



Biogeography and depth partitioning in deep-sea gastropods at the Pacific Costa Rica Margin

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Funding information

American Malacological Society; National Science Foundation

Abstract

Aim: Understanding the global distribution of biodiversity and the factors that influence it are among the central goals of biogeography. How abiotic and biotic factors limit species' ranges has been investigated across a variety of environments and taxonomic groups. However, such investigations across oceanic depths remain underrepresented, particularly for chemosynthetic environments such as hydrocarbon seeps. The depth differentiation (DD) hypothesis suggests that steep environmental gradients in the upper depths of the ocean may limit species' ranges over relatively short vertical distances. The present study aims to investigate the evidence for the DD hypothesis at hydrocarbon seep sites along the Pacific Costa Rica Margin (CRM) by investigating the biogeographic distributions of gastropods sampled there and the factors that influence them.

Location: Costa Rica.

Taxon: Mollusca, Gastropoda, Provannidae, Genus: *Provanna* (Dall, 1918).

Methods: 1813 *Provanna* snails were collected across 1300 metres (m) of depth from Costa Rican hydrocarbon seeps. To test the DD hypothesis, species partitioning across the depth range was investigated. In addition to gradients in oceanographic conditions, such as temperature, oxygen, and salinity, other factors potentially responsible for filtering species were also considered as alternative hypotheses, including the availability of biogenic substrate, the availability of and reliance on chemosynthetic fluids, and specific preferences for certain environmental conditions.

Results and Main Conclusions: Three species of *Provanna* were identified from the CRM and exhibited partitioning across depth. For the two dominant species (*P. laevis* and *P. goniata*), the data suggest that depth-associated oceanographic conditions are more likely than any other factor investigated to explain their observed partitioning across depth, supporting the DD hypothesis. This study also presents novel evidence that such partitioning may be unilaterally determined, wherein species with narrower depth ranges may limit the local depth ranges of others. This study represents an extension of biogeographic range limit literature into chemosynthetic habitats and across oceanic depths.

KEY WORDS

biogeography, competition, depth differentiation hypothesis, depth partitioning, evolutionary ecology, gastropoda, range limits



1 | INTRODUCTION

Range limits are an observable expression of species' ecology and life history and have been the subject of biological investigation since Darwin's time. In theory, it is possible for a species to adapt to live anywhere given sufficient time (Case et al., 2005), gradual enough environmental gradients (Bridle et al., 2009), balanced dispersal allowing for local adaptation and expansion (Haldane, 1956; Kirkpatrick & Barton, 1997; Skellam, 1951), and sufficient genetic variation (Antonovics, 1976; Holt & Gomulkiewicz, 1997). While such cosmopolitan distributions exist, they are not common in every habitat. Addressing the question of why species occur in certain places and not others is an overarching goal of biogeography (Arntzen & Espregueira Themudo, 2008; Kirkpatrick & Barton, 1997; Sexton et al., 2009).

Species range limits are determined by both biotic and abiotic factors in their environments (Case & Taper, 2000; Kirkpatrick & Barton, 1997; Louthan et al., 2015; Sexton et al., 2009) such as the presence of predators (Aragón & Sánchez-Fernández, 2013; DeRivera et al., 2005; Holt & Barfield, 2009), competitor species (Arif et al., 2007; Bullock et al., 2000; Case & Taper, 2000; Pigot & Tobias, 2013; Tilman, 1982; Van der Putten et al., 2010; Wisz et al., 2013), steep environmental gradients (Case & Taper, 2000; Pulliam, 2000), and barriers to dispersal (Goldberg & Lande, 2007). In general, biotic factors have been found to exert greater influence on a species' range in low-stress environments, while abiotic factors exert more influence in high-stress environments (Dobzhansky, 1950; Louthan et al., 2015; MacArthur, 1984). In aquatic ecosystems, much of the seminal work testing species range limits has been conducted across latitude in marine invertebrates (Fischer, 1960; Pianka, 1966) and freshwater fishes (Barbour & Brown, 1974), on barnacles (Connell, 1961a, 1961b) and mussels (Paine, 1974) within marine rocky intertidal zones, or on aquatic plants (e.g. Spence, 1967) and fishes (e.g. Barbour & Brown, 1974) across depth in freshwater lakes.

More recent work has extended such investigations across oceanic depths. Such studies are analogous to those conducted across altitudinal gradients in the terrestrial realm, such as those investigating abiotic influence on the range limits of alpine vascular plants (Ettlinger et al., 2011; Normand et al., 2009; Webster, 1961) and animal species (Stevens, 1992). In the upper layers of the ocean, oceanographic conditions such as oxygen, temperature, salinity, and nutrient availability may change rapidly with depth (Fiedler & Talley, 2006). The effects of such environmental gradients led to the formation of the depth differentiation (DD) hypothesis, which describes the pattern seen where genetic isolation and species turnover occurs more readily across short vertical distances than even large horizontal ones in the upper depths of the ocean, owing to these steep environmental gradients (Cordes et al., 2007; Etter et al., 2005; Jennings et al., 2013; Quattrini et al., 2015).

Often occurring in upper oceanic depths, chemosynthetic ecosystems represent biodiversity hotspots on the ocean floor (Levin et al., 2016). Chemosynthetic ecosystems occur where chemical-laden fluids are emitted from the seafloor into the water column

through geothermal heating (hydrothermal vents) or mantle dewatering processes such as plate subduction (hydrocarbon seeps) (Corliss et al., 1979; Suess, 2014). These fluids may fuel chemosynthetic primary producers (Cavanaugh et al., 1981) and may determine the range limits of foundational taxa, such as Siboglinid vestimentiferan tubeworms and Bathymodiolin mussels (Cordes et al., 2009; Cordes, Hourdez, & Roberts, 2010; Levin et al., 2016). These organisms may subsequently influence the diversity and composition of the established communities (Cordes et al., 2006; Cordes, Cunha, et al., 2010; Portail et al., 2015). While differences in depth-associated oceanographic conditions have been suggested as being more influential than differences in chemosynthetic context in determining species distributions across chemosynthetic sites (Krylova & Sahling, 2006; Ogura et al., 2018; Sahling et al., 2005), this is often difficult to assess without chemosynthetic or geographic differences confounding results.

Hydrocarbon seeps at the Costa Rica Margin (CRM) present a unique case with which to test the DD hypothesis, as changes in depth occur across relatively short horizontal distances. Therefore, the present study aims to test the DD hypothesis at the CRM by assessing whether steep environmental gradients associated with depth result in depth partitioning of species endemic to chemosynthetic habitats. Species of the gastropod genus *Provanna* (Dall, 1918) were selected as the model organisms for this study as they are among the primary grazers at these sites and are found worldwide at chemosynthetic environments (Amano & Little, 2012; Johnson et al., 2010; Linse et al., 2019). Furthermore, *Provanna* snails were collected from the entire depth range sampled at the CRM, were found in high abundance, exhibited notable phenotypic variation, and are unable to migrate between sites once they settle out of their larval stages. All of these factors support their use as model organisms for biogeographic investigation at these sites. Furthermore, while depth has been suggested as being an important factor influencing the distributions of *Provanna* in other regions, such as in the Okinawa Trough (Ogura et al., 2018) and Sagami Bay (Chen, Watanabe, & Sasaki, 2019; Okutani et al., 1992), little research has been conducted on any of the gastropods at the CRM.

