Helfman GF, Collette BB, Facey DE, Bowen BW. 2009. The diversity of fishes: biology, evolution, and ecology. 2nd ed. West Sussex (UK): Wiley-Blackwell.

Hudson RR, Boos DD, Kaplan NL. 1992. A statistical test for detecting geographic subdivision. Mol Biol Evol 9:138–51.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–55.

Ingrasci MJ. 2011. Molecular systematics of the coffee snakes, genus *Ninia* (Colubridae: Dipsadinae) [MSc Thesis]. University of Texas at Arlington.

Keogh JS. 1998. Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. Biol J Linn Soc 63:177–203.

Kropach C. 1971. Sea snake (*Pelamis platurus*) aggregations on slicks in Panama. Herpetologica 27:131–35.

Kropach C. 1975. The yellow-bellied sea snake, *Pelamis*, in the eastern Pacific. In: Dunson WA, editor. The biology of sea snakes. Baltimore (MD): University Park Press. p. 185–213.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–52.

Lillywhite HB, Solórzano A, Sheehy CM III, Ingley S, Sasa M. 2010. New perspectives on the ecology and natural history of the yellow-bellied sea snake (*Pelamis platurus*) in Costa Rica: does precipitation influence distribution? IRCF Reptiles Amphibians 17: 69–72.

Lukoschek V, Keogh JS. 2006. Molecular phylogeny of sea snakes reveals a rapidly diverged adaptive radiation. Biol J Linn Soc 89:523–39.

Lukoschek V, Waycott M, Marsh H. 2007. Phylogeography of the olive sea snake, *Aipysurus laevis* (Hydrophiinae) indicates Pleistocene range expansion around northern Australia but low contemporary gene flow. Mol Ecol 16:3406–22.

Maddison DR, Maddison WP. 2005. MacClade 4: analysis of phylogeny and character evolution. Version 4.08a. http://macclade.org.

Minton SA. 1975. Geographic distribution of sea snakes. In: Dunson WA, editor. The biology of sea snakes. Baltimore (MD): University Park Press. p. 21–31.

Ramos-Onsins E, Rozas J. 2002. Statistical properties of neutrality tests against population growth. Mol Biol Evol 19:2092–100.

Sanders KL, Lee MSY. 2008. Molecular evidence for a rapid late-Miocene radiation of Australasian venomous snakes (Elapidae, Colubroidea). Mol Phylogenet Evol 46:1180–88.

Sanders KL, Lee MSY, Leys R, Foster R, Keogh JS. 2008. Molecular phylogeny and divergence dates for Australasian elapids and sea snakes (hydrophiinae): evidence from seven genes for rapid evolutionary radiations. J Evol Biol 31:682–95.

Sanders KL, Mumpuni, Lee MSY. 2010. Uncoupling ecological innovation and speciation in sea snakes (Elapidae, Hydrophiinae, Hydrophiini). J Evol Biol 23:2685–93.

Schmidt DN. 2007. The closure of the Central American seaway: evidence from isotopes and fossils to modern molecules. In: Haywood WM, Gregory AM, Schmidt DN, editors. Deep-time perspectives on climate change: marrying the signal from computer models and biological proxies. The Micropalaeontological Society Special Publications. London: The Geological Society. p. 429–44.

Solórzano A. 2011. Variación de color de la serpiente marina *Pelamis platura* (Serpentes: Elapidae) en el Golfo Dulce, Puntarenas, Costa Rica. Cuadernos de Investigación UNED 3:89–96.

Svendsen H, Rosland R, Myking S, Vargas JA, Lizano OG, Alfaro EJ. 2006. A physical-oceanographic study of Golfo Dulce, Costa Rica. Rev Biol Trop 54:147–70.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–39.

Tu AT. 1976. Investigation of the sea snake *Pelamis platurus* (Reptilia, Serpentes, Hydrophiidae) on the Pacific coast of Costa Rica, Central America. J Herpetol 10:13–18.

Ukuwela KDB, Sanders KL, Fry BG. 2012. *Hydrophis donaldi* (Elapidae, Hydrophiinae), a highly distinctive new species of sea snake from northern Australia. Zootaxa 3201:45–57.