

RESEARCH ARTICLE

Diel activity correlates with colour pattern morphology of heterobranch sea slugs

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Abstract

1. Visually hunting predators drive the evolution and maintenance of bold visual defences, including aposematic signalling. Such visual signals must be encountered by predators in lighting conditions where the perception of colour patterns is possible. Therefore, aposematic colouration is predicted to evolve in species encountered by visually hunting predators during daytime, with diurnal species most at risk of visual predation. However, colour patterns in diurnal and nocturnal species are highly diverse, and little is known about the relationships between prey colouration and daytime activity.
2. To investigate correlations between daytime activity and colour pattern phenotypes across species, we quantified colour patterns in 45 species ($n = 346$ individuals) of eastern Australian sea slugs (Gastropoda: Heterobranchia).
3. We used 158 descriptors of colour pattern morphology using the Quantitative Colour Pattern Analysis (QCPA) framework, which models pattern appearance using the visual system of a potential predator, a triggerfish (*Rhinecanthus aculeatus*). We then used phylogenetic factor analysis to identify a single factor that was strongly correlated with daytime activity, comprising 55 individual colour pattern descriptors. This dimension of pattern variation identified daytime activity in 87% of species in our dataset.
4. We found that daytime activity in heterobranch sea slugs was associated with a distinct, highly descriptive set of spatiochromatic image statistics. Specifically, diurnal species were more boldly patterned than nocturnal species, exhibiting increased colour, luminance and pattern contrast, indicating the presence of visual signalling and, thus, visual predation as a key driver of heterobranch sea slug phenotype.

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5. Our study sheds light on the ecology and evolution of defensive colouration in sea slugs and provides a novel and robust workflow for comparative phylogenetic studies considering colour pattern spaces described by high-dimensional datasets, which can be applied to other species and ecosystems.

KEYWORDS

animal colouration, aposematism, colour pattern space, comparative phylogenetics, Heterobranchia, phylogenetic factor analysis, quantitative colour pattern analysis, visual modelling

1 | INTRODUCTION

Animal visual signals, such as those used for aposematic signalling, are shaped by both the biotic and the abiotic environment in which they evolve (Cronin et al., 2014; Cummings & Endler, 2018; Endler, 1991; Kemp et al., 2023). Therefore, understanding the constraints provided by the environment is crucial for understanding the evolution of these signals. Biotic factors shaping the ecology and evolution of defensive animal colouration, such as the role of signal receivers and conspecifics, have received much consideration (Briolat et al., 2019; Ruxton et al., 2018). In contrast, how abiotic properties of the environment, such as temperature and light, shape the ecology and evolution of aposematic colouration remains comparatively understudied (but see Dalrymple et al., 2018; Lindstedt et al., 2009; Nokelainen et al., 2022).

Differing light environments can impact the perception of aposematic colour signals, as the efficacy of visual signals requires stable and sufficient environmental illumination (Cronin et al., 2014). Indeed, Nokelainen et al. (2022) found that blue tits (*Cyanistes caeruleus*) preyed on different colour pattern morphs of wood tiger moths (*Arctia plantaginis*) depending on the brightness of environmental light. Arenas et al. (2014) found colour pattern contrast variation in different species of ladybirds (Coleoptera: Coccinellida) across varying levels of daylight illumination when modelled using the visual system of a potential predator, the blue tit. Lastly, using human predators and artificial clay models in open versus covered forest environments, Rojas et al. (2014) suggested differential selection of their prey models to be influenced by differences in environmental lighting. However, the contribution of the light environment to predation rates was not investigated experimentally.

Aposematic colour patterns are therefore assumed to be limited to predominantly contemporary (or during their recent evolutionary history) diurnally active species (Daan, 1981; Merilaita & Tullberg, 2005). As a potential exception to this rule, some species, such as moths, can perceive colour in low-light conditions (Kelber et al., 2002), and visual signalling in darkness is possible with bioluminescence (De Cock & Matthysen, 1999; Guilford & Cuthill, 1989; Marek et al., 2011). However, little is known about correlations between daytime activity and the presence of bold colouration enabling aposematic signalling. Studies investigating correlations

between daytime activity and aposematic signalling remain largely theoretical (Merilaita & Tullberg, 2005), with only a few suggestive examples (Cott, 1940; Semlitsch & Pechmann, 1985; Wollenberg & John Measey, 2009). Crucially, these studies do not consider the visual abilities of ecologically relevant observers (e.g. Nokelainen et al., 2024). However, the ecology and evolution of aposematic animal colouration and visual signals are, in general, shaped by the properties of the visual systems of the intended signal receivers (Cummings & Endler, 2018; Endler, 1991, 1992; Ruxton et al., 2018). Therefore, comparative studies that consider the visual abilities of ecologically relevant observers to investigate the presence and diversity of defensive colouration in prey communities are lacking.

Furthermore, the limited research on correlations between daytime activity and the prevalence of aposematic signalling has focused almost exclusively on terrestrial systems (e.g. Merilaita & Tullberg, 2005; Nokelainen et al., 2024). However, the physical environment of aquatic habitats and the optical confounds on visual signalling differ dramatically from those on land (Cronin et al., 2014). Crucially, 80%–90% of animal phyla do not exist on land or, if so, are represented by a fraction of their taxonomic diversity (Hickman et al., 2000). This substantial bias highlights the need for investigations into aquatic and particularly marine, systems to investigate the prevalence and generalisability of correlations between daytime activity and warning signals, or even defensive signalling more broadly.

To investigate the relationship between colour pattern, environment, and receiver vision, spatiochromatic colour pattern analyses using calibrated digital imaging provide a significant quantitative and qualitative advance in visual ecology (van den Berg, Troscianko, et al., 2020). Such analyses can provide hundreds of phenotypic descriptors that incorporate the visual capabilities of ecologically relevant observers. However, harnessing the analytical power of such high-dimensional datasets in an 'agnostic' approach (i.e. without any prior selection of phenotype descriptors due to assumptions on underlying relationships) requires appropriate statistical tools (Kemp et al., 2023; van den Berg et al., 2022; van den Berg, Condon, Conradsen, White, & Cheney, 2024; van den Berg, Santon, et al., 2024; van den Berg, Santon, Endler, & Cheney, 2024; van den Berg, Troscianko, et al., 2020). Furthermore, many species have poorly or patchily resolved phylogenies, making the correct use of comparative analyses accounting for shared ancestry difficult. In combination with the effort needed to obtain and analyse suitable

calibrated image data, these factors likely contribute to the lack of comparative studies using more than a handful of pre-selected colour pattern descriptors (e.g. Allen et al., 2019; Arias et al., 2016; Cheney et al., 2014; Cortesi & Cheney, 2010) that often do not account for non-human visual perception (e.g. Alfaro et al., 2019; Nokelainen et al., 2024).

Here, we investigated the relationship between daytime activity and colour pattern morphology in 346 individuals belonging to 45 species of co-occurring marine heterobranch molluscs (Gastropoda: Heterobranchia). We quantified colour patterns by approximating chromatic and achromatic contrast sensitivity and spatial acuity of an ecologically relevant observer, a triggerfish (*Rhinecanthus aculeatus*), using Quantitative Colour Pattern Analysis (QCPA) (van den Berg, Troscianko, et al., 2020). Following colour pattern quantification, we used a modified Bayesian phylogenetic factor analysis (PFA) (Hassler et al., 2022; Tolkoff et al., 2018) and a customised approach for complementing partial phylogenetic trees with taxonomic classification to investigate correlations between 158 pattern statistics and daytime activity. We then used previously published measures of secondary defences (Winters et al., 2022) for eight species to investigate if colour pattern morphology associated with diurnal activity also coincides with the presence of secondary defences. Specifically, we hypothesised that the colour patterns of nocturnal species would appear notably different from those of diurnally active species when viewed from a close distance by an ecologically relevant observer. Furthermore, we expected colour pattern statistics associated with bold patterning to be correlated with daytime activity and the strength of secondary defences across species.

2 | MATERIALS AND METHODS

2.1 | Sampling

We captured calibrated images of 346 individuals belonging to 45 species of heterobranch sea slugs (Figure 2) (median individuals per species: 3, 96% CI [LL, UL]: [2, 7]) between March 2016 and June 2019 (Table S1). Calibrated images were acquired while SCUBA diving ($n=304$) or post-collection in a wet lab ($n=42$) during three consecutive field trips to Nelson Bay, New South Wales (Australia, 32.7207° S, 152.1415° E) at the following dive sites: Little Beach, Fly Point, Pipeline and Seahorse Garden (Table S1). Sampling was conducted during 38 dives, distributed roughly equally among sites and different times of the day (18 night/20 day) at depths between 2 and 20 m. Night dives were conducted after sunset (~6 pm) and before sunrise (~6 am). All dives were performed in groups of 2–4 divers, conducting roving diver sampling (Rassweiler et al., 2020). The species in this study represent 84% of all heterobranch sea slugs captured by systematic nighttime surveys along transects at the same location, and 87% of all animals found during daytime surveys (Larkin et al., 2018). This excluded *Aplysia* sp., *Elysia* sp., *Chelidonura* sp. and any species with external shells, which were omitted from our study.

2.2 | Photography

All images contained a custom-made colour and grey standard to allow for image linearisation and normalisation (Troscianko & Stevens, 2015). All standards were crafted from a Tiffen colour separation guide and grayscale (Q-13) (Tiffen, NY, USA), but cast into different materials. The standard for the first trip was cast into a plastic key ring, whereas the standard for the other trips was cast into clear resin and dried in a vacuum to remove any trapped air (detailed instructions can be found on www.empiricalimaging.com).