To test the DD hypothesis, we first set out to confirm the presence of environmental gradients associated with depth across the sampling range. We then characterize the distribution of *Provanna* species across this depth range. To support the significance of depth-associated gradients in species partitioning, other alternative hypotheses regarding what may be filtering species must be ruled out; specifically, we assess whether species at the CRM may instead be partitioned due to overlapping nutritional needs, substrate unavailability, or overlapping environmental preference and resulting competitive exclusion (Table 1). Given our main hypothesis, we expect that species partitioned across depth would display signs of resource partitioning in their nutritional needs and substrate preferences, such that they would theoretically be able to coexist in a shared habitat if not for the depth-associated oceanographic conditions between them. It is also expected that species partitioned across depth would display different morphological changes to



TABLE 1 The major factors that may explain partitioning among study sites. Variables measured in the present study that correspond to each factor and the expected results for each are given in the table. Expectations are based upon the depth differentiation hypothesis and are supported by the citations listed.

Factor	Measured variable(s)	Expected results	Citation
Depth-associated oceanographic conditions	Temperature (°C)	Temperature varies across the study range	Fiedler and Talley (2006)
	Salinity (PSU)	Salinity varies across the study range	Fiedler and Talley (2006)
	Oxygen ($\mu\text{M/L}$)	Oxygen varies across the study range	Fiedler and Talley (2006), Kwiecinski and Babbin (2021)
Nutritional needs	Stable isotopes ($\delta^{13}\text{C}$ & $\delta^{15}\text{N}$)	Species have different nutritional needs (i.e. stable isotope signatures differ)	Bergquist et al. (2007)
	Oxidative reductive potential (ORP)	Species occur at different proximities to active seepage (i.e. the strength of ORP anomalies differ)	Walker et al. (2007)
Substrate availability	Collection records	Species' preferred substrate is present and available across depth	Cordes et al. (2009), Levin et al. (2016)
Environmental preference	Morphological reactions to differing environmental conditions	Species have different reactions to similar environmental changes	Case et al. (2005), Chen, Watanabe, Nagai, et al. (2019)

similar environmental conditions; species that fill similar ecological roles in similar environments can theoretically be expected to have similar responses to environmental changes (Case et al., 2005). Thus, differences in morphological responses would suggest that partitioning across depth is the result of ongoing adaptation to different depth strata in these species. This work aims to further the study of biogeographic patterning across oceanic depths in chemosynthetic species and to rigorously test the DD hypothesis within a novel environmental context.

2 | MATERIALS AND METHODS

2.1 | Biological sampling

1813 *Provanna* specimens were collected from six hydrocarbon seeps between 700 and 2000 metres (m) depth along the Pacific CRM during four cruises from 2017 to 2019 (Figure 1, Table S1.1). Specimens were collected using the human-operated vehicle (HOV) *ALVIN* during the 2017 and 2018 cruises aboard the Research Vessel (R/V) *Atlantis*, while the remotely operated vehicle (ROV) *SUBASTIAN* was utilized during the 2019 cruise aboard the R/V *Falkor*. *Provanna* were collected in situ using sampling tools attached to the HOV and ROV, including manipulator arms and vacuum suction attachments. Detailed dive logs were taken by observers that included the time (GMT), depth (m), latitude, and longitude of each biological sampling event. Data on the substrate from which specimens were collected were verified using these dive logs, timestamps of sampling events, and the ROV or HOV video feed.

Upon being transported to the surface, *Provanna* were either placed promptly into >95% ethanol and stored at room temperature (20–25°C) or frozen at -80°C. Collections ultimately included three confirmed species: *P. laevis* ($n=1624$) (Warén & Ponder, 1991), *P. goniata* ($n=180$) (Warén & Bouchet, 1986), and *P. pacifica* ($n=9$) (Warén & Bouchet, 1986). *P. laevis* was sampled at The

Thumb ($n=815$), Mound 12 ($n=803$), and Jaco Summit ($n=6$); *P. goniata* was only sampled from Jaco Scar ($n=180$); and *P. pacifica* was found at Quepos Seep ($n=3$) and Mound 11 ($n=6$). *P. laevis* was sampled from mussel shells ($n=1016$), tubeworms ($n=488$), wood ($n=25$), and plastic chips ($n=11$). *P. goniata* was sampled from tubeworms ($n=89$), mussels ($n=51$), rock ($n=15$), and plastic chips ($n=5$). *P. pacifica* was collected from fallen wood ($n=6$) and shell hash ($n=3$).

2.2 | Environmental analyses

Within-site environmental data on salinity (ppt), temperature (ITS-90), conductivity (S/m), depth (m), sound velocity (m/s), time (GMT), latitude, and longitude were recorded by the HOV and ROV each time *Provanna* were collected (Table S1.2). Five shipboard conductivity-temperature-depth (CTD) casts were also used to collect data on temperature, salinity, oxygen, total alkalinity, and depth-adjusted pH trends across the water column in the study region.

Oxidative reductive potential (ORP, mV) and dissolved oxygen (DO, $\mu\text{M/L}$) were measured by the autonomous underwater vehicle (AUV) *Sentry* during R/V *Atlantis* cruises AT37-13 and AT42-03 (Table S1.1). ORP readings represent within-site oxidative anomalies, with lower readings indicating a larger ratio of reduced/oxidized species in the medium (Hem, 1985). Within the context of chemosynthetic environments, lower ORP readings may indicate the presence of greater concentrations of hydrocarbons and/or sulphide and are thus used as a proxy for nearness to a point source of active seepage (Hem, 1985; Walker et al., 2007). ORP data were collected 24 h to 5 months from the time of corresponding biological sampling. ORP readings taken more than 10 m above the seafloor were excluded from analyses. To characterize anomalies within each site, all remaining ORP readings were converted to z-scores by subtracting the site's mean ORP measurement and dividing by the site's standard deviation. Transformed ORP measurements and raw DO measurements

