Zoological Journal of the Linnean Society, 2025, **205(2)**, zlaf131
https://doi.org/10.1093/zoolinnean/zlaf131

OXFORD

Original Article

The Golfo Dulce yellow sea snake (Elapidae: *Hydrophis platurus xanthos*) from morphological and molecular perspectives

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ABSTRACT

The yellow sea snake *Hydrophis platurus xanthos* is found only in Costa Rica's South Pacific embayment of Golfo Dulce, confined to a <215-m-deep inner basin. This endemic population is geographically separated from the pelagic sea snake *Hydrophis platurus platurus* by >20 km and has distinctive morphological characters, suggesting potential phylogenetic divergence. Our study confirms morphological diagnosability of the Golfo Dulce population using coloration (predominantly yellow vs. dorsally black) and consistently small body size (<60 cm in total length). Several significant differences in cephalic and caudal scale counts are also documented. Seven preserved yellow specimens collected outside Golfo Dulce in the 1970s are morphologically consistent *H. p. xanthos*, suggesting that they originated from inside the gulf. Despite this, when we used reduced representation sequencing to examine single-nucleotide polymorphisms, targeted squamate conserved loci, and mined mitochondrial DNA, our molecular analyses provided no evidence that *H. p. xanthos* and *H. p. platurus* are separately evolving lineages. Indeed, we found near-complete lack of structure both within and between these populations. The absence of genetic differentiation, which suggests regular gene flow despite contrary morphological and biogeographical factors, creates an intriguing paradox. Recent separation and/or high selection pressure might be in effect.

Keywords: Central America; Costa Rica; phylogeography; adaptation; molecular phylogeny; Reptilia; morphometrics

INTRODUCTION

The Golfo Dulce yellow sea snake, *Hydrophis platurus xanthos* Bessesen & Galbreath, 2017 (hereafter, the yellow sea snake), has been described as a subspecies of the widely distributed pelagic sea snake *Hydrophis platurus platurus* Linnaeus, 1766 (Fig. 1A; hereafter, the pelagic sea snake). We use recognized trinomials for the purpose of comparing these morphologically distinct geographical populations but note that some authors here do not recognize subspecies as a valid taxonomic rank (see Burbrink *et al.* 2022). The yellow sea snake inhabits the narrow inlet of Golfo Dulce on the South-Pacific side of Costa Rica (Solórzano 2011, Bessesen 2012; Fig. 1B). Golfo Dulce is considered a 'tropical

Received 13 April 2025; revised 31 July 2025; accepted 15 August 2025

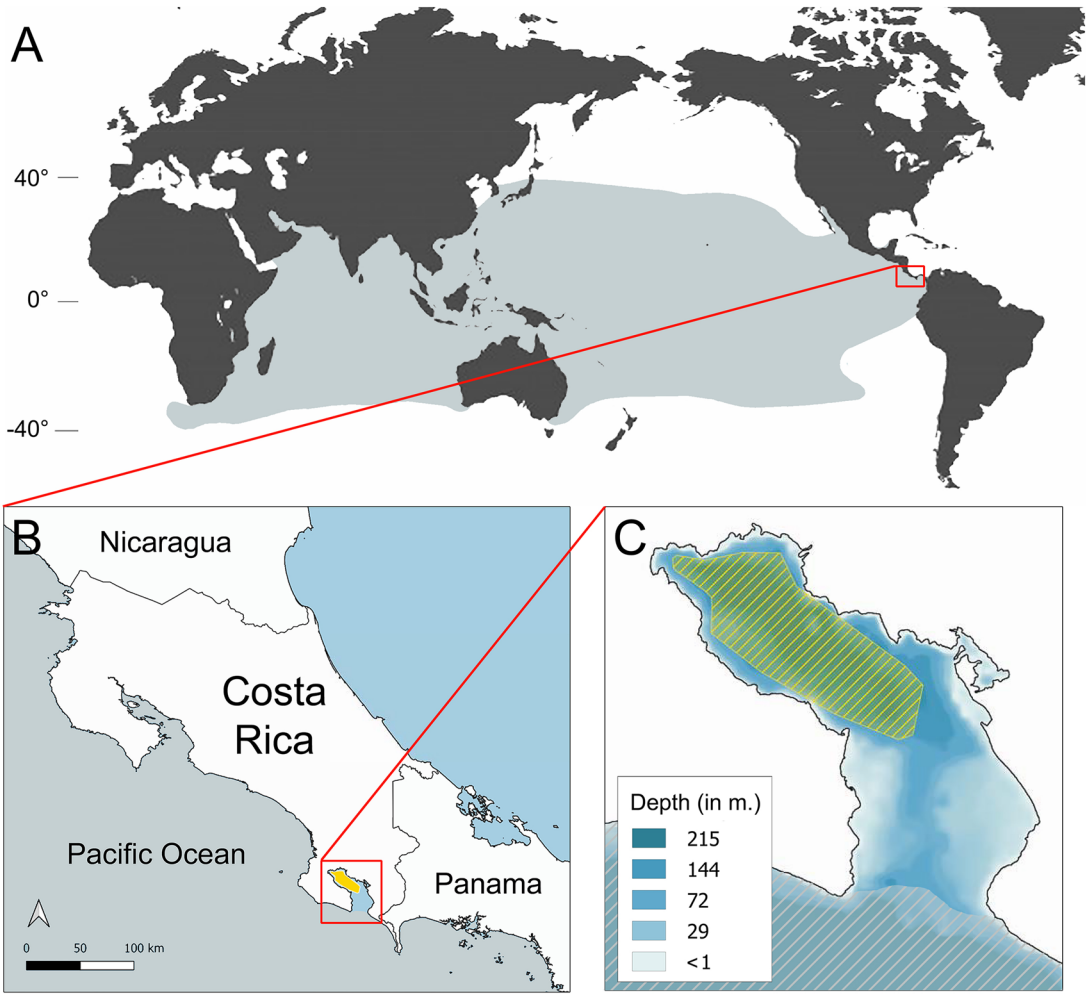

Figure 1. Distribution of the study species. A, *Hydrophis platurus platurus* ranging across the Indo-Pacific Ocean (grey; based on Brischoux *et al.* 2016). B, *Hydrophis platurus xanthos* inside Golfo Dulce in South-Pacific Costa Rica (yellow shading; based on Bessesen *et al.* 2024). C, a spatial gap between the two populations is marked by shallow waters with a complicated current structure.

fjord' because its mesopelagic inner basin has limited exchange with the coastal masses (Wolff *et al.* 1996), and the yellow sea snake is confined to that inner basin (Bessesen 2012, 2015, 2022, Solórzano and Sasa 2024, Lillywhite 2025), where a calm, estuarian circulation pattern prevails (Svendsen *et al.* 2006). Inhabiting a single area of occupancy of <260 km^2 (Bessesen *et al.* 2023; Fig. 1C), this endemic population is estimated at <30000 individuals (Bessesen *et al.* 2022). Importantly, it is geographically separated from the pelagic sea snake population by a spatial gap of >20 km (Bessesen 2012, 2022) characterized by shallow waters (≤30 m deep) and a complicated current structure (Svendsen *et al.* 2006, Morales-Ramírez *et al.* 2015).