The camera was equipped with two 12,000-lumen VK6 Pro Scubalamp and two 6500-lumen PV6S Scubalamp video lights, providing 37,000-lumen white LED light (van den Berg, Troscianko, et al., 2020). We calibrated an Olympus E-PL5 PEN camera with a 60 mm macro lens (van den Berg, Troscianko, et al., 2020) in an Olympus PT-EP10 housing. All pictures were taken in RAW format using manual aperture and shutter speed at a fixed ISO 200. We aimed to achieve slight underexposure, thus preventing the loss of pixel information due to overexposure. Animals collected during dives before images could be taken ($N=42$) were placed in a petri dish submerged in water before photography under white LED light. Nudibranchs were collected under the NSW Scientific Collection Permit P16/0052-1.0, with no ethics approval required. All photos were taken dorsally at an approximately 90-degree angle relative to each animal and its background, with the animals moving forward, stretched out in a more-or-less neutral position (i.e. neither contracted nor overly bent).

2.3 | Activity time

As Larkin et al. (2018) conducted standardised transect surveys, we used their data to determine the daytime activity of most species in our study (Table S1). For four species without data, we used the predominant time of day during which we encountered the species on our dives. Species with considerable sightings during both day and night were considered diurnal. Thus, only species predominantly found during nighttime were considered nocturnal.

2.4 | Taxonomy and phylogeny

Species identity was determined using available literature, field guides and internet resources. Taxa which could not be identified at the species level were described at the genus level (e.g. *Discodoris* sp., *Marionia* sp.). Animals categorised as *Discodoris* sp. could not be reliably distinguished between *Tayuva lilacina*, *Jorunna pantherina* or *Sebadoris fragilis*. However, all individuals were found exclusively during nighttime and shared a remarkable visual similarity. *Marionia* sp., *Rostanga* sp., *Onchidoris* sp. and *Goniobranchus* sp. consisted of a single, unidentified species. A phylogeny was constructed for those species for which genetic sequences were available ($n=33$, Figure S1). Available data for

COI and 16S genes were extracted from GenBank and aligned in Geneious Prime 2022.2 (Biomatters, Kearse et al., 2012) using the plug-in for MAFFT (Kato & Standley, 2013). Auto options were used for aligning COI, and 16S was aligned with the iterative algorithm FFT-NS-I $\times 1000$ using the default scoring matrix 200PAM. Primer regions were removed, and both datasets were concatenated and analysed (partitioned by gene) with maximum-likelihood in IQ-tree (Trifinopoulos et al., 2016).

To complement the primary (genetic) tree, we constructed a second tree using all species' taxonomic classification in the World Register of Marine Species (WoRMS, Ah Yong et al., 2024) as the input character matrix (Table S3; Figure S2). Species denoted with 'cf.' or 'Sp.' were identified at the genus level (Table S3; Figure S2) for the taxonomic tree. *Tayuva lilacina* in the genetic tree was treated as *Discodoris* sp. We then used the resulting classification tree to complement the phylogenetic tree using a modified approach from Schluessel et al. (2008) to build a 'supertree' (Figure S3). This supertree approach aims to maintain the information in the phylogenetic tree while introducing missing species with information from the taxonomic tree, assuming that the taxonomy encodes at least some phylogenetic information. Specifically, we used SuperTree0.85b (Salamin et al., 2002) to construct two character matrices using matrix representation with parsimony with Baum-Ragan encoding (Baum, 1992; Ragan, 1992), with unordered or irreversible characters. We analysed the resulting matrices using Phylip (program "mix", Felsenstein, 2005). The unordered character set was analysed with Wagner parsimony (Eck & Dayhoff, 1966; Kluge & Farris, 1969), and the irreversible character set was analysed using Camin-Sokal parsimony (Camin & Sokal, 1965). The quality of this composite supertree depends strongly on the contributing sub-trees and is by no means a true substitute for a completely resolved phylogenetic tree. For example, a supertree constructed with Matrix Representation with Parsimony will never have interpretable branch lengths and does not provide information about phylogenetic uncertainty. However, our approach aims to use the maximum possible phylogenetic information available and, by definition, provides a more accurate base for comparative analysis than assuming no phylogenetic structure to the data.

To ensure our supertrees were predominantly driven by the genetic data (i.e. the phylogeny), characters derived from the taxonomy were given a weight of 1. In contrast, characters derived from the phylogeny were given the highest possible weight (35) in Phylip. In addition, we rooted the tree with *Pleurobranchus peroni*, as pleurobranchs are consistently recovered as the sister group to Nudibranchia (e.g. Krug et al., 2022). We made 50,000 random additions to the tree in each run. The 100 most parsimonious trees were retained from each run, and we built two extended majority rule consensus trees using Phylip (program 'consense'). The resulting trees conserved monophyly and were highly congruent, so we combined both tree sets and computed a consensus tree based on the ordered and unordered analyses. Combining both sets of trees from the ordered and unordered analysis resulted in the retention of

generic monophyly and the maintenance of the dorids and aeolids as distinct sister taxa (Wägele & Willan, 2000). We used this tree with Grafen's (1989) arbitrary branch length, with Grafen's $\rho = 1$ in all subsequent comparative analyses (Figure 3). While it is ideal to use a phylogeny with time-calibrated branch lengths, comparative analyses are usually robust to misspecification of branch lengths (Díaz-Uriarte & Garland, 1998; Li et al., 2019; Stone, 2011).

2.5 | Colour pattern analysis

Images were analysed using the QCPA framework (van den Berg, Troscianko, et al., 2020), which is part of the 'Multispectral Image Calibration and Analysis' (MICA) toolbox (Troscianko & Stevens, 2015) in ImageJ (Mutterer & Rasband, 2012). Images were linearised and normalised using the 'estimate black point' option in MICA and a grey standard in each image. The images were then transformed into cone catch images as per the photoreceptor stimulation of a Picasso triggerfish (*Rhinecanthus aculeatus*) (Cheney et al., 2013) at a slightly greenish illumination (typical for NSW coastal waters) as measured at 5 m depth (van den Berg, Troscianko, et al., 2020). The same illuminant was used for analysis as all species are commonly found across the depth range (~2–20 m) considered in this study.

Rhinecanthus aculeatus was chosen as a representative ecologically relevant observer as they are abundant, active during daytime, omnivorous (including feeding on small invertebrates) (Kuwamura, 1997; Randall et al., 1997) and have a comparatively well-studied trichromatic visual system representative of many fish in shallow coastal waters (Cheney et al., 2022; Losey et al., 2003). Trichromatic fish (with spectral sensitivities lacking UV sensitivity) are plausible to be a common predator of heterobranch sea slugs across most of their past and present global distribution in shallow marine habitats, with the vast majority of colour patterning being visible to non-UV sensitive visual systems with spectral sensitivities covering 400–700 nm (e.g. Cortesi & Cheney, 2010). The amount of spatial information perceived by the fish was modelled using a combination of Gaussian acuity filtering followed by image smoothing using the receptor noise limited (RNL) ranked filter, both implemented in QCPA. Images were rendered assuming a spatial acuity of 3 cycles/degree (Champ et al., 2014), a 2 cm viewing distance and Weber fractions of 0.07:0.05:0.05:0.05 (sw:mw:lw:dbl) derived from a relative receptor abundance of 1:2:2 (sw:mw:lw) (Cheney et al., 2013), with luminance contrast perception mediated by the double cone receptors measured as $(mw + lw)/2$ (Siebeck et al., 2014). Also, 2 cm was chosen as an arbitrarily small viewing distance roughly corresponding to immediate prey subjugation at which the vast majority of colour pattern detail would be visible to a predator. Each animal was manually isolated from its visual background before acuity modelling using ImageJ's 'Region of Interest' (ROI) selection tool. This was followed by image segmentation into colour pattern elements using RNL clustering with a chromatic threshold of 2 ΔS (Green et al., 2022) and an achromatic threshold of 4 ΔS

(van den Berg, Hollenkamp, et al., 2020). A distance of 1 ΔS in RNL space equates to a theoretical discrimination success of 75% between two colour pattern elements under ideal viewing conditions (Kelber et al., 2003; van den Berg, Troscianko, et al., 2020; Vorobyev & Osorio, 1998).

To quantify each animal's visual appearance, 158 pattern parameters were selected from the QCPA output (Table S3). These included QCPA's modified versions of the colour adjacency analysis (CAA) (Endler, 2012), visual contrast analysis (VCA) (Endler & Mielke, 2005) and boundary strength analysis (BSA) (Endler et al., 2018) as well as the Gabor ratio contrast (GabRat) (Troscianko et al., 2017) and local edge intensity analysis (LEIA) (van den Berg, Troscianko, et al., 2020). These analyses provide a vast colour pattern descriptor space, quantifying colour, brightness, pattern geometry and their combination in the form of spatiochromatic measures of pattern contrast.

For a detailed description of each colour pattern statistic, please refer to Supporting Information (Table S5) and van den Berg, Troscianko, et al. (2020). In short, LEIA quantifies edge contrast in an image using RNL colour and luminance contrast at the scale of an edge-detecting visual field without clustering the image. CAA, VCA and BSA rely on an RNL clustered image and quantify colour pattern geometry (for CAA), visual contrast of colour patches (for VCA) and the boundaries between them (for BSA) using a transition matrix that counts pixel class transitions at the scale of an edge detecting receptive field (van den Berg, Troscianko, et al., 2020). GabRat quantifies the ratio of edge contrast along the outline of an animal in line with the outline (shape) of the animal and is a means of describing disruptive colouration (Troscianko et al., 2017). To account for animals photographed against artificial backgrounds, we ignored the GabRat values from those observations.