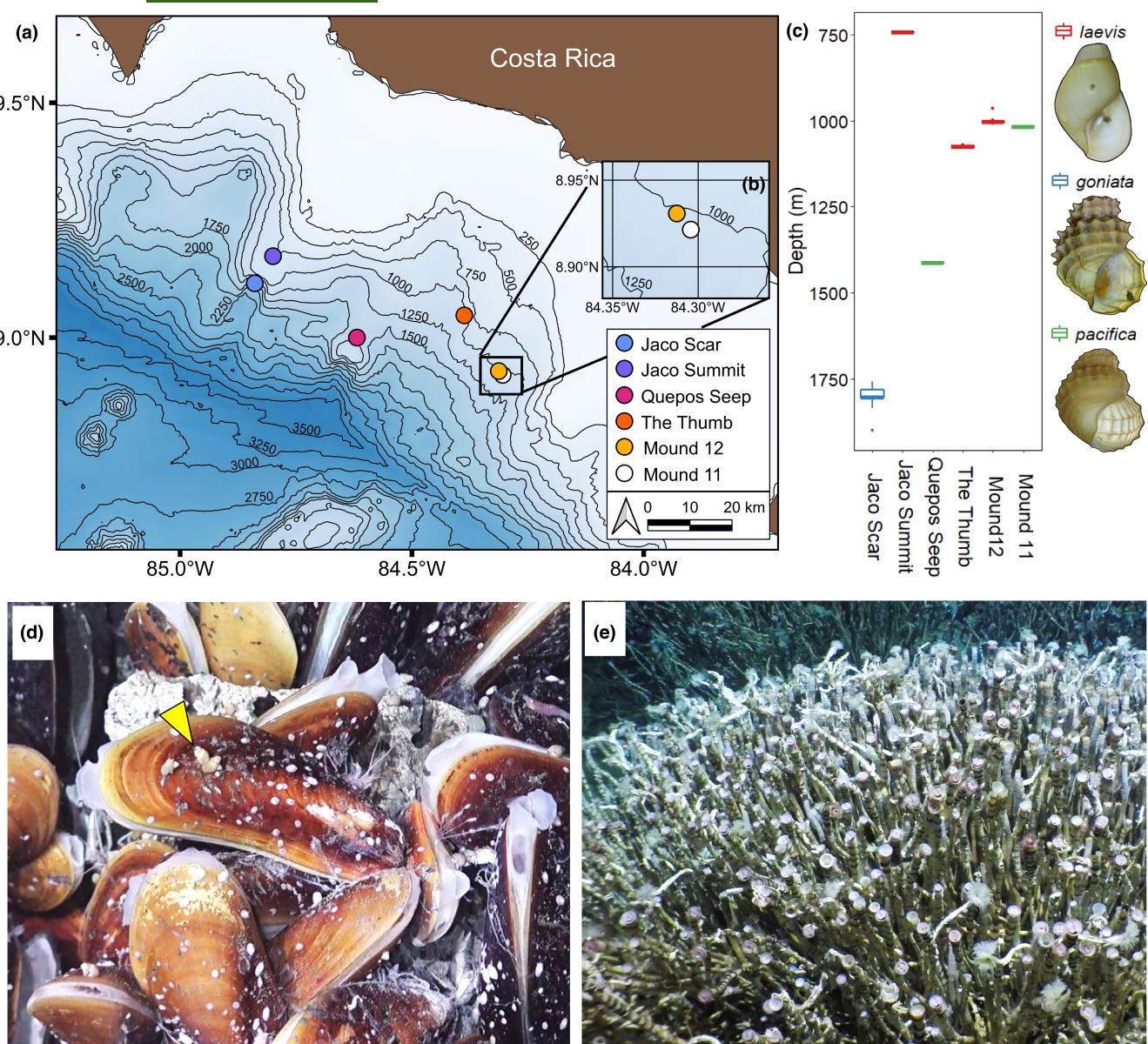


FIGURE 1 (a) Map of the Pacific Costa Rica Margin with sampling sites labelled. Numbers above black contour lines represent bottom bathymetry in metres (m). Contouring for every 250 m in depth is shown. (b) Close-up of the sites Mound 12 and Mound 11, which appear overlapping in the larger map. (c) Distribution of *Provanna* species across sites and depth. (d,e) The major foundational species found at the Costa Rica Margin: (d) Bathymodioli mussels, showing *Provanna laevis* grazing (yellow arrow), and (e) Siboglinid tubeworms.

were then loaded into QGIS (QGIS.org, 2022) (v3.16.11) where mosaic maps of ORP and DO readings were interpolated for each study site using inverse distance weighting (dist. coefficient = 2, output raster pixel size = 0.0001). ORP and DO data were then extracted from these interpolated maps for each location of biological sampling. If a specimen was collected outside of the interpolated map area, then no ORP or DO readings were assigned for that sample.

2.3 | Stable isotope analyses

Stable isotope analysis is a tool that is frequently used to discern trophic niches of organisms, particularly those in reducing

environments (Bergquist et al., 2007). Stable isotope signatures of our specimens were used to characterize their nutritional needs and preferences. As the two main sites hosting abundant foundational taxa were Jaco Scar and Mound 12, we limited our investigations to these sites, and thus to *P. laevis* and *P. goniata*. Tissue samples that had been stored at -80°C were rinsed with distilled water and oven-dried at 60°C before being homogenized into a fine powder and acidified using 10% HCl to remove inorganic carbon. Tissues were then redried and 0.5–1 mg of each sample was packaged into tin capsules before being sent to the Laboratory for Isotopes and Metals in the Environment (LIME) at Penn State University for analysis. Stable carbon and nitrogen isotopes were analysed using a Costech ECS 4010 combustion elemental analyser coupled to a Thermo Delta V isotope ratio



mass spectrometer (EA-IRMS) via ConFlo IV interface. Values are reported in delta notation as per mil (‰) deviations from internationally recognized standard reference materials (Vienna PeeDee Belemnite (VPDB) for carbon and atmospheric nitrogen (AIR) for nitrogen).

2.4 | Morphological analyses

To investigate how the environment may affect morphology, a subset of *Provanna* specimens representing the full size, depth, and geographic range of each species were selected for morphological analysis. Due to a deficiency of samples, the morphological traits of *P. pacifica* were not analysed. All measurement protocols and anatomical definitions are given in Figure S1.1. The following measurements were taken (in millimetres) for each individual selected: shell length, shell width, aperture length, aperture width, and shell thickness. Aperture and shell roundness were calculated by dividing width by length measurements, such that ratios closer to one indicate roundness and ratios closer to zero indicate obliqueness. Relative texture was calculated for shells displaying texturing by measuring the same five bumps/ridges on the body whorl of each shell, averaging them together, and then dividing by the shell length. This was done to give a measure of shell texture relative to size. Shell thickness was measured at the top of the aperture opening using digital callipers with a 0.01 mm resolution. All other morphological measurements were made from high resolution photographs taken using a mounted AmScope microscope adapter camera attached to a standard dissection microscope (Leica S6D, Leica Microsystems GmbH). Specimens were kept submerged in 1 cm of >95% ethanol while images were taken. Specimens were found to be small enough that distortion of measurements with altitude was negligible, and thus ignored. Measurements were then made digitally using the line measurement tool within AmScope (v4.7.14189.20190316 x64) calibrated to the same 1 mm marker each time the program was launched or the magnification was adjusted.

2.5 | Statistical analyses

Unless otherwise stated, all statistical analyses were conducted in R (v4.1.2) (R Core Team, 2022). For use in analyses, practical salinity (PSU) was calculated from conductivity and temperature using the Gibbs Seawater Oceanographic Toolbox package in R (package 'gsw') (Kelley, 2022). To assess the collinearity of variables, Pearson Correlation Coefficients were computed across all pairs of environmental variables and then across all pairs of morphological variables using the R package 'GGally' (Schlöerke et al., 2021).

To establish the existent gradients in oceanographic conditions across depth in the study region, depth profiles of environmental variables were visualized using Ocean Data View (v5.5.2) (Schlitzer, 2018). The data-interpolation variational analysis (DIVA) function was used to interpolate water column profiles from the CTD cast data. Colour scales representing each environmental

variable were z-adjusted to account for nonlinearity of the data and to make significant trends easier to see.

To assess whether the species at the CRM inhabit significantly different oceanographic conditions, one-way ANOVAs with post-hoc Tukey tests were conducted; species identity was used as a categorical predictor variable and data on depth, salinity, temperature, oxygen, and ORP were each used as response variables. Normality of response variables was assured using the qqPlot function in base R (Figure S1.2). As only a single datum was present for oxygen and ORP for *P. pacifica*, these environmental variables were only compared between *P. laevis* and *P. goniata*. Environmental differences were further assessed among species using a Principal Components Analysis (PCA, scaling=2), followed by Euclidean perMANOVAs (R Package 'vegan') (Oksanen et al., 2022) to assess the significance of centroid differentiation among the environmental conditions of each study species (10,000 permutations) (R Package 'pairwiseAdonis') (Martinez, 2017).