Habitat partitioning suggests allopatric distribution, and the yellow sea snake is distinct in both appearance and ecology. In addition to its xanthic coloration (predominantly yellow, lacking a solid black dorsum), a significant reduction in body size has been documented; notably, no yellow sea snake was found to reach the sexually mature length of female pelagic sea snakes (Bessesen and Galbreath 2017) reported as ≥60 cm (Kropach 1975, Vallarino and Weldon 1996). Environmental conditions might have contributed to these phenotypic changes, because water temperatures in Golfo Dulce are 2°C–4°C higher than in the open ocean (Rincón-Alejos and Ballestero-Sakson 2015, Bessesen *et al.* 2023). Pale integument has been proposed to help the yellow sea snake reduce overheating at the water surface (Solórzano 2011, Bessesen 2012), and smaller body size would allow the serpent to shed heat more readily (Bessesen and Galbreath 2017). Lighter colouring, however, could also lead to photosensitivity. The yellow sea snake evinces a nocturnal diel pattern, which is in direct contrast to the diurnal pattern of the pelagic sea snake (Bessesen and González-Suárez 2022). Feeding at night seems to require a higher tolerance for evening wave activity, and although the pelagic sea snake actively avoids rough waters (Rubinoff *et al.* 1986, Cook and Brischoux 2014), the yellow sea snake is commonly found in turbulent conditions, assuming a unique sinusoidal ambush posture that appears to have a stabilizing effect in the waves (Bessesen and Galbreath 2017). It also shows no association with drift lines (Lillywhite *et al.* 2015, Bessesen 2022), which are typically used by pelagic sea snakes for transport, feeding, and possibly reproduction (Kropach 1973, 1975). Visual cues are thought to play a role in drift line detection among pelagic sea snakes (Brischoux and Lillywhite 2011). Hence, the disassociation of the yellow sea snake from drift lines might relate to its nocturnal feeding strategy, because visibility is naturally inhibited at night (Bessesen 2022).

Given the conspicuous differences in morphology and ecology between the yellow and pelagic sea snakes, we considered the possibility that they could be separate species. The bathymetric barrier has long suggested that the yellow sea snake meets Mayr's (1942) biological species concept based on intrinsic reproductive isolation, and its divergent niche metrics meet the less-common ecological species concept (Van Valen 1976). Morphological characters were used for centuries to define and characterize taxonomic groups, including snakes (Underwood 1967). More recently, de Queiroz (2007) called for a unified approach to species delimitation, defining species as separately evolving metapopulation lineages and allowing previous species criteria to serve as secondary lines of evidence for the existence of separate species. Here, our objective was to examine evidence of divergence through morphological and molecular approaches.

To compare morphological characters between the populations, we examined both live and vouchered museum specimens. We also sought to determine the geographical origin of yellow sea snakes recorded off the coast of Central America. Voris *et al.* (1970) and Kropach (1971) were the first to report yellow sea snakes; the latter found 3% of the snakes collected outside the mouth of Golfo Dulce to be yellow. Additional researchers documented yellow sea snakes in the Pacific waters off Central America, although frequencies dropped precipitously the farther from Golfo Dulce they worked. For example, in northern Costa Rica, Tu (1976) collected 3077 sea snakes and found only four (0.1%) yellow specimens, and further to the south Kropach (1971) spotted one yellow snake among the tens of thousands of pelagic sea snakes recorded in Panama Bay. When the Golfo Dulce population was identified, it was hypothesized that yellow specimens seen in the open Pacific (hereafter, '1970s specimens') might have been swept out from the embayment (Bessesen 2015).

To test for genetic structure between populations, we undertook comparative molecular analyses of contemporary specimens using nuclear and mitochondrial DNA (mtDNA). Within the rapidly radiating *Hydrophis* clade, low variability of molecular markers can make gene trees challenging to resolve (Lukoschek and Keogh 2006, Rasmussen *et al.* 2011, Sanders *et al.* 2013). Yet, when examining allele frequencies across spatial gradients, a pattern of isolation by distance is often expected to emerge, whereby genetic similarity decreases as spatial distance increases (Wright 1943). Given the enormous east–west range of our study species, from the east coast of Africa to the west coast of the Americas (Hecht *et al.* 1974, Lillywhite *et al.* 2018), we anticipated finding shallow geographical variation across its oceanic distribution, but with more genetic changes attributed to the Golfo Dulce endemic, *H. p. xanthos*.

MATERIALS AND METHODS

Morphological data and analysis

From 2017 to 2024, we examined 124 yellow sea snakes from the inner basin of Golfo Dulce, including 93 free-ranging individuals briefly captured by net from a boat and 31 preserved specimens at the Zoological Museum of University of Costa Rica (UCR 20612, 20614–16, 20618–19, 20648–49, 20677, 20691, 20817–18, 20836–37, 20840, 21575, 21577, 21881, 21883, 21886, 21889, 21970, 21975–76, 21978, and six specimens yet to be catalogued). Following Bessesen and Galbreath (2017), we recorded measurements of weight, girth (circumference at thickest point), total length (TL; using the string technique), tail length (against a measuring stick), and paddle height (using callipers). We removed six particularly small (<38 cm TL) specimens from analyses of weight, girth, length, and tail dimensions to avoid possible age-related statistical bias. Because formalin and ethanol are known to cause dehydration in preserved squamate specimens (Vervust *et al.* 2009), we focused only on live weights for analysis. For cephalic scale counts (preoculars, postoculars, anterior temporals, supralabials, and infralabials), we followed Smith (1926). We counted ventral scales (atlas–axis to cloaca, excluding vent shield) and subcaudal scales (vent to tip) according to Dowling (1951). Following Rasmussen *et al.* (2014), scale rows (not including ventral scales) were counted one to four times around the neck (narrowest point), around the midbody (thickest point), and vertically across the flattened mid-paddle (unilaterally). On live snakes, scales were counted using high-resolution photography and using a system of red marks arranged on the skin *in situ*.

For comparison, we examined a total of 229 pelagic sea snakes from outside Golfo Dulce. Of those, 25 were live snakes captured and released following measurements of weight and TL. The remaining pelagic specimens came from institutional collections, including the Field Museum of Natural History (FMNH 9774–75, 16736, 16923–26, 41590, 69768, 79982–85, 97693, 105089, 140155, 140157, 142966, 154857, 154862, 154864–65, 154869, 154872–73, 154886–87, 163200, 163213, 165284, 171579–87, 171589–602, 171604–09, 171611–12, 171614–27, 171629–41, 171643–49, 171651–64, 171666–73, 171675–87, 171689–704, and 213669), Australian Museum (AMS 314, 1604, 3154, 3187, 3291, 3791, 3828, 4164, 4283, 6750, 7032, 8944, 8979, 9270, 9316, 10502, 13139, 13766, 13811, 15028, 16862, 19101, 44530, 45813, 92314, 107164, 178108, 178305, 188315–20, 202225–30, 202301, 202878, and one uncatalogued specimen), University of Colorado Museum of Natural History (UCM 58903–58907 and 58909), Natural History Museum of Denmark (ZMK R66143), Arizona State University Natural History Collections (ASUHEC 2617 and 29264), and two uncatalogued specimens preserved at Osa Conservation's Piro Research Station in Costa Rica. A majority of pelagic specimens ($N = 135$) were collected in Costa Rica; of those, 67% ($N = 90$) were found near the mouth of Golfo Dulce, making them proximate neighbours to *H. p. xanthos*. As with the yellow sea snakes, specimens <38 cm TL ($N = 39$) were removed from size-related analyses.

Finally, we examined seven 1970s specimens, all exhibiting xanthic coloration, from FMNH (171603), UCM (58900–02 and 58907), American Museum of Natural History (AMNH 106682), and United States National Museum of Natural History (USNM 192279). For a few of the 1970s specimens ($N = 4$) and pelagic sea snakes ($N = 4$), we examined rib/vertebrae counts and heart placement using radiographic techniques (Rasmussen 1989). All except one 1970s specimen had a metal pin marking the location of the heart, which allowed counts from atlas to heart to determine heart placement along the vertebral column; we also counted caudal vertebrae.

Descriptive statistics were performed in R v.4.2.1 (R Core Team 2022). We compared continuous variables between populations using Welch's two sample *t*-tests (t.test function in base R), and

frequencies of categorical variables (i.e. having one or more supralabials in contact with the ocular orbit) using Pearson's χ^2 tests (chisq.test function in base R) with a Yates' continuity correction. To control the increased familywise error rate caused by multiple comparisons, we applied a false discovery rate (FDR) correction (Benjamini and Hochberg 1995, Pike 2011).