2.6 | Statistical analysis

To meaningfully interpret the hundreds of colour pattern descriptors from the QCPA using dimensionality reduction analysis, we applied a phylogenetically corrected factor analysis (PFA) (Tolkoff et al., 2018). This enabled us to identify latent variables correlating with daytime activity in our dataset while accounting for shared ancestry. The 158 QCPA pattern parameters were normalised and used as inputs in a modified version of PFA (Hassler et al., 2022). Statistical analyses were done using R software (R Core Team, 2021) and Julia software (Bezanson et al., 2017). Specifically, phylogenies were handled using the R packages 'ape' (Paradis et al., 2004) and 'ggtree' (Yu et al., 2017).

Many QCPA parameters are organised into triplets comprising the horizontal (.hrz), vertical (.vrt), and combined parameters. We removed horizontal and vertical parameters >90% correlated with their combined measure to reduce model size and aid interpretability. This procedure removed 103 highly collinear parameters, resulting in 55 remaining for analysis (for residual collinearity, see

Figure S6). We adapted PFA to estimate the evolutionary correlation between the latent factors associated with the QCPA parameters and daytime activity. Specifically, discrete activity patterns were mapped to continuous space using a phylogenetic latent liability model (Cybis et al., 2015), and the QCPA factors and latent daytime traits were jointly modelled according to a multivariate Brownian diffusion (MBD) model (Felsenstein, 1985). Additionally, rather than taking the average QCPA value for each species, our PFA analysis used all 346 individuals across 45 species by appending zero-branch length polytomies to the phylogeny for each individual in each species.

We compared the posterior mean values for the first row of the PFA loadings matrix to determine four factors sufficient to explain the variation in our dataset (Figure S4). As Bayesian latent factor models (of which PFA is an example) can suffer from parameter non-identifiability (Anderson & Rubin, 1956), we post-processed the posterior distribution using generalised Procrustes analysis to induce parameter identifiability (Aßmann et al., 2016; Okada & Mayekawa, 2018). We then rotate all posterior samples so that only the first factor can (but not necessarily does) have a non-zero expected correlation with daytime activity. The correlation between two latent variables can be challenging to interpret. As such, we evaluated the ability of this factor to predict daytime activity via its receiver operating characteristic (ROC) and prediction accuracy. See the Supplement on Github for a complete repository of all code.

2.7 | Chemistry data

We discuss the putative signalling function of colour patterns using previously published toxicity and unpalatability assays on eight species of nudibranchs included in this study (Winters et al., 2022; Winters, White, et al., 2018) (Table S2). Winters et al. (2022) quantified unpalatability by measuring the concentration of whole-body extracts at which 50% of rockpool shrimps (*Palaemon serenus*) would reject feeding pellets (ED_{50}). Toxicity was measured by observing the concentration of dissolved whole-body extracts at which 50% of brine shrimp would die (LD_{50}). See the original publications for details on the precise methodology of how chemicals were extracted. In short, animals were collected on-site across Queensland and New South Wales in Australia, with many individuals originating from the same sites as those visited for this study. Individuals were stored at -20°C until processed. Using vertebrates for toxicity and palatability assays is difficult for ethical reasons and remains rare (e.g. Cortesi & Cheney, 2010; Mollo et al., 2005; Winters et al., 2022). Therefore, toxicity and palatability assays using invertebrates such as crustaceans are more common and considered broadly ecologically representative (e.g. Haber et al., 2010; Wägele et al., 2006; Winters et al., 2022), with the suitability of brine shrimp assays to indicate the toxicity of nudibranch chemical defences for fish predators having been investigated in detail by Chan et al. (2021). Given the highly uneven

spread of these values and to represent them in rough categories, we ranked them from highest to lowest by subtracting each LD₅₀ and ED₅₀ value from 1 and designating each species to an unpalatability score of 'none' ($1 - ED_{50}/LD_{50} = 0$), 'weak' ($1 - ED_{50}/LD_{50} = 0.01$ to 0.24), 'medium' ($1 - ED_{50}/LD_{50} = 0.25$ to 0.74) and 'high' ($1 - ED_{50}/LD_{50} = 0.75$ to 1).

3 | RESULTS

We chose a four-factor model as the most parsimonious model that captures the most variation in our data (Figure S4). As the post-processing described above means only factor 1 can be associated with daytime activity, we focused exclusively on presenting this factor. To understand what visual attributes define factor 1, we need to understand the loadings of the associated colour pattern statistics (Figure 1). We focus our discussion on parameters with the strongest loadings as these contribute the most to factor 1. For a detailed overview of each colour pattern parameter, see the Supplement (Table S5).

Local edge intensity analysis (LEIA): Diurnal species had stronger and more variable luminance contrast edges and weaker saturation contrast edges compared to nocturnal species.

Factor 1 was positively associated with multiple measures of visual contrast elicited by luminance and colour LEIA contrast (Figure 1). Positive loadings included the mean luminance LEIA contrast (*Lum.mean*), the standard deviation of luminance LEIA contrast (*Lum.sd*), the kurtosis of the edge intensity histogram for chromatic edges (*Col.kurtosis*), the skew of the histogram for both chromatic and achromatic edges (*Col.skew*, *Lum.skew*), as well as the coefficient of variation for both chromatic and achromatic edges (*col.CoV*, *Lum.CoV*). Therefore, species with high positive factor 1 scores appear to express more striking internal achromatic and chromatic contrast, with overall increased amounts of achromatic edges. However, factor 1 is not clearly associated with the standard deviation of chromatic edges (*Col.sd*) and the mean chromatic LEIA contrast (*Col.mean*). See the supplement for additional discussion of LEIA factor loadings.

Colour adjacency analysis (CAA): Diurnal species were more elongated and/or stripey and more regularly patterned.

Factor 1 was positively associated with the Shannon and Simpson transition diversity (*CAA.Ht/CAA.St*), meaning that species with a high loading of factor 1 either express more different kinds of colour pattern elements or, for the same number of colour pattern elements, have them arranged more regularly. Factor 1 was also

positively associated with an overall decrease in transition regularity (*CAA.Jt*), indicating that animals with high factor 1 scores are more 'deliberately' (less randomly/mottled) patterned. However, the inverse was the case for the Shannon regularity of transitions across the horizontal axis (*CAA.Qt.hrz*), which indicates that patterns with high factor 1 scores are more likely to display all possible transitions in a colour pattern across the horizontal body axis. The reduction in aspect ratio (*CAA.Asp*) associated with factor 1 indicates that, overall, animals or patterns with high factor 1 scores are more elongated along the anterior-posterior axis. However, this could be caused by posture or patterns such as stripes instead of spots. See the supplement for additional discussion of CAA factor loadings.

Boundary strength analysis (BSA): Diurnal species had more contrasting and variable achromatic colour pattern edges, yet showed differential intensity and variability of saturation and hue boundary contrast.

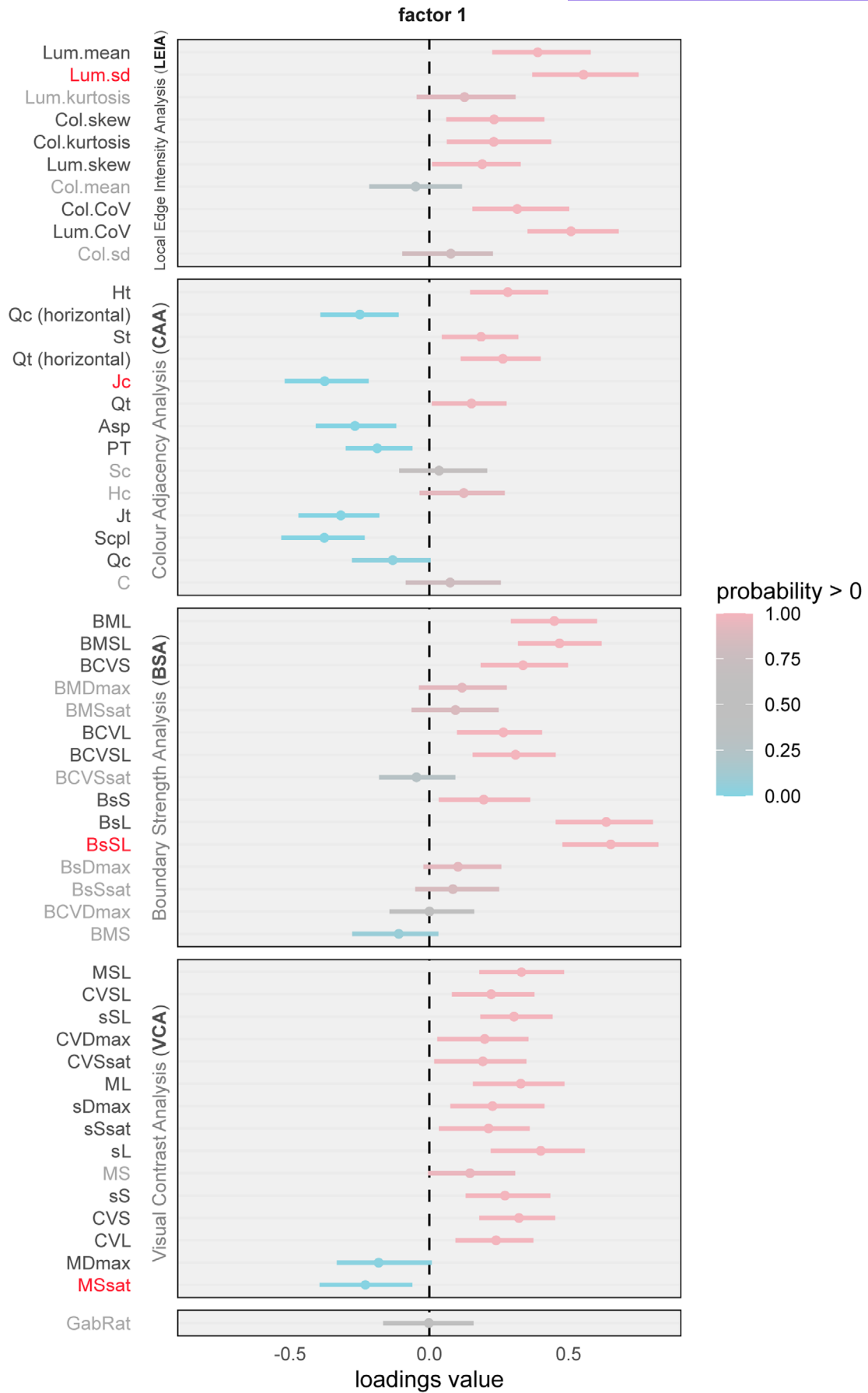
The loadings of BSA parameters largely mirror those of LEIA. For example, colour pattern boundaries in animals associated with factor 1 were overall brighter, independent of how the average luminance contrast of boundaries was assessed (*BSA.BML/BSA.BMSL*). Factor 1 was more associated with the standard deviation of luminance contrast of colour pattern boundaries when measured using BSA (*BSA.BsL/BSA.BsSL*) than LEIA (*Lum.sd*). Similar to the strong correlation with factor 1 for the variation of luminance boundary contrast relative to the mean luminance of animals (*Lum.CoV*) when measured by LEIA, this was also true for the analogue BSA metrics (*BSA.BCVL/BSA.BCVSL*).