To investigate how morphological traits vary along in situ environmental data, redundancy analyses (RDA) were conducted for *P. laevis* and *P. goniata* (Capblancq & Forester, 2021). Shell width, shell length, aperture length, and aperture width all displayed high collinearity (Pearson Coefficients > +0.90 for all pairs). Thus, shell size is represented by aperture length alone in analyses. Aperture length, shell thickness, aperture roundness, shell roundness, and, for *P. goniata*, relative shell texture were all assessed in response to depth, salinity, oxygen concentration, temperature, and ORP. All data included in analyses were z-transformed prior to loading into the RDA. Finally, to assess how the shell traits of *P. laevis* and *P. goniata* varied across different substrates, two-way ANOVAs with post-hoc Tukey tests were conducted to examine how shell morphology differed within and among species on different substrates. Normality of response (morphological) variables were assessed using the qqPlot function in base R (Figure S1.3).

3 | RESULTS

3.1 | Environmental analyses

Environmental conditions varied across the study sites (Table. S1.2). Characterization of the water column found that measures of temperature and oxygen correlated strongly with depth (Pearson Coefficients: -0.99 and +0.98, respectively). Oxygen levels in the study region reached their minimum between 200 and 1000 m in a well-defined oxygen minimum zone (OMZ), in agreement with published literature (Figure 2a) (Fiedler & Talley, 2006; Kwiecinski & Babbin, 2021). Temperatures in the study region steadily decreased from around 30°C at the surface to just above freezing around 2000 m (Figure 2b). Measures of salinity showed a band of very high salinity between 50 and 500 m depth, likely due to evaporation of near-surface waters that occurs at low latitudes in the Eastern Pacific (Figure 2c) (Fiedler & Talley, 2006). Measures of total alkalinity were similar between 1000 and 2000 m depth (2360 vs. 2373) (Figure 2d), while depth-adjusted pH increased from around 7.63 to 7.77 from 1000 to 2000 m (Figure 2e).

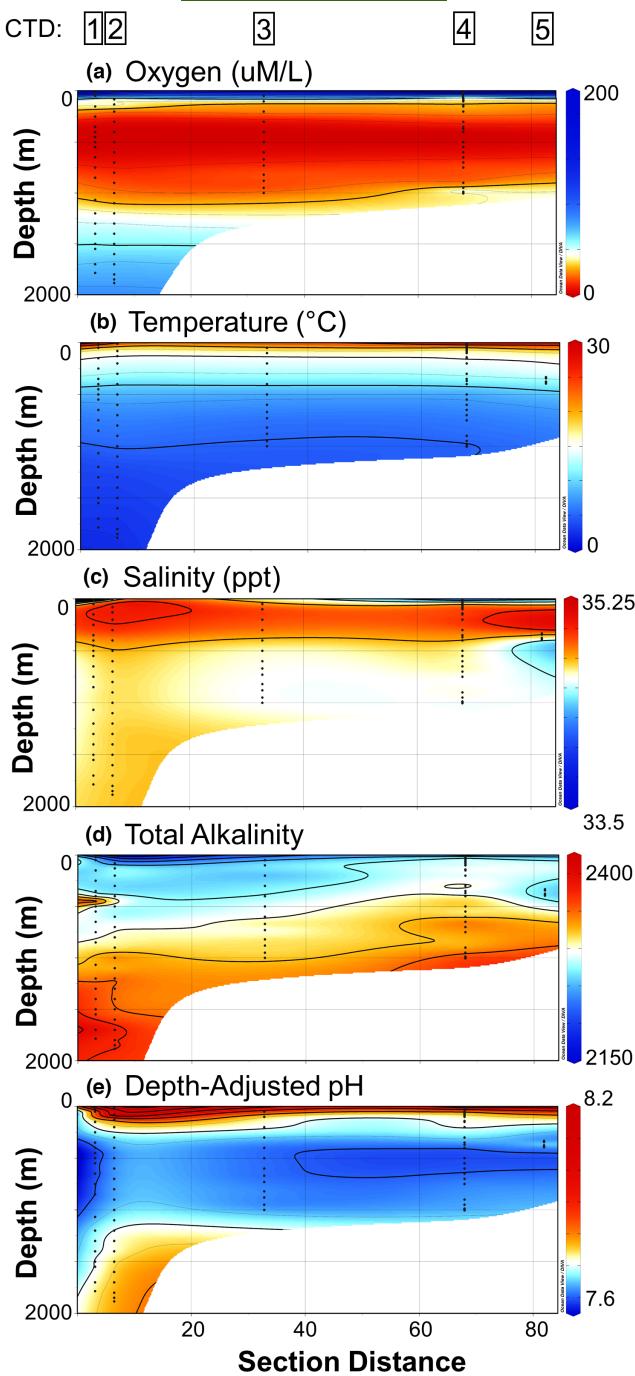


FIGURE 2 Water column profiles for (a) dissolved oxygen, (b) temperature, (c) salinity, (d) total alkalinity, and (e) depth-adjusted pH data from five CTD casts taken during AT37-13. Numbers 1–5 at the top of the figure refer to these five CTD casts: 1=near Jaco Scar {9.12°N, 84.84°W}, 2=near Jaco Scar {9.09°N, 84.83°W}, 3=near Quepos Seep {8.97°N, 84.63°W}, 4=near Mound 12 {8.93°N, 84.31°W}, 5=offsite {8.85°N, 84.22°W}. Depths at which bottles were triggered are indicated by black dots on the graphs. Seafloor bathymetry is not shown. Note that colour scales are z-adjusted for each variable to make significant trends easier to see.

Specimens of *P. laevis* were collected from 742 to 1075 m depth, *P. goniata* from 1757 to 1899 m, and *P. pacifica* from 1017 to 1413 m (see Figure 1c). Results of our one-way ANOVAs supported

that *P. laevis*, *P. goniata*, and *P. pacifica* all occur at significantly different average depths ($p < 0.001$ for all pairs; R^2 -adj. = 0.97; std. errors = 23.4, 17.3, and 45.9, respectively; t -values = −34.2, 104, and −11.5, respectively) and significantly different average temperatures ($p < 0.001$ for all pairs; R^2 -adj. = 0.93; std. errors = 0.09, 0.07, and 0.18, respectively; t -values = 24.5, 37.0, and 6.77, respectively). Thus, *P. laevis* were consistently collected from lower salinities than *P. goniata* ($p < 0.001$; R^2 -adj. = 0.63; std. errors = 6.70e−3 and 4.97e−3, respectively; t -values = −8.59 and 6967, respectively) and lower oxygen levels than *P. goniata* ($p < 0.001$; R^2 -adj. = 0.94; std. errors = 2.85 and 1.95, respectively; t -values = −22.7 and 48.9, respectively). *P. pacifica* was also collected from lower salinities than *P. goniata* ($p < 0.05$; R^2 -adj. = 0.63; std. errors = 1.31e−2 and 4.97e−3, respectively; t -values = −2.43 and 6967, respectively).

Measures of ORP did not correlate with any other environmental variable (Pearson correlations: −0.18 with depth, −0.19 with oxygen, and 0.20 with temperature) and accurately reflected the expected anomalies in water chemistry at these chemosynthetic ecosystem sites, demonstrating the effectiveness of this sensor in characterizing the seep environment. *P. goniata* was collected at greater within-site ORP anomalies (nearer to active seepage) than *P. laevis*, with an average ORP score of −0.1678 compared to *P. laevis*' average of 0.0153, though this difference was not significant. *P. laevis*, however, was found at a wider range of ORP anomalies (Min: −1.3978, Max: 1.1836) than *P. goniata* (Min: −0.5792, Max: 0.0706). Only a single ORP score could be assigned to *P. pacifica* (−0.1822) which was comparable to the other species.