Molecular data and analysis

In 2023, we collected tissue samples (tail biopsies) from 50 yellow sea snakes and 25 pelagic sea snakes briefly captured from adjacent populations in the inner basin and immediately outside Golfo Dulce, respectively. Collection from yellow sea snakes was limited to the densest 34 km^2 portion of their range, whereas pelagic sea snake collection occurred within 79 km^2; there was a spatial gap of 33 km between collection areas. Samples were stored in 95% ethanol. We extracted DNA following the DNeasy Blood and Tissue Kit (Qiagen) protocol and quantified the extracts using a Quantus fluorometer to obtain >500 ng of DNA at concentrations of 50 ng/mL. Samples were concentrated using a Centrivap DNA concentrator, if required, and checked for quality using gel electrophoresis. The DNA extractions for each of the two sequencing processes described below were done separately but with identical protocols.

First, we sought to capture single-nucleotide polymorphisms (SNPs), which are biological markers commonly used to detect genetic variation between individuals and populations. For this line of testing, we aggregated genetic samples for 27 yellow and 21 pelagic sea snakes from Costa Rica with 15 additional pelagic specimens obtained at various locations across the Indo-West Pacific (IWP; which ranges from Sri Lanka to the East Coast of Australia) and the United Arab Emirates (UAE; Supporting Information, Table S1). The samples were sent to Diversity Arrays Technology Pty Ltd (Canberra, ACT, Australia), where SNP genotyping was conducted using a proprietary genome complexity reduction pipeline with a pair of restriction enzymes (PstI and HpaII; Kilian *et al.* 2012, Georges *et al.* 2018). After initial digestion/ligation reactions and amplification, samples were sequenced on an Illumina HiSeq 2500. One-third of samples were sequenced a second time for use as technical replicates. We obtained raw demultiplexed reads from DArTseq, which we checked for quality using FastQC (Andrews 2010), then filtered out adapters and quality trimmed the reads using BBduk (Bushnell 2014). We filtered out potential microbial and human contamination using Kraken2 (Lu *et al.* 2022). We then assembled loci and called SNPs using ipyrad v.0.9.85 (Eaton and Overcast 2020) run on the University of Adelaide Phoenix HPC. Filtered and demultiplexed reads were assembled *de novo*, setting the cluster threshold to .90, mindepth (statistical and majority rule) to five and maxdepth to 10000. We retained one SNP per locus to reduce the effects of linkage, using the --thin command in VCFtools (Danecek *et al.* 2011).

We used two datasets for SNP population genetic analyses: Dataset 1 included 54 samples from across the full range of the study species *H. platurus*, with no missing loci ($N = 498$), whereas Dataset 2 included 43 samples from the adjacent Costa Rican populations, with 614 unlinked loci. We generated principal component analysis plots using the SNP data for each grouping separately. Using the HIERFSTAT package (Goudet 2005) in R, we estimated observed (H_o) and expected (H_e) heterozygosity and *F*-statistics (F_{IS}, the inbreeding coefficient; and F_{ST}, the proportion of differentiation attributable to genetic structure). We also estimated population ancestry coefficients using the program sNMF (Frichot *et al.* 2014) implemented in the R package LEA v.3.5.0 (Frichot and François 2015). We tested various combinations to find the best regularization (ε) and tolerance (α) values, settling on .0001 and 100, respectively, which minimized cross-entropy values. To find the optimal number of populations (K) we ran 100 repetitions from $K = 1$ to $K = 5$ to obtain the optimal value of K with the lowest cross-entropy value.

As a secondary approach, we undertook target sequence capture of squamate conserved loci (SqCL), which uses bait sequences to target particular genomic regions. For these analyses, samples for 15 yellow and 9 pelagic sea snakes from Costa Rica (Supporting Information, Table S1) underwent library preparation for sequence capture at Daicel Arbor Biosciences (Ann Arbor, MI, USA). Samples were optimized for capture using the SqCL v.2 probe set (Singhal *et al.* 2017), which targets ultra-conserved elements (UCEs; Faircloth *et al.* 2012), anchored hybrid enrichment (AHE; Lemmon *et al.* 2012), and other traditional squamate loci (Singhal *et al.* 2017). Sequencing was conducted on an Illumina Hi-Seq platform. For outgroups, we downloaded raw SqCL sequences from the National Center for Biological Information (NCBI) Sequence Read Archive (dataset: Hills and Singhal 2023) via the SRA toolkit v.3.1.0: *Hydrophis kingii* Boulenger, 1896 (SRR23022445), *Hydrophis macdowelli* Kharin, 1983 (SRR23022444), *Aipysurus duboisii* Bavay, 1869 (SRR23022465), *Emydocephalus annulatus* Krefft, 1869 (SRR230224499), and *Laticauda colubrina* Schneider, 1799 (SRR23022443). After raw reads were cleaned using illumiprocessor (Faircloth 2013) and trimmed with trimmomatic (Bolger *et al.* 2014), contigs were assembled using SPAdes (Prjibelski *et al.* 2020) with default parameters. We then matched our contigs to the SqCL v.2 probe set (Singhal *et al.* 2017) and aligned them using MAFFT v.1.5.0 (Katoh *et al.* 2002). We processed the data in PHYLUCE v.1.7.3 (Faircloth 2016) run on the Field Museum Grainger Bioinformatics Center Phoebe HPC. A concatenated file with the data matrix at 95% completeness was used to run a maximum likelihood analysis and estimate a gene tree in IQ-TREE v.2 (Minh *et al.* 2020); the best model fit was TVM+F+I+R10, as determined by the ModelFinder method (Kalyaanamoorthy *et al.* 2017). The PHYLUCE pipeline was then rerun with only the populations adjacent in Costa Rica to make a species-specific network in SplitsTree4 v.4.19.2 (Huson and Bryant 2006).

Finally, because the faster mutation rates in mtDNA could potentially allow a stronger signal of population structure to be recovered, we sought to capture mtDNA from our nuclear sequences. To mine the mtDNA, we used Geneious Prime v.2024.0.3 (https://www.geneious.com) with default settings. We imported raw paired (forward and reverse) fastq target-capture reads and trimmed those reads using BBduk v.38.84 (Bushnell 2014). We then imported our raw trimmed DArTseq reads, including technical replicates. From the NCBI GenBank database, we downloaded a complete mitogenome for *H. platurus* (MK775530 from South Korea, 18101 bp; Kim *et al.* 2020) and, setting it as the reference genome in the 'Map to Reference' tool in Geneious, we mapped each sample independently. For any snake that was sequenced for both DArTseq and target capture and/or had replicate DArTseq sequences, we aligned those and generated an individual consensus

sequence (with the 'Highest Quality' setting). Four specimens were removed from further analysis owing to insufficient data, resulting in a final mtDNA dataset of 33 yellow and 20 pelagic sea snakes (Supporting Information, Table S1). Using MAFFT in the Multiple Align tool (default settings), the mapped sequences were then aligned with the reference genome, in addition to several other complete sea snake mitochondrial genomes downloaded from NCBI GenBank to serve as outgroups: *Hydrophis curtus* Shaw, 1802 (MT712129; Zhang and Yan 2020), *Hydrophis melanocephalus* Gray, 1849 (MK775532; Yi *et al.* 2020), *Hydrophis ornatus* Gray, 1849 (NC_066233; Xiaokaiti *et al.* 2022), *Aipysurus eydouxii* Gray, 1849 (NC_062614; NCBI Genome Project), *Emydocephalus ijimae* Stejneger, 1898 (MK775531, Yi *et al.* 2019), and *Laticauda colubrina* (NC_036054; NCBI Genome Project). A gene tree was generated using RAxML v.8 (Stamatakis 2014) and a GTR GAMMA model with bootstrapping ($N = 500$; random seed = 1) and rooted with *Laticauda colubrina*.