Visual contrast analysis (VCA): Diurnal species have small colourful patches contrasting against larger achromatic colour pattern elements.

The contrast between colour pattern elements in animals with high factor 1 scores was overall higher, as LEIA and BSA would imply. However, while the contrast between colour pattern elements in animals with high factor 1 scores was notably less saturated on average (*VCA.MDmax/VCA.MSsat*), it was slightly positively associated with hue (*VCA.MS*). This is opposite to the BSA results and highlights the differences between considering colour pattern boundaries and patches.

Despite being overall less saturated in appearance (*VCA.Mssat*), the variation in chromaticity (*VCA.sS*) and saturation (*VCA.sSsat*) contrast between colour pattern elements was larger in animals with high factor 1 scores, even when considering how colourful or saturated animals are on average. This suggests that animals with high

FIGURE 1 Factor 1 loadings ($n=55$) with auto-correlated factors ($n=103$) removed. The values indicated with red and blue bars indicate the probability that a colour pattern parameter is associated with factor 1, with the loadings value indicating the strength and direction of a parameter's association with factor 1. Greyed-out parameters are unlikely to contribute to factor 1. Parameters highlighted in red are represented visually for their minimum and maximum values in Supporting Information (Figure S7).



factor 1 scores express colour patterns with small, relatively colourful colour pattern elements contrasting with more dominating colour pattern elements of low saturation, such as white or black. See the supplement for additional discussion of factor loadings.

3.1 | Using factor 1 to determine daytime activity

Using the posterior of the covariance matrix from the MBD process on the tree, we estimated the evolutionary correlation between this first factor and daytime activity patterns to be 0.37 with a 95% highest posterior density interval of 0.23–0.50. This correlation indicates a statistically significant, phylogenetically corrected association between colour patterns and daytime activity. By selecting an optimal threshold (i.e. the value at which false classification is minimal), this factor correctly predicted the activity pattern of 39 out of 45 (87%) taxa (Figure 2). It achieved an area under the ROC curve of 0.91 (Figure S5), which is considered 'outstanding' predictive performance (Hosmer et al., 2013).

3.2 | Phylogenetic patterns of daytime activity

Mapping the factor 1 scores of each species onto the consensus tree revealed highly heterogeneous values within and among clades (Figure 3, median \pm SD). Specifically, we found generally uniform positive factor 1 scores for the chromodorid nudibranch superfamily (Chromodoridoidea) (0.06 ± 0.17). In contrast, Doridoidea (-0.47 ± 0.24) and Phyllidoidea (-0.53 ± 0.31) showed consistently low factor levels. However, the superfamily Polyceroidea was highly variable in its factor values (-0.22 ± 0.47), even at the family level, showing high, neutral and negative correlations with factor 1 (Figure 3). Finally, the superfamily of Aeolidoidea showed very little positive or negative correlation with factor 1 (Figure 3) (-0.03 ± 0.16).

3.3 | Level of chemical defence

All species with known chemical defences in our dataset ($n=5$) were diurnal, whereas species with a known absence of chemical defences ($n=3$) were both diurnal and nocturnal (Figure 3; Table S1). Species with chemical defences had slightly positive scores for factor 1 (median = 0.09, SD = 0.19). Species without chemical defences ($n=3$) (Figure 3; Table S1), on average, had slightly negative factor 1 scores (median = -0.25 , SD = 0.24).

4 | DISCUSSION

Our study investigated whether daytime activity correlates with the colours and patterns of heterobranch sea slugs according to the visual system of a potential predator. Using a customised pipeline of imaging, vision modelling, colour pattern analysis, and subsequent

phylogenetic and statistical tools, we identified a highly descriptive latent factor strongly correlated with daytime activity. The factor comprised 55 distinct colour pattern descriptors, allowing us to explore differences in the visual phenotype of animals coinciding with variations in diel activity. Specifically, diurnal species had a more positive association with factor 1 than nocturnal species (Figures 2 and 3).

Among the most notable differences in colour patterning between diurnal and nocturnal species was that highly positive scores for factor 1 were associated with colour patterns that were lower in saturation and brighter in appearance to a potential trichromatic fish predator (Figure 1). A strong positive association with factor 1 indicated a higher proportion of achromatic colour pattern elements, such as white, greys and black, or long-wavelength colours such as red, which appear dark to *Rhinecanthus aculeatus* due to short wavelength-shifted spectral sensitivities (Losey et al., 2003) (Figure 2). This increase in achromatic patches corresponded with a higher presence of colour pattern boundaries with greater average luminance contrast and increased variability of luminance contrasts in diurnal species. Species positively associated with factor 1 exhibited smaller colour pattern elements with significantly contrasting chromaticity, saturation and hue compared to more achromatically contrasting colour pattern elements. These species displayed bolder, more patterned visual phenotypes, contrasting species with more mottled patterns or colours and shades of luminance that were more evenly mixed rather than arranged in structured, geometric patterns.

Many species use colour patterns to disrupt their body outline and achieve camouflage (Stevens & Cuthill, 2006). Our data indicate that species with high factor 1 scores exhibit colour patterns that generally follow the elongated shape of an animal and are therefore likely to enhance the appearance of nudibranch body shape, rather than break it up. However, this patterning does not seem to occur exclusively along the body outline (as is common in some highly aposematic chromodorid nudibranchs), but also appears in internally contrasting pattern elements, as suggested by the low GabRat loading. In summary, we demonstrate that the correlations between colour pattern design and daytime activity are complex, and capturing these intricate relationships requires a highly differentiated quantitative analysis that goes beyond a few pre-determined colour pattern descriptors (Kemp et al., 2023; van den Berg et al., 2022; van den Berg, Troscianko, et al., 2020).

Overall, the systematic shifts in colour pattern morphology indicated by factor 1 align with well-established mechanisms underlying the strategic and tactical design of aposematic signals and camouflage (Rowe, 2013; Ruxton et al., 2018). Nocturnal animals tended to have a mottled or drab colouration, with a notable absence of strongly contrasting, bold colour patterns. These findings suggest that bold, aposematic colouration is likely limited to species active during the day, a conclusion supported by the presence of various chemically defended species with high factor 1 loadings (Figure 3). For example, chromodorid nudibranchs are well-known for their vibrant colour patterns paired with potent chemical defences (Cheney et al., 2016; Rudman, 1991; Winters, White, et al., 2018).