PCA results yielded significant centroid differentiation between *P. goniata* and *P. laevis* ($p < 0.001$; R^2 = 0.72) and marginal differentiation between *P. goniata* and *P. pacifica* ($p < 0.06$; R^2 = 0.44) (Figure 3). *P. laevis* and *P. pacifica* did not show significant centroid differentiation from each other ($p > 0.10$; R^2 = 0.01), which was expected given that they overlap in their distributions around 1000 m (see Figure 1c). PC1 explained 74.69% of the variance in the data and was captured by temperature in the negative direction and depth, oxygen, and salinity in the positive direction. This was consistent with temperature showing highly negative Pearson correlations with depth (−0.99), oxygen (−0.99), and salinity (−0.74). Temperature was found to have the greatest species score along this axis (PC1 = −1.58; scaling = 2), followed closely by oxygen (PC1 = 1.56; scaling = 2), implicating these as potentially the most influential environmental conditions. *P. goniata* was notably separated from the other two species along this axis, consistent with its habitation of deeper, more oxygenated, and saltier water. PC2 explained 19.15% of the variance in the data and was captured mainly by ORP. Species thus exhibited more environmental partitioning along PC1 (depth and its associated variables) than PC2 (seep-associated variable).

3.2 | Stable isotope analyses

Tissue stable $\delta^{13}\text{C}$ of *P. laevis* collected at Mound 12 were distinct from *P. goniata* collected from Jaco Scar (Figure 4). $\delta^{13}\text{C}$ values of *P.*

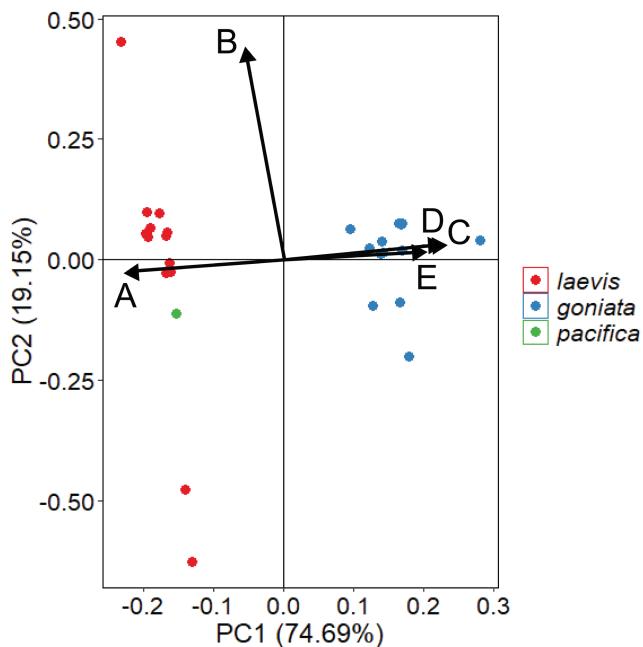


FIGURE 3 Principal component analyses for *Provanna* species comparing their environmental conditions (z-transformed): (A) Temperature, (B) oxidative reductive potential, (C) dissolved oxygen, (D) depth, (E) salinity. Numbers along the axes represent the proportion of variance explained. Scaling=2.

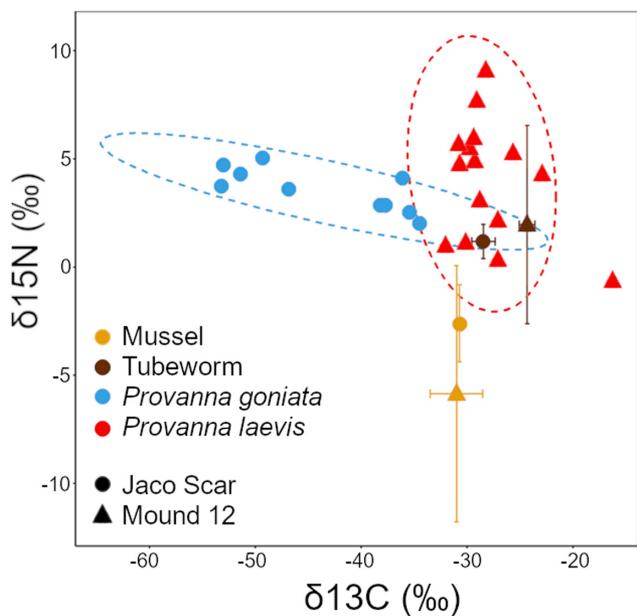


FIGURE 4 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *Provanna laevis* and *Provanna goniata* at Mound 12 and Jaco Scar. Ellipses denote 95% confidence intervals. Mean tubeworm and mussel $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are also displayed from each location. Error bars denote standard deviation.

goniata specimens were highly negative ($-43.6 \pm 7.8\text{\textperthousand}$; $N = 10 \pm 1 \text{ SD}$) compared to *P. laevis* ($-27.8 \pm 3.9\text{\textperthousand}$; $N = 15 \pm 1 \text{ SD}$). These negative isotope values indicate greater incorporation of methane-derived carbon, which has been shown to range between $-62\text{\textperthousand}$ and $-50\text{\textperthousand}$ as reported from methane in the water column above point sources

of fluid at Jaco Scar (Mau et al., 2014). Although direct measurements are not available, the end-member methane C-isotope values are likely similar between these two sites, as the $\delta^{13}\text{C}$ of both chemosynthetic tubeworms and mussels collected from Mound 12 and Jaco Scar were very similar to each other (tubeworms: $-24.3 \pm 1.9\text{\textperthousand}$, $-28.4 \pm 1.1\text{\textperthousand}$, mussels: $-31 \pm 2.4\text{\textperthousand}$, $-30.7 \pm 1.1\text{\textperthousand}$, respectively). Mean $\delta^{15}\text{N}$ values for both *P. laevis* and *P. goniata* were quite depleted ($4 \pm 2.7\text{\textperthousand}$, $3.6 \pm 1\text{\textperthousand}$, respectively). However, *P. laevis* had a notably wider range of $\delta^{15}\text{N}$ values than *P. goniata*.

3.3 | Morphological analyses

151 total individuals, representing the full size, depth, and geographic range of *P. laevis* ($n = 96$) and *P. goniata* ($n = 55$) were selected for morphological characterization (Table S1.3). To assess how morphological traits vary along environmental gradients, redundancy analyses were conducted on *P. laevis* (Figure 5a) and *P. goniata* (Figure 5b). Increasing calcification would be evidenced by thicker, larger, and more oblique shells. Our results for *P. laevis* found that the overall redundancy model was significant ($p < 0.001$; variance = 0.65; $F = 2.63$) and that oxygen ($p < 0.01$; variance = 0.25; $F = 4.08$) and depth ($p < 0.001$; variance = 0.31; $F = 5.07$) were significant within that model. *P. laevis* shells were found to be rounder, thinner, and larger at greater oxygen concentrations and depths (Figure S1.4). However, the model overall seemed inadequate in describing these data, as over 80% of the variance in the morphological traits was left unexplained by the environmental variables tested. This indicates that the conclusions drawn from these analyses may be tenuous, and that some other variable, such as genetic relatedness or the magnitude of environmental fluctuations (Verhaegen et al., 2018), may be influencing morphology. Our results for *P. goniata* found that the overall redundancy model was also significant ($p < 0.01$; variance = 0.90; $F = 2.16$) and that oxygen ($p < 0.05$; variance = 0.24; $F = 2.82$) and ORP ($p < 0.05$; variance = 0.23; $F = 2.78$) were significant within that model. *P. goniata* shells were thinner, smaller, and more textured at greater oxygen concentrations (Figure S1.5). They were also more oblique, thinner, and larger nearer to active seepage (lower ORP values). Similar to *P. laevis*, about 82% of the variance in the data was unexplained by the model, also indicating that some other factor besides the environment may be influencing intraspecific morphological variation.