RESULTS

Morphology

All 124 yellow sea snakes from the inner basin of Golfo Dulce are predominantly yellow. Signs of melanin are not entirely absent, however, because most have one or more tiny black dots or specks located on the body or head, often near the supraocular and/or parietal scales (Fig. 2A). Only 14% of examined specimens have black marks >1 cm on the body, including some with narrow dorsal dashes (Fig. 2B) and one with a thin mottled stripe. None has a solid black dorsum or black bands across the tail paddle. Irises are consistently light in colour, usually pale grey green (Fig. 2C). Descriptive measurements of the yellow sea snake (Table 1) are recorded as follows: live weight, 22–90 g (mean = 45.5 g); girth, 4–6.5 cm (mean = 5.2 cm); TL, 39–59.3 cm (mean = 49.5 cm); snout–vent length, 34.25–53.3 cm (mean = 44.1 cm); tail length, 4.25–7 cm (mean = 5.5 cm); and paddle height, .8–1.4 cm (mean = .98 cm). Cephalic scales (Fig. 2D–F) are represented by

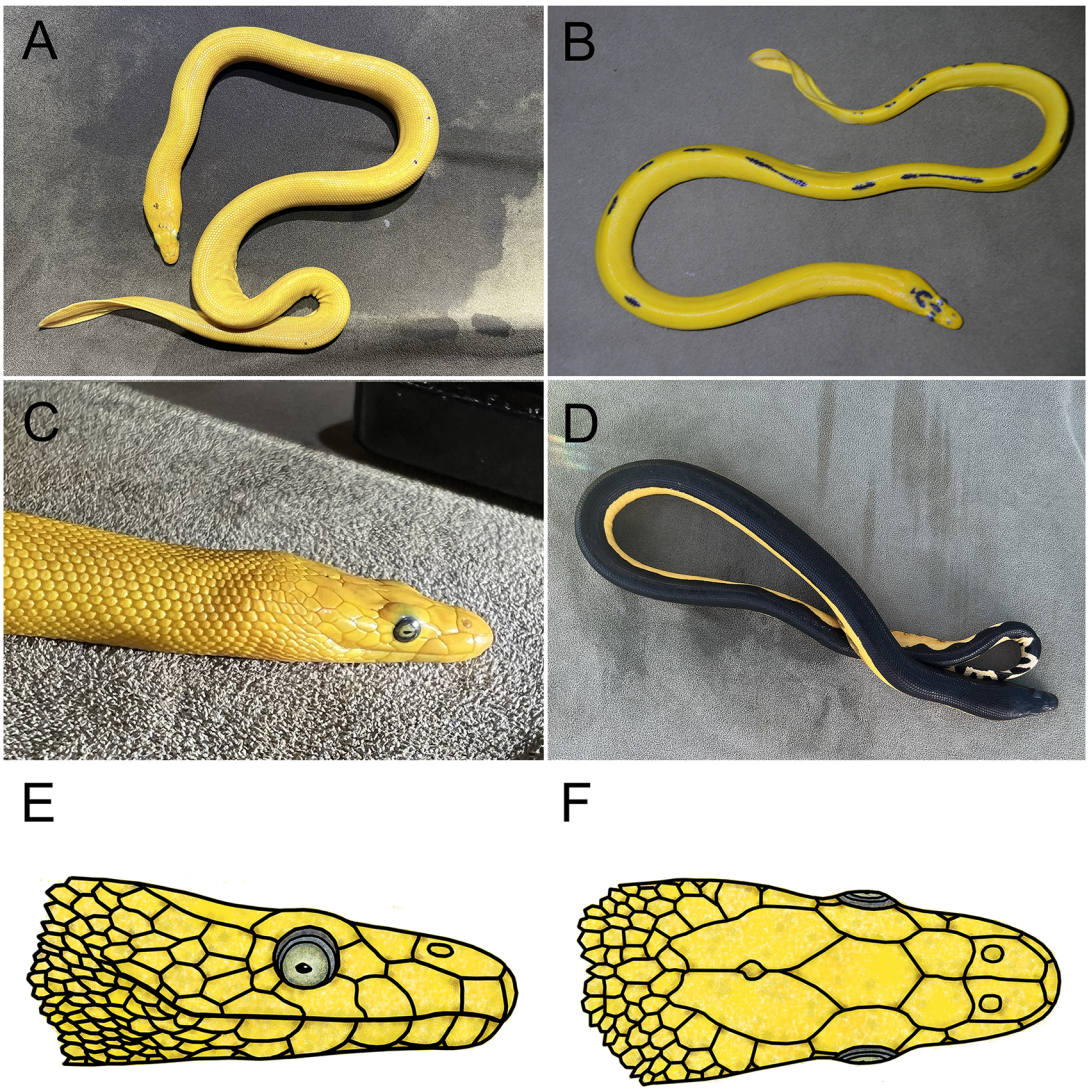

Figure 2. A–C, diagnosable xanthic coloration of *Hydrophis platurus xanthos*: predominantly yellow with a few black dots (A); more rarely, with dashed dorsal lines or thin strip (B); and light iris (C). D, typical coloration of *Hydrophis platurus platurus*, with solid black dorsum and lateral tail markings. E, F, illustrated cephalic scalation of *H. p. xanthos* in lateral view (E; note lack of subocular scale creates labial-to-orbit contact) and dorsal view (F). Photographs and illustrations: B. Bessesen.

Table 1. Morphological characters for *Hydrophis platurus xanthos* and test results of comparisons with the 1970s specimens (yellow sea snakes collected from the Eastern Tropical Pacific Ocean) and *Hydrophis platurus platurus*.

			Compared with									
	H. p. xanthos		1970s specimens					*H. p. platurus*				
Character	***N***	**Mean ± SD**	***N***	**Mean ± SD**	***t*-value**	***P*-value**	***q*-value**	***N***	**Mean ± SD**	***t*-value**	***P*-value**	***q*-value**
Live weight (g)	91	45.3 ± 13.1	NA	NA	NA	NA	NA	24	75.7 ± 29.7	−4.9	**<.001**	**<.001**
Girth (cm)	51	5.2 ± .8	5	4.1 ± .4	5.2	**.001**	**.021**	87	8.7 ± 2.5	−12.5	**<.001**	**<.001**
SVL (cm)	117	44.1 ± 3.9	5	41.9 ± 7.2	.7	.547	.744	107	62.2 ± 17.2	−10.7	**<.001**	**<.001**
TL (cm)	118	49.5 ± 4.3	5	46.8 ± 7.9	.8	.476	.744	188	63.8 ± 17.1	−10.9	**<.001**	**<.001**
Tail length (cm)	117	5.4 ± .6	5	4.9 ± .8	1.4	.169	.608	107	7.2 ± 1.8	−9.8	**<.001**	**<.001**
Tail/TL ratio	117	.11 ± .01	5	.10 ± .01	.8	.296	.707	107	.11 ± .09	−.4	.701	.791
Paddle height (cm)	62	.98 ± .1	5	.92 ± .1	1.2	.300	.707	109	1.6 ± 1.8	−12.4	**<.001**	**<.001**
Paddle aspect ratio	62	.18 ± .02	5	.19 ± .05	−.6	.579	.744	106	.23 ± .19	−2.9	**.004**	**.008**
Preoculars	49	1.0 ± .1	6	1.0 ± .0	.6	.569	.744	97	1.2 ± .4	−3.9	**<.001**	**<.001**
Postoculars	48	1.9 ± .3	6	2 ± .0	−1.5	.128	.608	96	1.9 ± .3	−.4	.712	.791
Anterior temporals	50	2.6 ± .5	6	2.9 ± .5	−1.7	.142	.608	78	2.6 ± .4	−.8	.418	.597
Supralabials	49	8.4 ± .6	6	8.3 ± .8	.2	.858	.858	94	8.4 ± 1.4	−.3	.769	.809
Infralabials	48	11.0 ± .6	6	10.8 ± .5	1.1	.314	.707	97	11.0 ± .7	−.5	.605	.756
Neck scale rows	43	42.7 ± 3.0	7	38.4 ± 2.9	3.6	**.007**	.061	38	42.4 ± 3.1	−.7	.479	.639
Midbody scale rows	42	52.1 ± 3.3	7	51.1 ± 5.1	.5	.634	.761	36	54.0 ± 4.6	−2.0	.053	.082
Paddle scales	17	12.4 ± .5	7	12.1 ± .7	.7	.483	.744	76	13.4 ± 1.0	−6.2	**<.001**	**<.001**
Ventrals	27	314.4 ± 29.2	7	310.4 ± 40	.2	.813	.858	27	314.6 ± 57.6	−.0	.988	.988
Subcaudals	26	45.1 ± 3.4	7	46.7 ± 5.3	−.8	.475	.744	67	46.8 ± 4.2	−2.0	**.050**[*]	.082
Xanthic coloration	124	100%	7	100%	NA	NA	NA	229	0%	$\chi^2 = 348.6$	**<.001**	**<.001**
Labial touches orbit	57	73%	7	86%	$\chi^2 = 0.5$	.675	.858	194	51%	$\chi^2 = 5.5$	**.004**	**.008**