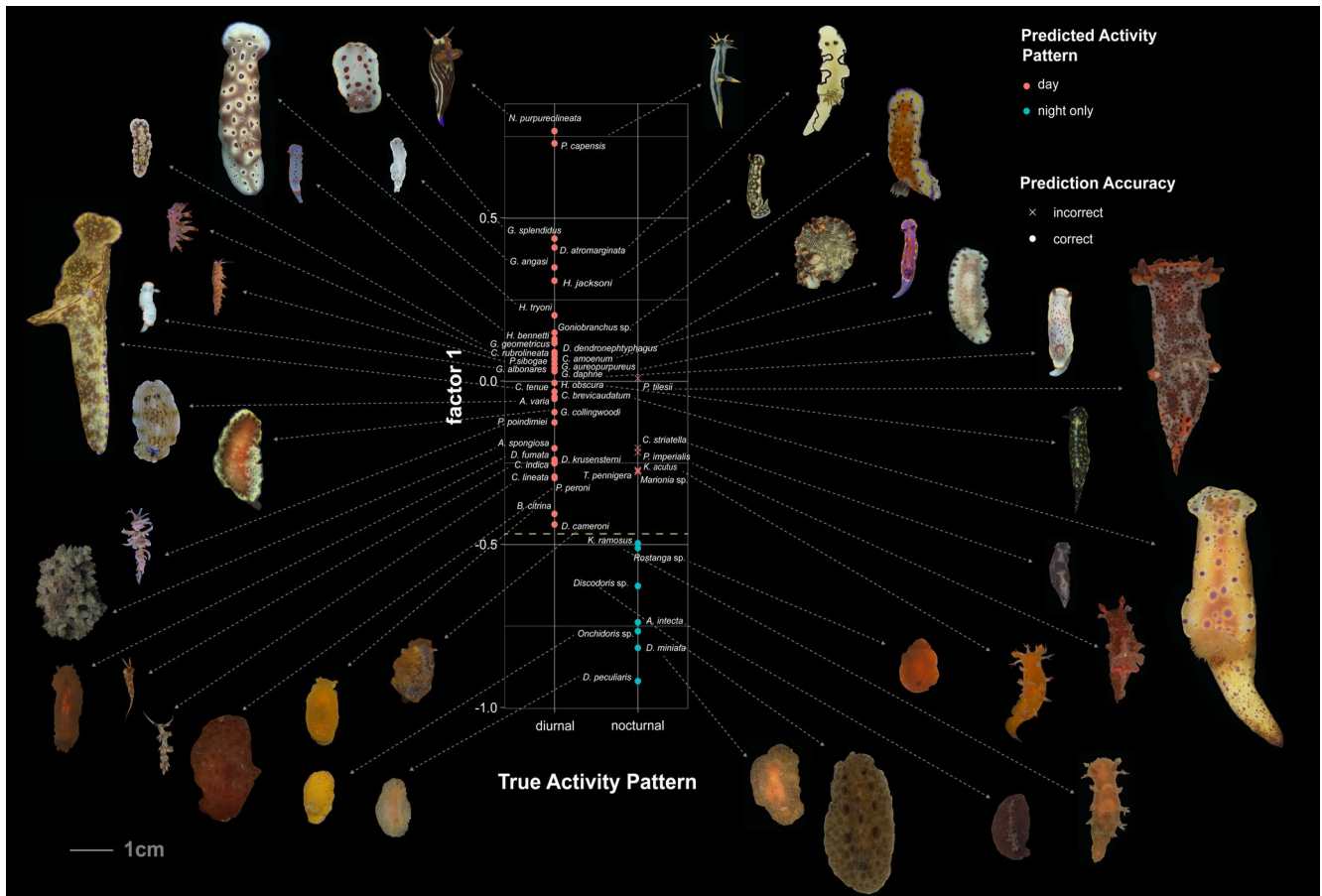


FIGURE 2 Prediction of daytime activity using factor 1, with each species visually represented. The yellow dashed line indicates the boundary between nocturnal and diurnal species. Factor 1 accurately predicted daytime activity for 87% of species (39 out of 45).

Additionally, none of the species in a putative red-spot mimicry ring present in the area (Rudman, 1991; Winters, White, et al., 2018) and represented in our study are nocturnal or have negative factor 1 values. These species included *G. splendidus*, *G. daphne*, *C. amoenum* and *H. bennetti* (Figures 2 and 3). This suggests that similar to aposematic signalling, colour pattern designs with a signalling purpose, such as mimicry, are less likely to be found in nocturnal heterobranch sea slugs. Our results also indicate that nocturnally active species seem less reliant on bold visual signalling, whether for aposematic signalling or mimicry.

However, visually hunting predators during the day may still find nocturnally active species. For example, *Plocamopherus imperialis* was readily encountered during daytime surveys in the benthic algae, despite being predominately nocturnal according to Larkin et al. (2018). Interestingly, species in the genera *Plocamopherus* and *Kaloplocamus* are known for producing bioluminescence, likely as a distraction or deimatic display (Vallès & Gosliner, 2006). The conspicuous swimming defence of *Plocamopherus tilesii* may complement these dynamic visual defences when disturbed. Additionally, *P. tilesii*'s relatively contrasting patterning (Figure 2; Figure S8) could further aid in deimatic defence against visual predators. This highlights that secondary defences in heterobranch sea slugs are not exclusively associated with boldly coloured phenotypes. The

evolution of chemical defences, possibly as a by-product of dietary specialisation, may have preceded the appearance of aposematism (e.g. Cortesi & Cheney, 2010; Winters et al., 2022). Nonetheless, our results generally support Larkin et al.'s (2018) findings that most nocturnal species are efficiently concealed from visual predators during the daytime.

Interestingly, aeolid species exhibit a relatively uniform (factor 1 value ≈ 0) association with factor 1 that is somewhat lower than that of chemically defended dorid species (Figure 3). The difference in body patterning between aeolids and non-aeolids may be driven by their distinct body plan and types of secondary defences. In aeolids, the majority of the visible dorsal area is covered by intricate, nematocyst-loaded cerata (Wägele, 2004), which, along with their generally smaller body size (but see *Pteraeolidia ianthina*, Figure 2), may prevent the expression of large, boldly contrasting patches commonly seen in other putatively aposematic diurnal heterobranch sea slugs. From a statistical perspective, the lack of a strong correlation with factor 1 might be due to the presence of multiple 'daytime' suites of traits that differ from each other. For example, there could be a suite of camouflage traits and another of aposematic traits. The methods we used cannot distinguish between these suites, so if one (e.g. aposematism) is more dominant in our dataset, species employing the other (e.g. camouflage) may not load onto the factor.

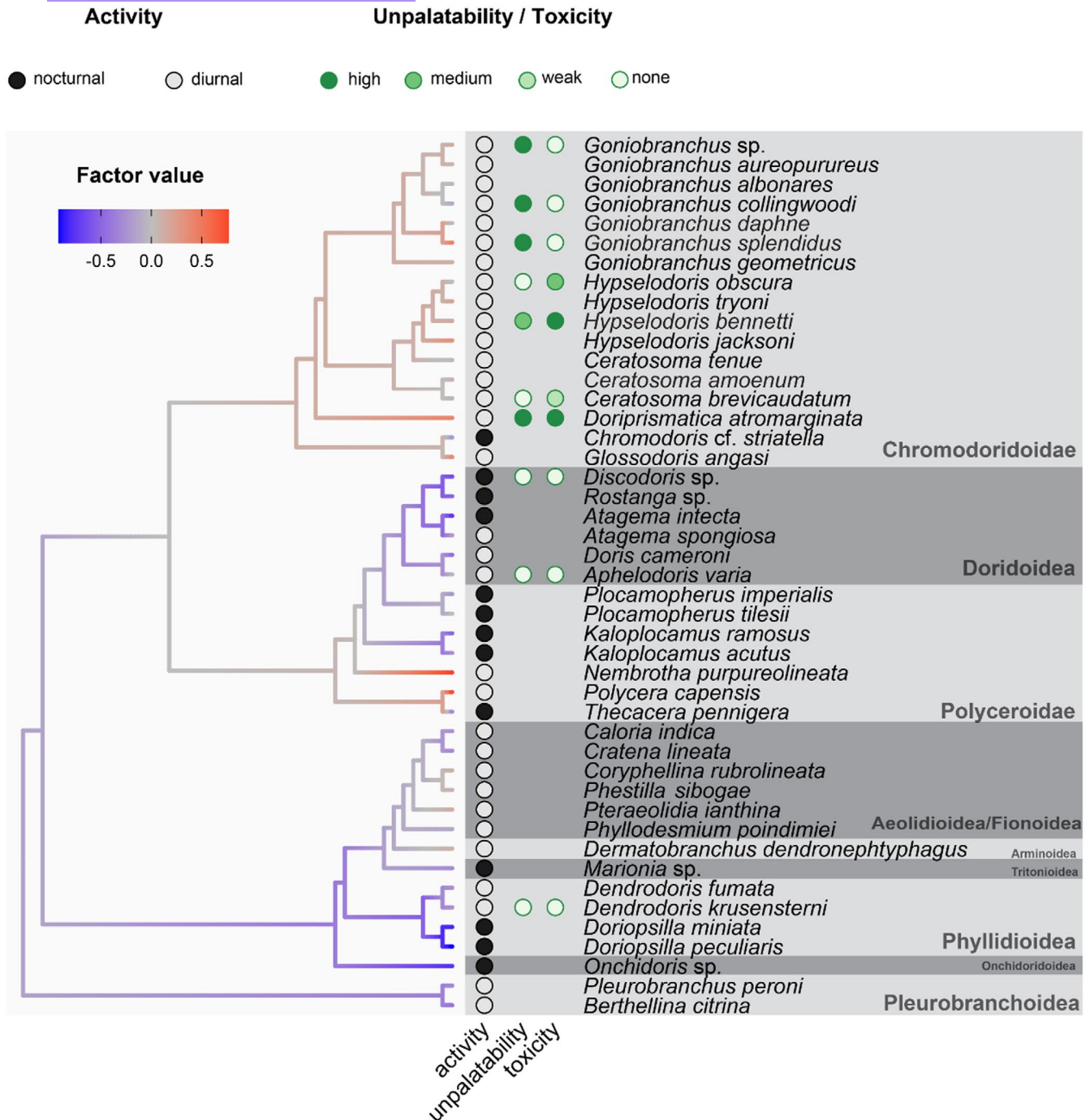


FIGURE 3 Visualisation of factor 1 on the consensus tree. Nocturnal species are indicated with a black dot. Chemistry data from Winters et al. (2022) was used to indicate the presence or absence of chemical defences. Superfamilies are indicated with grey overlays. Branch lengths correspond to Grafen's branch lengths (Grafen, 1989).

Nevertheless, a factor value of 0 is itself associated with diurnal activity (Figure 2).

Several critical gaps in knowledge prevent us from providing a more definitive explanation for the observed differences in colouration in our dataset. These gaps include a lack of understanding of predator-prey-specific interactions throughout species' evolutionary histories, the potential influence of animal-background interactions on the efficacy of visual defences, the diversity and function of primary and secondary defences beyond a few select families

of heterobranch sea slugs, and more detailed data on the relative abundance of predators and prey species across different environments and time periods. Notably, the presence of both, secondary defences (i.e. defences that act during physical interactions between predator and prey) and bold colouration, does not necessarily indicate aposematism. For example, bold colouration can also be used for camouflage, and co-occurring species may exploit honest signalling through Batesian or quasi-Batesian mimicry (see Summers et al., 2015; White & Umbers, 2021 for review). Additionally,

secondary defences have only been investigated in a fraction of the species considered in this study (e.g. Winters et al., 2022). Although Batesian and Müllerian mimicry have been repeatedly suggested in heterobranch sea slugs (Balfour et al., 2018; Haber et al., 2010; Winters, Wilson, et al., 2018), empirical evidence on the protective value of such mimicry remains largely suggestive (e.g. Green et al., 2018). Our dataset currently only contains a single nocturnal species with chemistry data, preventing any meaningful inferences between nocturnality and the lack of chemical defences.