In our assessment of morphological traits across different substrates, only marginal intraspecific differences were found (Figure 6). *P. goniata* shells were smaller on mussel shells than on tubeworms (Figure 6a, $p < 0.05$; $R^2\text{-adj.} = 0.11$; std. errors = 0.23 and 0.34, respectively; $t\text{-values} = 18.9$ and 2.44, respectively) as well as thinner on mussel shells than tubeworms (Figure 6b; $p < 0.05$; $R^2\text{-adj.} = 0.12$; std. errors = 0.03 and 0.04, respectively; $t\text{-values} = 10.0$ and 2.46, respectively). *P. laevis* shells were rounder on rock than on mussels (Figure 6c; $p < 0.05$; $R^2\text{-adj.} = 0.09$; std. errors = 0.02 and 4.07e-3, respectively; $t\text{-values} = 2.89$ and 160, respectively), and their apertures were marginally more oblique on rock than on tubeworms (Figure 6d; $p < 0.05$; $R^2\text{-adj.} = 0.06$;

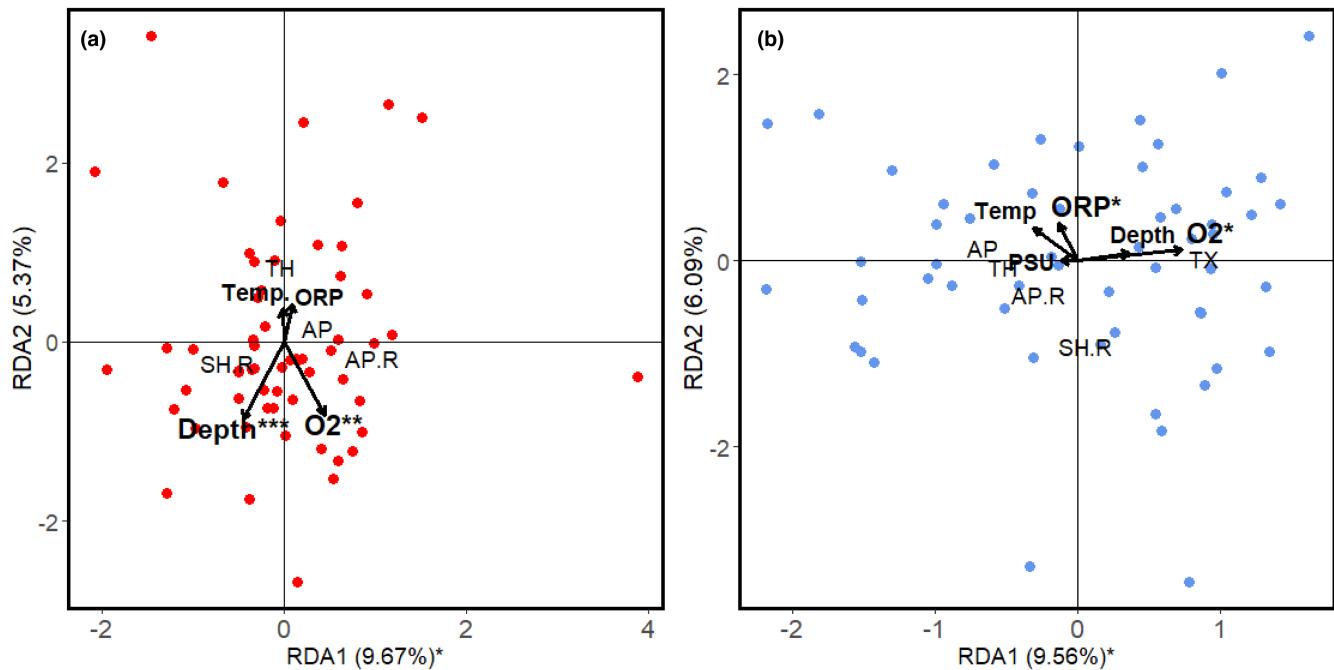


FIGURE 5 Redundancy analysis plots for (a) *Provanna laevis* and (b) *Provanna goniata* showing the interaction of environmental variables and shell traits (all variables z-transformed). Numbers next to the axis labels represent the proportion of total variance explained and the significance of that axis (p -value: *** <0.001 ; ** <0.01 ; * <0.05). Scaling = 2. AP, Aperture length; APR, Aperture roundness; O₂, Dissolved oxygen; ORP, Oxidative reductive potential; SH.R, Shell roundness; TH, Shell thickness; TX, Relative shell texture.

std. errors = 0.02 and 0.01, respectively; t -values = -1.83 and 1.82, respectively). Interspecific analyses between *P. laevis* and *P. goniata* on different substrates yielded few significant differences; *P. goniata* shells were more oblique than *P. laevis* shells on mussels ($p < 0.06$; std. error = 4.62e-3; t -value = 142), rock ($p < 0.001$; std. error = 0.02; t -value = 2.55), and tubeworms ($p < 0.05$; std. error = 0.01; t -value = 1.57) (Figure 6c; Model $p < 0.0001$; R^2 -adj. = 0.25). *P. goniata* also had significantly rounder apertures than *P. laevis* on mussels ($p < 0.05$; std. error = 5.08e-3; t -value = 126) (Figure 6d; Model $p < 0.005$; R^2 -adj. = 0.10). Notably, however, the trends observed between species on the same substrate were no different than their overall morphological differences as distinct species.

4 | DISCUSSION

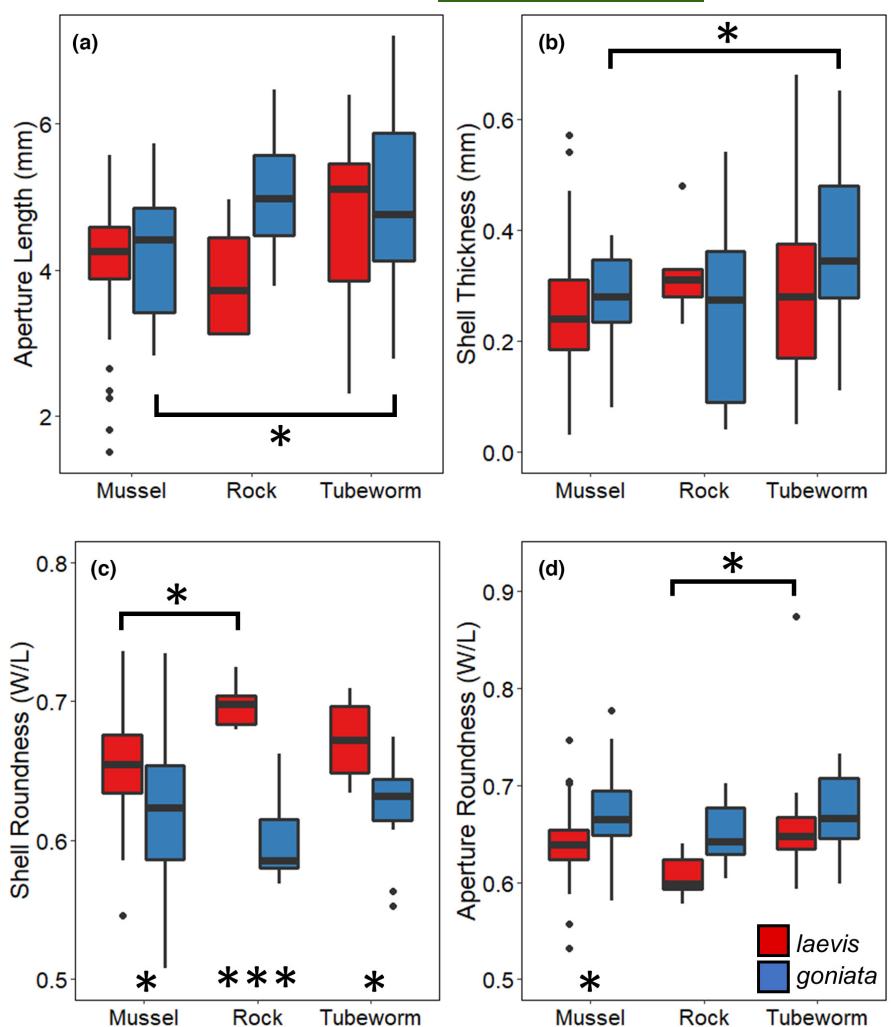
This study assessed the biogeographic range limits of chemosynthetic gastropod species across depth at the Costa Rica Margin (CRM). Evidence of depth partitioning among three species of deep-sea gastropods (*P. laevis*, *P. goniata*, and *P. pacifica*) was identified and investigated. This study is among the first to characterize the distribution of these gastropods in detail and to conduct a rigorous test of the depth differentiation (DD) hypothesis in this region. To address whether steep environmental gradients associated with depth may lead to depth partitioning among these species, we investigated not only the environmental differences associated with depth in this region, but also several alternative