Results of the correction (Benjamini and Hochberg 1995, Pike 2011) are presented as FDR-adjusted *P*-values (*q*-values); statistically significant results are bolded. Xanthic coloration is predominantly yellow, lacking a solid black dorsum; additional sources of morphological data for *H. p. platurus* can be found in the Supporting Information (Table S2). Abbreviations: FDR, false discovery rate; *N*, number of specimens; SVL, snout–vent length; TL, total length; NA, not available/applicable.
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2 nasal shields (touching; no internasals); 2 prefrontals (no loreals); 1 frontal; 2 parietals; unilaterally, preoculars, 0–2 (mode = 1); postoculars, 1–3 (mode = 2); anterior temporals, 2–3 (rarely 4); supralabials, 7–10 (mode = 8); infralabials, 10–13 (mode = 11, with the first 5 larger in size); and 2 anterior sublinguals (separated by small scales). In 73% of specimens, a supralabial (usually the fourth, occasionally the fifth, and rarely both) touched the ocular orbit. For body scales, we counted around the neck, 36–47 (mean = 42.7); around the midbody, 45–59 (mean = 52.1); vertical paddle, 11–13 (mode = 12); ventrals, 245–383 (mean = 314.4); and subcaudals, 38–53 (mean = 45.1).

The pale integument and light iris of the yellow sea snake contrasts with the solid black dorsum and dark eye of the pelagic sea snake (Fig. 2D). Yellow sea snakes also consistently lack the black spots or bands on the lateral tail paddle that are seen in pelagic sea snakes. Even taking a conservative approach for multiple comparison tests by applying an FDR correction, several morphological characters differ significantly between the two groups (Table 1; Supporting Information, Table S2). Overall, yellow sea snakes are smaller than pelagic sea snakes, as demonstrated by reductions in live weight, TL, tail length, and paddle height. Paddle aspect ratio (as tail height/length) indicates that the tail of the yellow sea snake is narrower, and we also find fewer paddle scale row counts. Cephalic scalation shows additional significant differences: yellow sea snakes are more likely than pelagic sea snakes to have one preocular scale rather than two and exhibit a higher frequency of labial-to-orbit contact (Fig. 2E).

Comparisons between contemporary yellow sea snakes and the 1970s specimens captured off-shore show near-perfect alignment (Table 1). After FDR correction, the only character showing significant difference is girth. All the 1970s specimens are within the recorded size range for the yellow sea snake, with even a higher percentage (86%) possessing at least one labial scale in contact with an eye.

We did not obtain rib counts for any yellow sea snakes; however, radiographs of four 1970s specimens and four pelagic sea snakes allowed counts of body ribs (1970s = 146–152, pelagic = 139–155), caudal vertebrae (1970s = 32–34, pelagic = 29–33) total counts (body–caudal; 1970s = 180–185, pelagic = 168–185), and atlas-to-heart counts (1970s = 44–47, pelagic = 39–47). Although minimum counts are consistently lower in the pelagic specimens, our sample size is small, and all counts overlap; additional study is needed to clarify whether genuine variations in rib/vertebrae counts can be linked with colour. Half ($N = 2$) of our radiographed pelagic sea snakes were gravid, which might have influenced heart position. One (ZMK R66143)

carries six embryotic offspring. The other (FMNH 165213) shows two fetuses no longer in well-formed embryonic sacs with one positioned more elongate, head pointed towards the caudal end of the mother's body, suggesting that it might have been moving through the oviduct; if so, it is possible that additional neonates were released prior to capture or preservation.

Genetics

For both SNP Dataset 1 (samples from across the full range of the study species) and SNP Dataset 2 (adjacent populations in Costa Rica), principal component analyses unexpectedly fail to show clustering between geographical regions or between the yellow and pelagic populations (Fig. 3A, B). Regional groups show similar patterns of within-population variation (H_o, H_e, and F_{IS}; Tables 2 and 3), and F_{ST} is close to zero across the species range (Table 4) and within the adjacent Costa Rican populations (−0.0006459). For both datasets, the best cross-entropy criterion from sNMF was obtained at $K = 1$ [0.202 and 0.237 for Dataset 1 (Fig. 4A) and Dataset 2 (Supporting Information, Fig. S1A), respectively], suggesting that all samples were best characterized as belonging to a single population. When testing higher values of K, ancestry coefficients did not correspond to any obvious geographical or taxonomic structuring for either Dataset 1 (Supporting Information, Fig. S1B–F) or Dataset 2 (Fig. 4B–D; Supporting Information, Fig. S2).

Likewise, when examining the SqCL sequence data, we find no evidence of genetic structure between the adjacent but geographically separated yellow and pelagic sea snakes. In the resulting gene tree, individuals from both populations are interspersed (Fig. 3C), and SplitsTree generates a starburst pattern of near-equal distance between all specimens (Fig. 3D). SqCL targets conserved loci that are unlikely to capture shallow or interspecific variation, for which mitochondrial data might be better suited. Our analysis of the mined mtDNA, however, returns similar results, with a gene tree suggesting a single, intermixed population (Fig. 3E). We note that the amount of mtDNA mapped from our SNPs and SqCL sequences to the *H. platurus* reference mitogenome was limited based on visual inspection and the Geneious statistics comparing the sequences within our final alignment (pairwise identity = 13.7%; identical sites = 4.4%).

DISCUSSION

We compared morphological and molecular markers of the yellow sea snake (*H. p. xanthos*) residing inside Golfo Dulce against the pelagic sea snake (*H. p. platurus*) ranging across the Indo-Pacific Ocean, with the expectation of describing a new species. As evidence of geographical isolation, comparative morphology illuminated multiple differences between the populations: not only does the yellow sea snake exhibit xanthic coloration with light irises and a reduction in all body size measurements, including live weight, but it also shows statistically significant shifts in tail morphology (paddle shape as aspect ratio and paddle scale count), preocular count, and the frequency of labial scales touching an eye.

Paradoxically, molecular analyses using SNPs, target-capture loci, and mined mtDNA failed to reveal genetic differentiation between populations. This is curious, because fine-scale structure within a single snake species can usually be detected across relatively short distances even without morphological differentiation (Marshall *et al.* 2009, Pernetta *et al.* 2011, Meister *et al.* 2012). Our results suggest intense selection for certain morphological features inside Golfo Dulce and/or an evolutionary split too recent to identify. Although our molecular data do not support the hypothesis of a separate species, this study raises important questions about the complexities of the evolutionary process.

As a species, *H. platurus* is known to be polymorphic. Smith (1926) published seven colour forms, all versions of dark above and light below (see Supporting Information, Fig. S3). In and near the Persian Gulf, some specimens appear whitish yellow, with a light brown dorsum and pale paddle markings (iNaturalist 2024; J. Crowe-Riddell, pers. comm.). However, unlike the mixed phenotypes seen elsewhere, the yellow sea snakes have 100% conformity in xanthic coloration. They are also consistently smaller in body size, and the aspect ratio of the tail suggests that it is narrower and has fewer scale rows. Perhaps bound to the relatively calm subsurface waters of the inner basin, the snakes in Golfo Dulce do not require as much paddle power as those diving the open ocean. The yellow sea snakes are also significantly more likely than their pelagic counterparts to have one prefrontal scale vs. two and at least one supralabial in contact with the ocular orbit (also see Tu 1976). Scale count variability and clinal changes between geographical areas are common in squamates (Dohm and Garland 1993), and it is unknown whether these cephalic traits have been increasing through selection owing to reduced space on a smaller animal or whether they are simply phenotypic variance derived through genetic drift. Either way, these characters might be moving towards fixation in the yellow population.