Despite decades of research, empirical studies on whether the diversity of colour patterns in heterobranch sea slugs correlates with chemical defences remain incomplete (e.g. Cortesi & Cheney, 2010; Haber et al., 2010; Rudman, 1991). This is partly due to limited data on the toxicity and palatability of secondary metabolites, despite extensive identification of chemical compounds in these species (e.g. Cheney et al., 2016; Dean & Prinsep, 2017; Haber et al., 2010; Yong et al., 2015). Furthermore, novel colour pattern analyses, such as those presented in this study, continue to offer increased differentiation and quantitative insights into prey colouration as perceived by ecologically relevant observers. However, whether and how these data translate to prey survival remains an area of ongoing research (see van den Berg et al., 2022; van den Berg, Santon, et al., 2024; van den Berg, Endler, & Cheney, 2023 for discussion). Therefore, addressing the scarcity of chemical data and the uncertainty surrounding functional mimicry and aposematism in nudibranch species are promising directions for future research, particularly when considering the potential for integrating these findings into large-scale comparative analyses.

Our results suggest that high values of factor 1 are an emergent, rather than ancestral, property of heterobranch sea slugs in our study. This aligns with existing literature on the coevolution of chemical defences and aposematic signalling (Cortesi & Cheney, 2010; Rudman, 1991). However, heterobranch taxonomy remains in flux, with the ancestry of many clades remaining poorly understood (Layton et al., 2020). The evolutionary origins of colour pattern traits in our study are further complicated by the use of a taxonomy-informed supertree rather than a highly resolved, fully calibrated phylogenetic tree. For example, genetic data place *Tayuva lilacina*, a Discodorid species possibly found in our dataset, within the Policeroidea (Figures S1 and S3). However, taxonomically, this species is classified as part of the sister clade to the Doridoidea (Figure S2), highlighting discrepancies between genetic data and traditional taxonomy and the challenges in using a taxonomically complemented supertree. Moreover, while our study accurately represents local taxa, it only captures a fraction of the thousands of described species globally (Wägele & Willan, 2000). Colour patterns can vary widely between and within taxa over relatively short evolutionary time scales (e.g. Ronco et al., 2021) or even without any contribution of genetic adaptation (Layton et al., 2018), making it difficult to draw conclusions about ancestral states of heterobranch colouration. This is particularly challenging given the inclusion of phylogenetically distant taxa in our study, which could result in undetected convergence.

Our study analysed 45 species of sea slugs, with a sample size of a few hundred individuals—significantly fewer than recent comparative studies on the ecology and evolution of defensive colouration

across various taxa (e.g. Ezray et al., 2019; Hoyal Cuthill et al., 2019; Nokelainen et al., 2024; Robinson et al., 2023). However, extensive comparative studies on the ecology and evolution of defensive colouration often do not account for the visual perception of ecologically relevant observers due to the challenges and cost of obtaining sufficient data, such as calibrated images or spectral measurements. As a result, it can be difficult to identify relationships between how predators perceive prey and the resulting selective pressures (e.g. predation) shaping prey phenotype. Despite the extensive size of the dataset (by comparison) and the depth of analysis provided in this study, we do not consider how (and if) the observed correlations between daytime activity and phenotype change with viewing distance. This would be an essential avenue for future research (van den Berg, Endler, & Cheney, 2023; van den Berg, Santon, Endler, Drummond, et al., 2024), in addition to considering prey phenotypes in the context of the respective visual background against which predators see them (van den Berg, Santon, Endler, & Cheney, 2024).

In conclusion, our study shows a distinct correlation between daytime activity and colour pattern morphology in a community of heterobranch sea slugs. The differences in appearance between nocturnal and diurnal species to a potential predator follow broadly generalisable patterns, suggesting bold colour pattern contrast and correlated visual signalling, such as aposematism or mimicry, are associated with daytime activity. Importantly, our study introduces a scalable approach for integrating partially complete phylogenies with extensive descriptive datasets of colour patterns. Our methodological pipeline demonstrates a comprehensive approach to phylogenetic comparative analyses, specifically tailored to examine correlations between high-dimensional datasets and explanatory variables, even in the context of incomplete phylogenetic data, significant autocorrelation, missing data, and variable numbers of observations across taxa. We hope our study encourages the broader use of high-dimensional, latent colour pattern spaces using visual modelling in comparative studies of organismal colouration. Finally, studies of visual signalling and defensive colouration, particularly in marine systems, are disproportionately underrepresented (Cronin et al., 2014; Ruxton et al., 2018). We aim to contribute to filling this gap by highlighting the potential of heterobranch sea slugs as a promising system for future research into the ecology and evolution of defensive animal colouration.

AUTHOR CONTRIBUTIONS

Cedric P. van den Berg: Original concept (lead), data collection, software, data analysis, writing, project administration. Gabriel W. Hassler: Data Analysis, software, writing, review and editing. Simone P. Blomberg: Data analysis, software, writing, review and editing. Nerida G. Wilson: Data analysis, review and editing. Marc A. Suchard: Software, review and editing. Karen L. Cheney: Original concept, writing, review and editing, project supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw colour pattern data are available at: <https://doi.org/10.48610/a36c277> (van den Berg, Hassler, Blomberg, Wilson, et al., 2024). The code used for statistical analysis is available at: <https://github.com/gabehassler/Nudibranch-Colour>.

STATEMENT ON INCLUSION

The images used in this study were collected alongside local citizen scientists, academics and sea slug experts, profiting from their local knowledge of the field sites. The data were partially collected during local community events (dive surveys of sea slugs) and reported/contributed to the annual Nelson Bay Sea Slug Census.

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REFERENCES

- Ahyong, S., Boyko, C. B., Bailly, N., Bernot, J., Bieler, R., Brandão, S. N., Daly, M., De Grave, S., Gofas, S., Hernandez, F., Hughes, L., Neubauer, T. A., Paulay, G., Boydens, B., Decock, W., Dekeyser, S., Vandepitte, L., Vanhoorne, B., Adlard, R., ... Zullini, A. (2024). *World register of Marine species (WoRMS)*. WoRMS Editorial Board. <https://doi.org/10.14284/170>
- Alfaro, M. E., Karan, E. A., Schwartz, S. T., & Shultz, A. J. (2019). The evolution of color pattern in butterflyfishes (Chaetodontidae). *Integrative and Comparative Biology*, 59(3), 604–615. <https://doi.org/10.1093/icb/icz119>
- Allen, W. L., Moreno, N., Gamble, T., & Chiari, Y. (2019). Ecological, behavioral, and phylogenetic influences on the evolution of dorsal color pattern in geckos. *Evolution*, 74(6), 1–15. <https://doi.org/10.1111/evo.13915>
- Anderson, T. W., & Rubin, H. (1956). Statistical inference in factor analysis. *Proceedings of the Third Berkeley Symposium on Mathematical Statistics and Probability*, 5, 111–150.
- Arenas, L. M., Troscianko, J., & Stevens, M. (2014). Color contrast and stability as key elements for effective warning signals. *Frontiers in Ecology and Evolution*, 2, 1–12. <https://doi.org/10.3389/fevo.2014.00025>
- Arias, M., Mappes, J., Théry, M., & Llaurens, V. (2016). Inter-species variation in unpalatability does not explain polymorphism in a mimetic species. *Evolutionary Ecology*, 30(3), 419–433. <https://doi.org/10.1007/s10682-015-9815-2>
- Aßmann, C., Boysen-Hogrefe, J., & Pape, M. (2016). Bayesian analysis of static and dynamic factor models: An ex-post approach towards the rotation problem. *Journal of Econometrics*, 192(1), 190–206. <https://doi.org/10.1016/j.jeconom.2015.10.010>
- Balfour, V. L., Aumont, C., Dougherty, L. R., & Shuker, D. M. (2018). The fitness effects of a pale mutant in the aposematic seed bug *Lygaeus simulans* indicate pleiotropy between warning coloration and life history. *Ecology and Evolution*, 8(24), 12855–12866. <https://doi.org/10.1002/ece3.4723>
- Baum, B. R. (1992). Combining trees as a way of combining data sets for phylogenetic inference, and the desirability of combining gene trees. *Taxon*, 41(1), 3–10. <https://doi.org/10.2307/1222480>
- Bezanson, J., Edelman, A., Karpinski, S., & Shah, V. B. (2017). Julia: A fresh approach to numerical computing. *SIAM Review*, 59(1), 65–98. <https://doi.org/10.1137/141000671>
- Briolat, E. S., Burdfield-Steel, E. R., Paul, S. C., Katja, H. R., Seymoure, B. M., Stankowich, T., & Stuckert, A. M. M. (2019). Diversity in warning coloration: Selective paradox or the norm? *Biological Reviews*, 94(2), 388–414. <https://doi.org/10.1111/brv.12460>
- Camin, J. H., & Sokal, R. R. (1965). A method for deducing branching sequences in phylogeny. *Evolution*, 19(3), 311. <https://doi.org/10.2307/2406441>
- Champ, C. M., Wallis, G., Vorobyev, M., Siebeck, U., & Marshall, J. (2014). Visual acuity in a species of coral reef fish: *Rhinecanthus aculeatus*. *Brain, Behavior and Evolution*, 83(1), 31–42. <https://doi.org/10.1159/000356977>
- Chan, W., Shaughnessy, A. E. P., van den Berg, C. P., Garson, M. J., & Cheney, K. L. (2021). The validity of brine shrimp (*Artemia* Sp.) toxicity assays to assess the ecological function of Marine natural products. *Journal of Chemical Ecology*, 47(10–11), 834–846. <https://doi.org/10.1007/s10886-021-01264-z>
- Cheney, K. L., Cortesi, F., How, M. J., Wilson, N. G., Blomberg, S. P., Winters, A. E., Umanzör, S., & Marshall, N. J. (2014). Conspicuous visual signals do not coevolve with increased body size in marine sea slugs. *Journal of Evolutionary Biology*, 27(4), 676–687. <https://doi.org/10.1111/jeb.12348>
- Cheney, K. L., Hudson, J., de Busserolles, F., Luehrmann, M., Shaughnessy, A., van den Berg, C., Green, N. F., Marshall, N. J., & Cortesi, F. (2022). Seeing Picasso: An investigation into the visual system of the triggerfish *Rhinecanthus aculeatus*. *Journal of Experimental Biology*, 225(7), jeb243907. <https://doi.org/10.1242/jeb.243907>
- Cheney, K. L., Newport, C., McClure, E. C., & Marshall, N. J. (2013). Colour vision and response bias in a coral reef fish. *The Journal of Experimental Biology*, 216(15), 2967–2973. <https://doi.org/10.1242/jeb.087932>
- Cheney, K. L., White, A., Mudianta, I. W., Winters, A. E., Quezada, M., Capon, R. J., Mollo, E., & Garson, M. J. (2016). Choose your weaponry: Selective storage of a single toxic compound, latrunculin a, by