hypotheses which may explain species partitioning, including overlapping nutritional needs, substrate availability and preference, and overlap in environmental preference. *P. goniata* and *P. laevis* were found to be the most abundant *Provanna* species and exhibited complete species turnover over a short horizontal (Min: 8 km) and vertical distance (Max: 1150 m). *P. pacifica*, meanwhile, were found at low abundances on fallen wood and empty mussel shells. Thus, these specimens likely represented opportunistic settlers, rather than established populations. These three species exhibited distinct depth ranges across the 1300 m of depth investigated, providing initial support for our main hypothesis.

The first alternative hypothesis investigated was whether overlapping nutritional needs may be responsible for species turnover across depth (Bergquist et al., 2007). To support the DD hypothesis, species partitioned across depth were expected to exhibit distinct nutritional needs, such that they would be capable of coexisting within a single site if not for the depth-associated oceanographic conditions between them. In our stable isotope analysis, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the two most abundant species, *P. laevis* and *P. goniata*, indeed differed greatly. The highly negative $\delta^{13}\text{C}$ values in *P. goniata* tissues (-53.2 to -34.5‰) suggest a significant proportion of their biomass is being fuelled by methane-derived carbon, either by direct grazing on methanotrophic bacterial mats or through symbiosis with chemosynthetic and/or methanotrophic bacteria. Conversely, the more enriched $\delta^{13}\text{C}$ values of *P. laevis* (-30.1 to -16.2‰) suggest a different nutritional source. The majority of *P. laevis* specimens had $\delta^{13}\text{C}$ values around -29‰, reflecting $\delta^{13}\text{C}$ values of the vestimentiferan



FIGURE 6 Morphological comparisons between *Provanna laevis* and *Provanna goniata* across substrates. Resultant *p*-values from one-way ANOVAs between species are denoted by floating stars without brackets (*p*-value: *** < 0.001 < ** < 0.01 < * < 0.05). Resultant *p*-values from one-way ANOVAs within each species are denoted by stars with brackets. The number of individuals of *P. laevis* incorporated into the ANOVAs are as follows: Mussels ($n=65$), rock ($n=5$), tubeworms ($n=15$). For *P. goniata*: Mussels ($n=21$), rock ($n=8$), and tubeworms ($n=16$).



tubeworms that are known to have thiotrophic symbionts, indicating that products from symbiosis with sulphide-oxidizing bacteria may be fuelling part of their biomass. Values of $\delta^{15}\text{N}$ had a greater range for *P. laevis* than *P. goniata*, which may suggest a more generalist approach to grazing. This divergence in nutrition acquisition would perhaps suggest the capacity for these two species to coexist within a single site, albeit at different proximities to point sources of fluid. However, this was never observed.

To further investigate the nutritional needs of each study species, we also examined nearness to active seepage. We found that differences in the strength of ORP anomalies from which *P. laevis*, *P. goniata*, and *P. pacifica* were sampled were not sufficient to differentiate these species with confidence in comparison to other environmental conditions. Interestingly, the ORP data obtained for *P. laevis* also suggests a more generalist approach to grazing, as they inhabited the greatest range of ORP anomalies. However, we cannot rule out that the context of seepage at these sites may still play a role. Jaco Scar has been called a 'hydrothermal seep' in that it exhibits certain vent-like qualities, such as elevated concentrations of hydrogen sulphide and warmer-than-ambient pore fluids (Max: 9°C (pers. obs.); Ambient: 2.4–2.6°C (pers. obs.)), but lacks others, such as heavy $\delta^{13}\text{C}$ signatures and elevated concentrations

of heavy metals (Bergquist et al., 2007; Levin et al., 2012). However, current literature suggests that differences in water conditions associated with venting and seepage are rarely responsible for species filtering, with differences in oceanographic conditions associated with depth suggested as being more influential in recruitment success (Krylova & Sahling, 2006; Ogura et al., 2018; Sahling et al., 2005). Indeed, published records of *P. laevis* confirm that it inhabits both active vents and seeps, and thus their exclusion from Jaco Scar based on chemosynthetic context is unlikely (OBIS, 2023).

The second alternative hypothesis considered whether substrate availability or preference may be responsible for the species turnover observed among sites. To support the DD hypothesis, each species' preferred substrate(s) were expected to be both present and available at sites separated by depth, such that this would not be a factor responsible for their partitioning. As gastropods at hydrocarbon seeps are reliant on grazing, the presence of hard substrate, such as mussel shells, carbonate rocks, or tubeworms, may be considered necessary for the successful establishment of these species. Quepos Seep and Mound 11 are largely sedimented seeps, dominated by carbonate rocks, bacterial mats, fallen wood, and shell hash. Jaco Scar, Jaco Summit, the Thumb, and Mound 12,



meanwhile, host dense aggregations of foundational tubeworms and mussels. Thus, while a lack of suitable substrate may explain the reciprocal exclusion of *P. laevis* and *P. goniata* from the range of *P. pacifica* (Quepos Seep and Mound 11), it does not explain their exclusion from each other's habitats. Both *P. laevis* and *P. goniata* were found primarily on mussels and tubeworms, both of which were abundant at their respective sites. This affinity for the same biogenic substrate may implicate competitive exclusion as a factor partitioning these species; although, given the differences in their nutritional needs and the adaptability of *P. laevis'* grazing behaviour, these two species would likely not compete for the same space on that substrate. Thus, a lack of suitable substrate is also not supported as the driver of partitioning between these two species.