Our morphological data provide evidence that the 1970s specimens captured off Central America were likely to have originated from inside Golfo Dulce, because they aligned closely with the yellow sea snake. One of the 1970s specimens was found ~500 km from Golfo Dulce, in the Gulf of Panama (Kropach 1971), but no yellow sea snakes have been reported beyond Central America. The survival rate of yellow sea snakes that transition to open ocean, where waters are colder and contain higher salt content, is unknown. Although our sample size was small, a reduction in average girth among our 1970s specimens might suggest weight loss (McCue *et al.* 2012), possibly owing to reduced feeding in an unfamiliar environment.

Owing to the bathymetry of Golfo Dulce and its fiord-like characteristics (Svendsen *et al.* 2006), the potential for frequent contact between adjacent yellow and pelagic sea snake populations of Costa Rica is low. They appear separated by a >20 km spatial gap centred over shallow waters (mostly 10–30 m deep; Bessesen 2022), with a complicated current structure (Svendsen *et al.* 2006, Morales-Ramírez *et al.* 2015). Among nearly 900 recorded observations of Golfo Dulce yellow sea snakes over a 15 year period, only one occurred in that shallow zone (Bessesen 2015). These snakes being weak swimmers that spend their time diving and floating without active horizontal movements (Kropach 1973, Graham *et al.* 1987, Rubinoff *et al.* 1988) further reduces the likelihood of crossing such a sizeable space. We know yellow sea snakes are sometimes swept out of the embayment (Bessesen 2015, 2022), and pelagic sea snakes may occasionally wash in

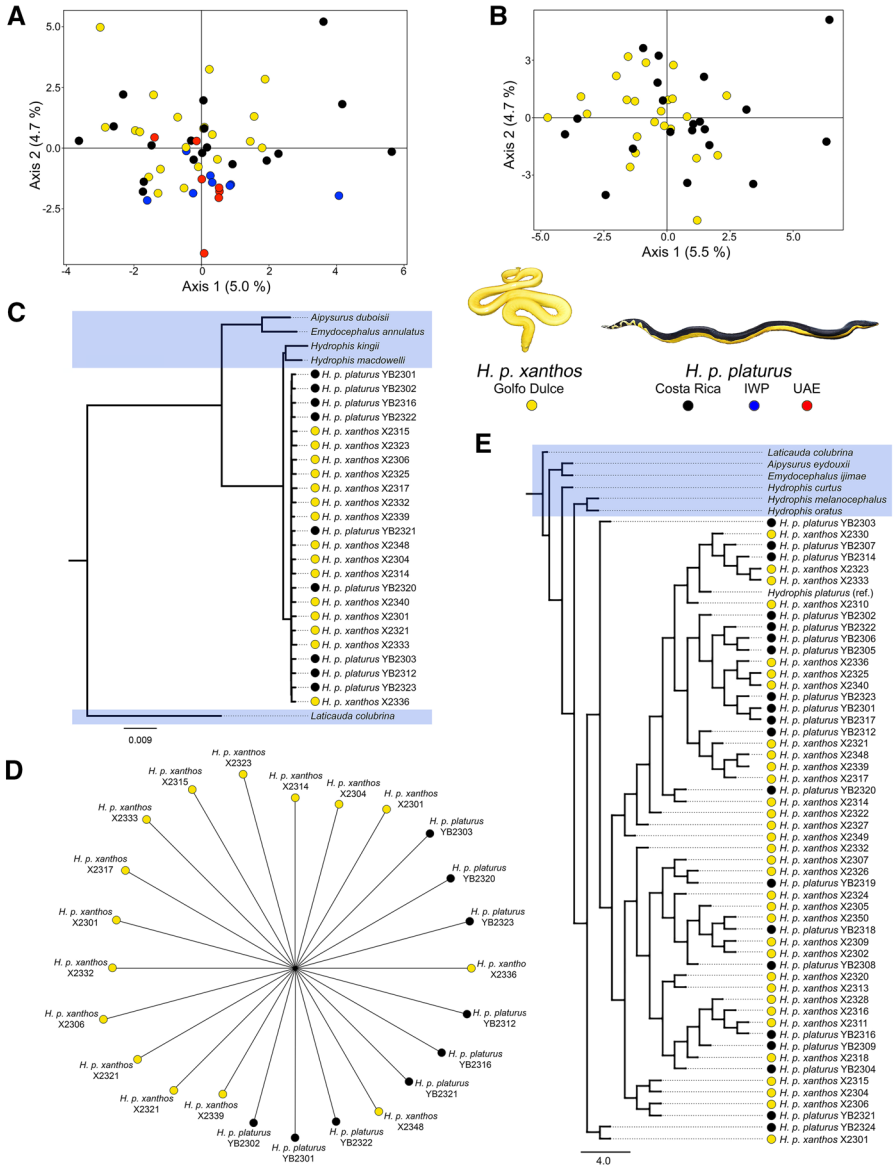

Figure 3. Genetic analyses comparing *Hydrophis platurus xanthos* and *Hydrophis platurus platurus*. A, principal component analysis for single-nucleotide polymorphism samples from across the full range of the species (no missing loci) divided as *H. p. platurus* from Costa Rica, Indo-West Pacific (IWP), and United Arab Emirates (UAE), and *H. p. xanthos* (Golfo Dulce). B, principal component analysis of Costa Rican single-nucleotide polymorphism samples. C, IQ-TREE (model = TVM+F+I+R10) of squamate conserved loci (SqCL) sequences, including outgroups (shaded in blue), at a 95% matrix. D, SplitsTree network of SqCL sequences from Costa Rican samples of *H. p. xanthos* ($N = 15$) and *H. p. platurus* ($N = 9$) at a 95% matrix. E, RAxML tree with bootstrapping ($N = 500$; random seed = 1) of mapped mitogenomes of *H. p. xanthos* ($N = 33$) and *H. p. platurus* ($N = 20$), plus the reference *H. platurus* and outgroups (shaded in blue).

(Bessesen and Galbreath 2017, Solórzano and Sasa 2024). However, the latter do not appear to survive long term, as evinced by an absence of typical black-and-yellow individuals in the inner basin (Solórzano 2011, Bessesen 2015, Lillywhite *et al.* 2015, Bessesen 2022). The effects of elevated thermal conditions might offer insight. Surface temperatures in Golfo Dulce reach at least 32.5°C (Rincón-Alejos and Ballestero-Sakson 2015, Bessesen *et al.* 2023). In laboratory experiments, every pelagic sea snake held in water heated to 32°C stayed at the bottom of the tank (Graham *et al.* 1971), and in waters ≥33°C, none survived longer than 2 days (Dunson and Ehlert 1971). Solórzano and Sasa (2024) published a photograph of an interbreeding event in the inner basin of Golfo Dulce, but because the coupling is reported to be a male yellow sea snake and female pelagic, reproductive success is unlikely given the improbable odds that she could withstand the environment to parturition. Successful interbreeding within the distribution area of the yellow sea snake, where thermal conditions and other hydrological characteristics appear unsuitable to 'outsiders' (Bessesen 2022, Bessesen *et al.* 2023), is likely to require a male pelagic sea snake carried in on a rogue wave or current to mate with a female yellow sea snake adapted to survive there for her gestation period of 6–8 months (Savage 2002).