- closely related nudibranch molluscs. *PLoS One*, 11(1), 1–16. <https://doi.org/10.1371/journal.pone.0145134>
- Cortesi, F., & Cheney, K. L. (2010). Conspicuousness is correlated with toxicity in marine opisthobranchs. *Journal of Evolutionary Biology*, 23(7), 1509–1518. <https://doi.org/10.1111/j.1420-9101.2010.02018.x>
- Cott, H. B. (1940). Animal coloration and natural selection. *Nature*, 146, 144–145.
- Cronin, T. W., Johnsen, S., Marshall, N. J., & Warrant, E. J. (2014). *Visual ecology*. Princeton University Press.
- Cummings, M. E., & Endler, J. A. (2018). 25 years of sensory drive: The evidence and its watery bias. *Current Zoology*, 64(4), 471–484. <https://doi.org/10.1093/cz/zoy043>
- Cybis, G. B., Sinsheimer, J. S., Bedford, T., Mather, A. E., Lemey, P., & Suchard, M. A. (2015). Assessing phenotypic correlation through the multivariate phylogenetic latent liability model. *Annals of Applied Statistics*, 9(2), 969–991. <https://doi.org/10.1214/15-AOAS821>
- Daan, S. (1981). Adaptive daily strategies in behavior. In J. Aschoff (Ed.), *Biological rhythms* (pp. 275–298). Springer US. https://doi.org/10.1007/978-1-4615-6552-9_15
- Dalrymple, R. L., Flores-Moreno, H., Kemp, D. J., White, T. E., Laffan, S. W., Hemmings, F. A., Hitchcock, T. D., & Moles, A. T. (2018). Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. *Ecological Monographs*, 88(2), 204–224. <https://doi.org/10.1002/ecm.1287>
- De Cock, R., & Matthyssen, E. (1999). Aposematism and bioluminescence: Experimental evidence from glow-worm larvae (Coleoptera: Lampyridae). *Evolutionary Ecology*, 13(7–8), 619–639. <https://doi.org/10.1023/A:1011090017949>
- Dean, L. J., & Prinsep, M. R. (2017). The chemistry and chemical ecology of nudibranchs. *Natural Product Reports*, 34(12), 1359–1390. <https://doi.org/10.1039/c7np00041c>
- Díaz-Uriarte, R., & Garland, T. (1998). Effects of branch length errors on the performance of phylogenetically independent contrasts. *Systematic Biology*, 47(4), 654–672. <https://doi.org/10.1080/106351598260653>
- Eck, R. V., & Dayhoff, M. O. (1966). Evolution of the structure of ferredoxin based on living relics of primitive amino acid sequences. *Science*, 152(3720), 363–366. <https://doi.org/10.1126/science.152.3720.363>
- Endler, J. A. (1991). Interactions between predators and prey. In J. R. Krebs & N. B. Davies (Eds.), *Behavioural ecology* (pp. 169–196). Blackwell Scientific.
- Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *The American Naturalist*, 139, S125–S153. <https://doi.org/10.1086/285308>
- Endler, J. A. (2012). A framework for analysing colour pattern geometry: Adjacent colours. *Biological Journal of the Linnean Society*, 107(2), 233–253. <https://doi.org/10.1111/j.1095-8312.2012.01937.x>
- Endler, J. A., Cole, G. L., & Kranz, A. M. (2018). Boundary strength analysis: Combining colour pattern geometry and coloured patch visual properties for use in predicting behaviour and fitness. *Methods in Ecology and Evolution*, 9(12), 2334–2348. <https://doi.org/10.1111/2041-210X.13073>
- Endler, J. A., & Mielke, P. W. (2005). Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society*, 86(4), 405–431. <https://doi.org/10.1111/j.1095-8312.2005.00540.x>
- Ezray, B. D., Wham, D. C., Hill, C. E., & Hines, H. M. (2019). Unsupervised machine learning reveals mimicry complexes in bumblebees occur along a perceptual continuum. *Proceedings of the Royal Society B: Biological Sciences*, 286(1910), 20191501. <https://doi.org/10.1098/rspb.2019.1501>
- Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, 125(1), 1–15.
- Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package) (3.6). Distributed by the author.
- Grafen, A. (1989). The phylogenetic regression. *Philosophical Transactions of the Royal Society of London, Series B*, 326(1233), 119–157. <https://doi.org/10.1098/rstb.1989.0106>
- Green, N. F., Guevara, E., Osorio, D. C., Endler, J. A., Marshall, N. J., Vorobyev, M., & Cheney, K. L. (2022). Colour discrimination thresholds vary throughout colour space in a reef fish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology*, 225(7), jeb243533. <https://doi.org/10.1242/jeb.243533>
- Green, N. F., Urquhart, H. H., van den Berg, C. P., Marshall, N. J., & Cheney, K. L. (2018). Pattern edges improve predator learning of aposematic signals. *Behavioral Ecology*, 29, 1481–1486. <https://doi.org/10.1093/beheco/ary089>
- Guilford, T., & Cuthill, I. (1989). Aposematism and bioluminescence. *Animal Behaviour*, 37(PART 2), 339–341. [https://doi.org/10.1016/0003-3472\(89\)90126-7](https://doi.org/10.1016/0003-3472(89)90126-7)
- Haber, M., Cerfeda, S., Carbone, M., Calado, G., Gaspar, H., Neves, R., Maharajan, V., Cimino, G., Gavagnin, M., Ghiselin, M. T., & Mollo, E. (2010). Coloration and defense in the nudibranch gastropod *Hypselodoris fontandraui*. *Biological Bulletin*, 218, 181–188. 218/2/181 [pii].
- Hassler, G. W., Gallone, B., Aristide, L., Allen, W. L., Tolkoff, M. R., Holbrook, A. J., Baele, G., Lemey, P., & Suchard, M. A. (2022). Principled, practical, flexible, fast: A new approach to phylogenetic factor analysis. *Methods in Ecology and Evolution*, 13(10), 2181–2197. <https://doi.org/10.1111/2041-210X.13920>
- Hickman, C. P., Keen, S. L., Larson, A., & Eisenhour, D. J. (2000). *Animal diversity* (9th ed.). Mc-Graw.
- Hosmer, D. W., Lemeshow, S., & Sturdivant, R. X. (2013). *Applied logistic regression*. Wiley. <https://doi.org/10.1002/9781118548387>
- Hoyal Cuthill, J. F., Guttenberg, N., Ledger, S., Crowther, R., & Huertas, B. (2019). Deep learning on butterfly phenotypes tests evolution's oldest mathematical model. *Science Advances*, 5(8), 1–12. <https://doi.org/10.1126/sciadv.aaw4967>
- Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kelber, A., Balkenius, A., & Warrant, E. J. (2002). Scotopic colour vision in nocturnal hawkmoths. *Nature*, 419(6910), 922–925. <https://doi.org/10.1038/nature01065>
- Kelber, A., Vorobyev, M., & Osorio, D. C. (2003). Animal colour vision—Behavioural tests and physiological concepts. *Biological Reviews of the Cambridge Philosophical Society*, 78(1), 81–118. <https://doi.org/10.1017/S1464793102005985>
- Kemp, D. J., Reznick, D. N., Arendt, J., van den Berg, C., & Endler, J. A. (2023). How to generate and test hypotheses about colour: Insights from half a century of guppy research. *Proceedings of the Royal Society B: Biological Sciences*, 290(2000), 20222492. <https://doi.org/10.1098/rspb.2022.2492>
- Kluge, A. G., & Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Systematic Biology*, 18(1), 1–32. <https://doi.org/10.1093/sysbio/18.1.1>
- Krug, P. J., Caplins, S. A., Algosó, K., Thomas, K., Valdés, Á. A., Wade, R., Wong, N. L. W. S., Eernisse, D. J., & Kocot, K. M. (2022). Phylogenomic resolution of the root of Panpulmonata, a hyperdiverse radiation of gastropods: New insight into the evolution of air breathing. *Proceedings of the Royal Society B: Biological Sciences*, 289(1972), 20211855. <https://doi.org/10.1098/rspb.2021.1855>
- Kuwamura, T. (1997). Evolution of female egg care in harem triggerfish, *Rhinecanthus aculeatus*. *Ethology*, 103(12), 1015–1023. <https://doi.org/10.1111/j.1439-0310.1997.tb00143.x>
- Larkin, M. F., Smith, S. D. A., Willan, R. C., & Davis, T. R. (2018). Diel and seasonal variation in heterobranch sea slug assemblages within