Our third alternative hypothesis examined whether differences in environmental preference may explain partitioning. This was examined using morphological trait variation across environmental gradients. To support the DD hypothesis, it was expected that these *Provanna* species have adapted to fill different depth strata, and thus their morphological traits were expected to show differing changes across similar environmental gradients. The redundancy models for both *P. laevis* and *P. goniata* were found to be significant but left a large amount of the variance in the data unexplained (>80% of the variance for both). For both *P. laevis* and *P. goniata*, species' shells significantly varied with oxygen concentrations. While *P. laevis* shells were found to be thinner, larger, and rounder at greater oxygen concentrations, *P. goniata* shells were instead found to be thinner, smaller, and more textured. Both, therefore, showed two shell traits exhibiting decreased calcification (*P. laevis*: thinner and rounder; *P. goniata*: thinner and smaller) and one trait showing increased calcification (*P. laevis*: larger; *P. goniata*: more textured) with increasing oxygen. This may suggest that these species allocate energy differently as oxygen increases, with *P. laevis* prioritizing size and *P. goniata* prioritizing texture. In both, however, at least one morphological benefit is seen with increasing oxygen. Contrary to expectations, this result suggests that both species would perhaps benefit from living at greater depths, where oxygen is more plentiful. *P. goniata* traits were also found to vary along gradients of active seepage. Notably, seepage did not relate to any morphological trait in *P. laevis*. This evidence agrees with the stable isotope and ORP data, in that *P. goniata* appears to be more greatly influenced by nearness to seepage than *P. laevis*. The morphological trends observed for *P. goniata* nearer to active seepage (more oblique, thinner, and larger shells) suggest that *P. goniata* may utilize seep fluids to facilitate an increase in body size.

Intraspecific morphological differences across substrate were found to be marginal. Morphological differences between *P. laevis* and *P. goniata* did not vary across different substrates, even if the magnitude and significance of those differences did. These data suggest that these species may suitably inhabit a variety of substrates and that the morphological traits investigated are taxonomically constrained. Taken together, shell variation, which is environmentally influenced in other gastropod groups, such as freshwater mudsnails (Verhaegen et al., 2018) and deep-sea Pectinodontid limpets (Chen, Watanabe, Nagai, et al., 2019), may be less so in *Provanna*. Rather,

they showed few clear or consistent trends along the environmental variables tested. As this investigation is one of the first to examine *Provanna* shell variation in detail, this is a notable result that may hopefully guide future studies.

Finally, environmental gradients across depth in this region were found to be steep. Temperature, oxygen, and salinity all differed greatly among species, with *P. laevis* and *P. goniata* inhabiting significantly different abiotic environments. For example, *P. laevis* was found to inhabit oxygen deficient regions at the CRM (~30 µmol/L) from which *P. goniata* was never sampled. Notably, current global biogeographic distribution of these species also agrees with these findings. *P. goniata* has been exclusively sampled from depths at or below 2000 m in the Gulf of California (OBIS, 2023). Meanwhile, *P. laevis* has been sampled from a much larger depth range (1100–2700 m) and a larger geographic distribution (OBIS, 2023). This depth range expands even further when one considers that *P. laevis* and another recognized species, *P. glabra* (Okutani et al., 1992) from the Western Pacific, may represent one molecular taxonomic unit (MOTU) (Chen, Watanabe, Nagai, et al., 2019; Linse et al., 2019). The maximum sequence divergence of the cytochrome oxidase 1 (CO1) gene for these two species falls well below 3%, which is the threshold generally accepted for species distinction across a variety of deep-sea invertebrate groups (Johnson et al., 2008). If *P. laevis* and *P. glabra* were considered one MOTU as these published data suggest, then the resultant biogeographic range of this MOTU would span more than 13,000 km and a depth range of 440–3600 m (OBIS, 2023). Not only does this highlight the importance of proper taxonomic identification in drawing conclusions about a species' biogeography, but it also suggests that the partitioning seen at the CRM is likely driven by the depth tolerance of *P. goniata*, rather than *P. laevis*.

Taken together, the data suggest that the two most abundant *Provanna* species at the CRM (*P. laevis* and *P. goniata*) may have evolved along different evolutionary pathways. *P. goniata* appears to inhabit a relatively narrow range of abiotic conditions in amenable depths below 2000 m. In contrast, *P. laevis* (and its sister species *P. glabra*), inhabits a wide range of depths, habitats, and environmental conditions, including those that may be considered stressful for a deep-sea species, such as low salinities and near-anoxic conditions. This suggests two distinct evolutionary trajectories: one maximizing competitiveness and/or resource acquisition within a narrow, preferred range (*P. goniata*), and one maximizing tolerance to a wide variety of environments, allowing one to avoid competition with sister taxa (*P. laevis*). Thus, we suggest that the partitioned distribution of these species across depth represents the fundamental niche of *P. goniata* and the realized niche of *P. laevis*, which may be unable to compete with or establish populations alongside *P. goniata* despite evidence that it can and may prefer to live in deeper water. Significantly, the local data obtained during this investigation align well with known species distributions, highlighting the capacity for local investigations to explain and support global biogeographic patterns.

In summary, this study finds evidence in support of the DD hypothesis. The steep environmental gradients associated with depth at the CRM were identified as being more likely than any other



variable tested (nutritional needs, substrate availability, and environmental preferences) to partition the dominant species across the study sites. While one species (*P. pacifica*) is likely partitioned from the others by its distinct substrate use, the alternative hypotheses that *P. laevis* and *P. goniata* are partitioned due to overlapping nutritional needs or substrate use were not supported. Preference for deeper water was potentially identified for both *P. laevis* and *P. goniata*. However, while *P. goniata* appears to be restricted to deeper water and to locations nearer to active seepage, *P. laevis* does not. This suggests that while *P. laevis* may not have been partitioned across the study region on its own, the presence of *P. goniata* and its intolerance to shallower water may work to limit the range of *P. laevis* across depth. These results introduce a vital, directional component to the DD hypothesis, such that the depth tolerance of one species may impact the realized depth niche ultimately inhabited by another. Future investigations incorporating additional statistical and modeling methods or testing additional variables that may influence species range limits (e.g. predation pressure, oceanic circulation, other components of water chemistry) may help to further elucidate the direct and indirect influences on species range limits in the deep ocean. These and future studies are essential to biogeographic literature, as the deep ocean is the largest habitat on Earth yet remains relatively under-sampled and under-explored compared to shallow-water and terrestrial ecosystems (Webb et al., 2010). Thus, the deep ocean presents an ongoing opportunity to apply well-known biogeographic theories to novel environmental contexts.

ACKNOWLEDGEMENTS

We thank those who helped fund this research, including the National Science Foundation (OCE 1635219), Temple University, and the American Malacological Society. We thank the crews of the R/V Falkor during FK19-0106, the R/V Atlantis during AT37-10, AT37-13, and AT42-03, as well as the operating teams of HOV ALVIN, ROV SUBASTIAN, and AUV Sentry from 2017-2019. We thank Greg Rouse and Charlotte Seid at Scripps Institute of Oceanography for loaning us vital specimens used in this study. We thank Blair Hedges and Rachel Spigler for their advice and guidance during this project. We would also like to thank the editors of the *Journal of Biogeography* and two anonymous reviewers, whose feedback significantly improved this manuscript. Finally, we thank the government and people of Costa Rica for allowing us to conduct this research in their national waters. Necessary permits and licences required to conduct this research were obtained through the Costa Rican Ministry of the Environment and Energy (RESOLUCION SINAC-CUS-PI-R-035-2017, R-070-2018-OT-CONAGEBIO, INCOPESCA-CIP-003-12-2018, SINAC-CUSBSE-PI-R-032-2018, SINAC-SE-064-2018).

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

All raw morphological and environmental data, as well as the R code used in statistical analyses, are freely available on Github

(Repository: <https://github.com/melissajbetters/Depth-Partitioning-in-Provanna>.) or through correspondence with the lead author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Betters, M., Stabbins, A., Keller, A., & Cordes, E. (2023). Biogeography and depth partitioning in deep-sea gastropods at the Pacific Costa Rica Margin. *Journal of Biogeography*, 00, 1–13. <https://doi.org/10.1111/jbi.14722>