Population structure has been studied in sea snakes (Lukoschek and Shine 2012, Sheehy *et al.* 2012, Bech *et al.* 2016, Nitschke *et*

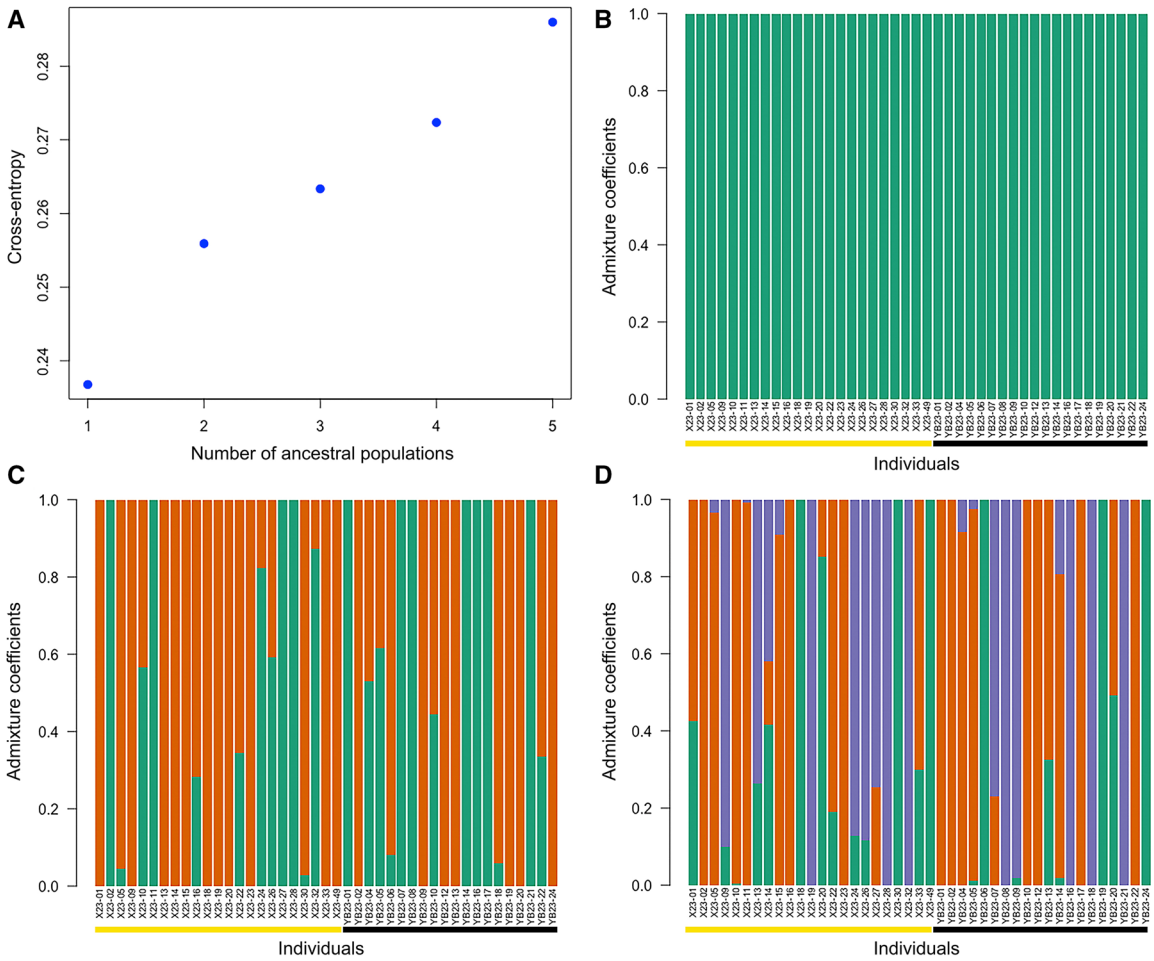

Figure 4. A, plot of minimum cross-entropy from $K = 1$ to $K = 5$ from 100 repetitions for single-nucleotide polymorphism Dataset 2 (adjacent sea snake populations in Costa Rica) using masked values generated using sNMF. B–D, ancestry coefficient plots for *Hydrophis platurus xanthos* (individuals labelled X; yellow bar) and *Hydrophis platurus platurus* (individuals labelled YB; black bar) from $K = 1$ to $K = 3$, respectively, estimated using sNMF.

Table 2. Population genetic metrics for samples (number in parentheses) with no missing loci from across the full range of the species, including *Hydrophis platurus platurus* from Costa Rica, Indo-West Pacific, and United Arab Emirates, and *Hydrophis platurus xanthos* from inside Golfo Dulce.

Population	H_o	H_e	F_{IS}
Costa Rica (18)	.0497	.0582	.0835
Indo-West Pacific (8)	.059	.0624	.035
United Arab Emirates (7)	.0476	.0567	.0875
H. p. xanthos (21)	.0517	.0574	.0636

Abbreviations: F_{IS}, inbreeding coefficient; H_e, expected heterozygosity; H_o, heterozygosity.

Table 3. Population genetic metrics for samples from the Costa Rican population of *Hydrophis platurus platurus* and *Hydrophis platurus xanthos* from inside Golfo Dulce.

Population	H_o	H_e	F_{IS}
H. p. platurus (20)	.0614	.0736	.062
H. p. xanthos (23)	.0621	.0688	.0979

Abbreviations: F_{IS}, inbreeding coefficient; H_e, expected heterozygosity; H_o, heterozygosity.

al. 2018, Ludington and Sanders 2021). Notably, the *Aipysurus–Emydocephalus* clade showed defined geographical genetic patterns, including intraspecific splits, whereas the *Hydrophis* clade showed weak population differentiation, suggesting that rapid distribution and speciation might have reduced phylogenetic signal across their range (Nitschke *et al.* 2018). The DArTseq methods used in our study have, nevertheless, detected population structure in >12 other *Hydrophis* species, even over small geographical distances (author J.H. Nankivell, pers. comm.), hence it is curious that we failed to find any clear structure across the near-global pelagic sea snake. The species reportedly diverged 5–7 Mya, spreading across oceans from its Indo-Australian origins (Lee *et al.* 2016). Although it seems to have reached the Americas after the formation of the Isthmus of Panama obstructed access to the Atlantic Ocean (Lillywhite *et al.* 2018), as recently as 2.8 Mya (O'Dea *et al.* 2016), a much more recent arrival would help to explain the low genetic variation between eastern and western sides of the Pacific, with some level of continued gene flow across

Table 4. The *F*-statistic F_{ST} (allele variation within relative to between subpopulations) for samples with no missing loci from across the full range of the species, including *Hydrophis platurus platurus* from Costa Rica, Indo-West Pacific, and United Arab Emirates, and *Hydrophis platurus xanthos* from inside Golfo Dulce.

	CR	IWP	UAE
IWP	.006698		
UAE	.010871	.004178	
H. p. xanthos	.000715	.012546	.011157

Abbreviations: CR, Costa Rica; IWP, Indo-West Pacific; UAE, United Arab Emirates.

oceans. Population structure can be reduced or lacking in marine fauna that live a pelagic lifestyle (Pfaller *et al.* 2019) and/or form exceedingly large and far-ranging populations (Palumbi 1994, Anderson *et al.* 2020). Nevertheless, panmixia across oceans should be interpreted with caution, because samples rarely cover the full range of the taxa, making subtle genetic differentiation challenging to detect, especially within recently diverging populations (Grosberg and Cunningham 2001).

We also found no genetic differentiation between the yellow and pelagic populations. Although our mined mtDNA sequences were incomplete, they were in accord with a mitochondrially based phylogeography study by Sheehy *et al.* (2012) and further supported by SNP and SqCL data. Reduced gene flow is often associated with geographical barriers (Gruber *et al.* 2013), and there is a sizeable shallow zone separating the yellow and pelagic sea snake populations (Bessesen 2022). Moreover, the inner basin of Golfo Dulce might have been fully cut off from the Pacific Ocean during some period of its geological history (T. Garner, pers. comm.). By 125 kya, the Osa landmass was being uplifted by subduction, forming an unbroken peninsula (Gardner *et al.* 2013) and potentially capturing a group of pelagic sea snakes within the boundaries of Golfo Dulce. During the Last Glacial Maximum, nominal eustatic curves suggest that sea levels dropped <130 m (Lambeck *et al.* 2014). Given that present-day Golfo Dulce has a sill at 60 m depth and outer basin generally <30 m deep (the shallow zone that today separates the yellow from the pelagic sea snake; Bessesen 2022), it is conceivable that low sea levels isolated the deep inner basin of Golfo Dulce for tens of thousands of years (T. Gardner, pers. comm.). The enclosed basin, essentially a brackish lake, would have offered a considerably different habitat from the adjacent Pacific, with the potential to accelerate differentiation. When sea levels rose, allowing for sporadic interbreeding events, a recombination of genes might have removed evidence of an earlier, more complete separation. However, even with admixture and recombination we would expect some sign of population structure. An alternative hypothesis provides for a weather event or anomalous wave having swept tens to thousands of pelagic sea snakes into the inner basin, but the founder effect should have produced a genetic signature of rapid population growth typically following a range expansion.

Although undetected in our analyses, a modest number of nuclear genes related to colour, size, tail shape, and/or scalation may have changed (Dohm and Garland 1993, Karsenty and Wagner 2002, Aubret 2015, Ullate-Agote *et al.* 2020). While the colour of a snake can be determined by a single gene mutation (Ullate-Agote *et al.* 2020), body size is more complex, generally driven by multiple genes, in addition to gene regulators and phenotypic plasticity associated with feeding and/or environmental factors (Karsenty and Wagner 2002, Aubret 2015). The anatomy and physiology of the yellow sea snake could also be influenced by feeding at night (Bessesen and González-Suárez 2022) and inhabiting waters with elevated temperatures, lower salinity, and limited dissolved oxygen compared with the open Pacific Ocean (Bessesen *et al.* 2023). Epigenetic gene regulation is known to influence body plan diversification in reptiles (Martín-del-Campo *et al.* 2019), and snakes are well known for their phenotypic plasticity (Aubret *et al.* 2004). The New Caledonia sea krait (*Laticauda saintgironsi*) presents clinal phenotypic variation across known colonies with differing habitats but with no apparent genetic variation (Bech *et al.* 2016). Likewise, Shine *et al.* (2012) found considerable phenotypic differences between two colonies of turtle-headed sea snakes (*Emydocephalus annulatus*) inhabiting adjacent bays. Forsman (2015) argues that irreversible developmental plasticity should be considered within the framework of quantitative genetics because it is fundamentally similar to gene expression and includes genetic components. Having demonstrated developmental plasticity in sea snakes, Bonnet *et al.* (2021) made the point that genetic homogeneity between spatially defined phenotypes does not negate the possibility of speciation but rather supports the idea of plasticity as a mechanism to facilitate speciation through the establishment of distinct, environmentally influenced subpopulations.

CONCLUSION

To elucidate the genetic underpinnings of the morphological differences seen between the yellow and pelagic sea snakes better, whole-genomic based work might prove useful (Nater *et al.* 2015, Streicher and Ruane 2018, Card *et al.* 2023), in addition to the examination of particular genes for which selection could be strong in Golfo Dulce. In addition, future research has potential to offer insight into the genetic and/or epigenetic mechanisms of the following: thermal tolerance (the yellow sea snake inhabits waters that can exceed the reported thermal maximum for the pelagic sea snake); visual acuity (nocturnality in the yellow sea snake might have led to improved night vision or reduced reliance on vision); osmoregulation (the yellow sea snake inhabits low-saline waters, which could reduce its ability to shield or excrete salts from the body); and/or blood oxygen-carrying capacity (the yellow sea snake inhabits waters with reduced dissolved oxygen).

Our study points to a dynamic evolutionary process. From a morphological perspective, the yellow sea snake is distinct, with unambiguous xanthic coloration and multiple changes to body size, weight, tail shape, and scalation. Those changes also coincide with considerable ecological shifts (Supporting Information, Table S3; for a review, see Bessesen 2022). Such findings suggest that the yellow sea snake is on a unique trajectory, and yet our genetic work offers no clear sign of evolutionary divergence. Genetic relationships among sea snakes can be difficult to resolve, especially for rapidly radiating hydrophiids, which present inadequate molecular resolution (Lukoschek and Keogh 2006, Rasmussen *et al.* 2011, Sanders *et al.* 2013); and to complicate matters,

snakes exhibit high levels of developmental plasticity, and morphological shifts may not always or entirely be linked to genetics (Burbrink *et al.* 2020). Nevertheless, the complete lack of population structure between our two study populations is both unexpected and difficult to reconcile. We have done our best to consider potential causes and leave it to our readers and future researchers to interpret the implications of this work. Perhaps the yellow sea snake was once fully isolated but has been hybridizing with the pelagic sea snake since the end of the Last Glacial Maximum. Perhaps it became more recently isolated and is in the early stages of speciation, its evolutionary trail yet undefined.

ACKNOWLEDGEMENTS

Support for this research was provided by the University of Reading, University of Adelaide, and the Field Museum of Natural History (FMNH), including the Grainger Bioinformatics Center and the Pritzker Laboratory for Molecular Systematics and Evolution. Fieldwork was conducted under Costa Rican Ministry of Environment and Energy (MINAE) research permit SINAC-ACOSA-D-PI-R-049-2022 and genetic materials permit R-001-2023-OT-CONAGEBIO. Tissue collection protocols were approved by the University of Reading School of Biological Sciences' Ethics Committee (reference number SBS21-22 19). Our deepest thanks to Osa Conservation for providing institutional sponsorship for the permits, to Luis Nieto Vásquez for vital assistance in the field, and to the team at Saladero Ecolodge for boarding and support. We owe tremendous gratitude to the numerous museum curators and collection managers who provided access to preserved specimens and tissue samples: Rachunliu Kamei and Josh Mata at the FMNH, Federico Bolanos, Adrián García, and Gerardo Chaves at the Zoological Museum of University of Costa Rica, Dane Trembath at the Australian Museum, Sally South at Australian Biological Tissue Collection, Gavin Dally at the Museum and Art Gallery of the Northern Territory, Emily Bracker at University of Colorado Museum of Natural History, Peter Rask Møller and Mark D. Scherz at the Natural History Museum of Denmark, Frank Burbrink and David Kizirian at the American Museum of Natural History, Kevin de Queiroz and Esther Langan at the National Museum of Natural History, Dakota Rowsey at the Arizona State University Natural History Collections, and Andy Whitworth at Osa Conservation Piro Station. Additional thanks to Gary Galbreath, Kevin Swagel, Jeffery Frederick, Diego Santana, and Yukun Sun for essential project support, to Harold Voris and William Dunson for insights and advice, and to Thomas Gardner for his invaluable knowledge of Osa geology.

AUTHOR CONTRIBUTIONS

Brooke Bessesen (study conception and design, data collection, laboratory preparation, data analysis, manuscript writing and revision), Manuela González-Suárez (funding, study design, data analysis), Guido Saborío-Rodríguez (permit acquisition, data collection), Edward Myers (data analysis), Balázs Buzás (data collection), Csaba Géczy (data collection), Arne Rasmussen (study design, data collection), Kate Sanders (funding, study design), Sara Ruane (funding, laboratory preparation), and James Nankivell (study design, data collection, laboratory preparation, data analysis, manuscript writing and revision). All authors, except B. Buzás and C. Géczy, contributed to the interpretation of results, and all authors reviewed this manuscript.

SUPPLEMENTARY DATA

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

BB and MGS were supported by the University of Reading Research Development Fund and the School of Biological Sciences, JHN and KLS were supported by the University of Adelaide, BB and SR were supported by the Field Museum of Natural History, specifically the Women's Board (including Field Dreams) and the Negaunee Integrative Research Center, and ARR was supported by the Carlsberg Foundation (grant CF 23-1842).

DATA AVAILABILITY

The sequences analyzed for this study can be accessed in the NCBI database (https://www.ncbi.nlm.nih.gov; BioProject: PRJNA1307641); remaining data, including .vcf files, are available through the public repository Figshare: https://doi.org/10.6084/m9.figshare.28800956.

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