- an embayment in temperate eastern Australia. *Marine Biodiversity*, 48(3), 1541–1550. <https://doi.org/10.1007/s12526-017-0700-9>
- Layton, K. K. S., Carvajal, J. I., & Wilson, N. G. (2020). Mimicry and mitochondrial discordance in nudibranchs: New insights from exon capture phylogenomics. *Ecology and Evolution*, 10(21), 11966–11982. <https://doi.org/10.1002/ece3.6727>
- Layton, K. K. S., Gosliner, T. M., & Wilson, N. G. (2018). Flexible colour patterns obscure identification and mimicry in Indo-Pacific *Chromodoris nudibranchs* (Gastropoda: Chromodorididae). *Molecular Phylogenetics and Evolution*, 124, 27–36. <https://doi.org/10.1016/j.ympev.2018.02.008>
- Li, D., Trotta, L., Marx, H. E., Allen, J. M., Sun, M., Soltis, D. E., Soltis, P. S., Guralnick, R. P., & Baiser, B. (2019). For common community phylogenetic analyses, go ahead and use synthesis phylogenies. *Ecology*, 100(9), 1–15. <https://doi.org/10.1002/ecy.2788>
- Lindstedt, C., Lindström, L., & Mappes, J. (2009). Thermoregulation constrains effective warning signal expression. *Evolution*, 63(2), 469–478. <https://doi.org/10.1111/j.1558-5646.2008.00561.x>
- Losey, G. S., McFarland, W. N., Loew, E. R., Zamzow, J. P., Nelson, P. A., & Marshall, N. J. (2003). Visual biology of Hawaiian coral reef fishes. I. Ocular transmission and visual pigments. *Copeia*, 2003(3), 433–454. <https://doi.org/10.1643/01-053>
- Marek, P., Papaj, D., Yeager, J., Molina, S., & Moore, W. (2011). Bioluminescent aposematism in millipedes. *Current Biology*, 21(18), 1–6. <https://doi.org/10.1016/j.cub.2011.08.012>
- Merilaita, S., & Tullberg, B. S. (2005). Constrained camouflage facilitates the evolution of conspicuous warning coloration. *Evolution*, 59(1), 38–45. <https://doi.org/10.1111/j.0014-3820.2005.tb00892.x>
- Mollo, E., Gavagnin, M., Carbone, M., Guo, Y. W., & Cimino, G. (2005). Chemical studies on Indopacific *Ceratosoma nudibranchs* illuminate the protective role of their dorsal horn. *Chemoecology*, 15(1), 31–36. <https://doi.org/10.1007/s00049-005-0289-5>
- Mutterer, J., & Rasband, W. (2012). *ImageJ macro language programmer's reference guide v1.46d*. RSB Homepage, 1–45.
- Nokelainen, O., De Moraes Rezende, F., Valkonen, J. K., & Mappes, J. (2022). Context-dependent coloration of prey and predator decision making in contrasting light environments. *Behavioral Ecology*, 33(1), 77–86. <https://doi.org/10.1093/beheco/arab111>
- Nokelainen, O., Silvasti, S. A., Strauss, S. Y., Wahlberg, N., & Mappes, J. (2024). Predator selection on phenotypic variability of cryptic and aposematic moths. *Nature Communications*, 15(1), 1678. <https://doi.org/10.1038/s41467-024-45329-5>
- Okada, K., & Mayekawa, S. i. (2018). Post-processing of Markov chain Monte Carlo output in Bayesian latent variable models with application to multidimensional scaling. *Computational Statistics*, 33(3), 1457–1473. <https://doi.org/10.1007/s00180-017-0759-6>
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20(2), 289–290. <https://doi.org/10.1093/bioinformatics/btg412>
- R Core Team. (2021). *R: A language and environment for statistical computing (4.1)*. R Foundation for Statistical Computing. <http://www.r-project.org/>
- Ragan, M. A. (1992). Phylogenetic inference based on matrix representation of trees. *Molecular Phylogenetics and Evolution*, 1(1), 53–58. [https://doi.org/10.1016/1055-7903\(92\)90035-F](https://doi.org/10.1016/1055-7903(92)90035-F)
- Randall, J. E., Allen, G. R., & Steene, R. C. (1997). *Fishes of the great barrier reef and coral sea*. Crawford House Publishing.
- Rassweiler, A., Dubel, A. K., Hernan, G., Kushner, D. J., Caselle, J. E., Sprague, J. L., Kui, L., Lamy, T., Lester, S. E., & Miller, R. J. (2020). Roving divers surveying fish in fixed areas capture similar patterns in biogeography but different estimates of density when compared with belt transects. *Frontiers in Marine Science*, 7, 1–14. <https://doi.org/10.3389/fmars.2020.00272>
- Robinson, M. L., Weber, M. G., Freedman, M. G., Jordan, E., Ashlock, S. R., Yonenaga, J., & Strauss, S. Y. (2023). Macroevolution of protective coloration across caterpillars reflects relationships with host plants. *Proceedings of the Royal Society B: Biological Sciences*, 290(1991), 20222293. <https://doi.org/10.1098/rspb.2022.2293>
- Rojas, B., Devillechabrolle, J., & Endler, J. A. (2014). Paradox lost: Variable colour-pattern geometry is associated with differences in movement in aposematic frogs. *Biology Letters*, 10(6), 20140193. <https://doi.org/10.1098/rsbl.2014.0193>
- Ronco, F., Matschiner, M., Böhne, A., Boila, A., Büscher, H. H., El Taher, A., Indermaur, A., Malinsky, M., Ricci, V., Kahmen, A., Jentoft, S., & Salzburger, W. (2021). Drivers and dynamics of a massive adaptive radiation in cichlid fishes. *Nature*, 589(7840), 76–81. <https://doi.org/10.1038/s41586-020-2930-4>
- Rowe, C. (2013). Receiver psychology: A receiver's perspective. *Animal Behaviour*, 85(3), 517–523. <https://doi.org/10.1016/j.anbehav.2013.01.004>
- Rudman, W. B. (1991). Purpose in pattern—The evolution of color in chromodorid nudibranchs. *Journal of Molluscan Studies*, 57(1991), 5–21.
- Ruxton, G. D., Allen, W. L., Sherratt, T. N., & Speed, M. P. (2018). *Avoiding attack* (Vol. 1). Oxford University Press. <https://doi.org/10.1093/oso/9780199688678.001.0001>
- Salamin, N., Hodkinson, T. R., & Savolainen, V. (2002). Building supertrees: An empirical assessment using the grass family (Poaceae). *Systematic Biology*, 51(1), 136–150. <https://doi.org/10.1080/106351502753475916>
- Schluessel, V., Bennett, M. B., Bleckmann, H., Blomberg, S., & Collin, S. P. (2008). Morphometric and ultrastructural comparison of the olfactory system in elasmobranchs: The significance of structure-function relationships based on phylogeny and ecology. *Journal of Morphology*, 269(11), 1365–1386. <https://doi.org/10.1002/jmor.10661>
- Semlitsch, R. D., & Pechmann, J. H. K. (1985). Diel pattern of migratory activity for several species of pond-breeding salamanders. *Copeia*, 1985(1), 86. <https://doi.org/10.2307/1444794>
- Siebeck, U. E., Wallis, G. M., Litherland, L., Ganeshina, O., & Vorobyev, M. (2014). Spectral and spatial selectivity of luminance vision in reef fish. *Frontiers in Neural Circuits*, 8, 118. <https://doi.org/10.3389/fncir.2014.00118>
- Stevens, M., & Cuthill, I. C. (2006). Disruptive coloration, crypsis and edge detection in early visual processing. *Proceedings of the Royal Society B*, 273, 2141–2147. <https://doi.org/10.1098/rspb.2006.3556>
- Stone, E. A. (2011). Why the phylogenetic regression appears robust to tree misspecification. *Systematic Biology*, 60(3), 245–260. <https://doi.org/10.1093/sysbio/syq098>
- Summers, K., Speed, M. P., Blount, J. D., & Stuckert, A. M. M. (2015). Are aposematic signals honest? A review. *Journal of Evolutionary Biology*, 28(9), 1583–1599. <https://doi.org/10.1111/jeb.12676>
- Tolkoff, M. R., Alfaro, M. E., Baele, G., Lemey, P., & Suchard, M. A. (2018). Phylogenetic factor analysis. *Systematic Biology*, 67(3), 384–399. <https://doi.org/10.1093/sysbio/syx066>
- Trifinopoulos, J., Nguyen, L. T., von Haeseler, A., & Minh, B. Q. (2016). W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, 44(W1), W232–W235. <https://doi.org/10.1093/nar/gkw256>
- Troschianko, J., Skelhorn, J., & Stevens, M. (2017). Quantifying camouflage: How to predict detectability from appearance. *BMC Evolutionary Biology*, 17(1), 7. <https://doi.org/10.1186/s12862-016-0854-2>
- Troschianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox—A free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution*, 6(11), 1320–1331. <https://doi.org/10.1111/2041-210X.12439>
- Vallès, Y., & Gosliner, T. M. (2006). Shedding light onto the genera (Mollusca: Nudibranchia) *Kaloplocamus* and *Plocamopherus* with description of new species belonging to these unique bioluminescent dorids. *Veliger*, 48(3), 178–205.
- van den Berg, C. P., Condon, N. D., Conradsen, C., White, T. E., & Cheney, K. L. (2024). Automated workflows using quantitative colour pattern analysis (QCPA): a guide to batch processing and downstream

- data analysis. *Evolutionary Ecology*, 38(3), 0123456789. <https://doi.org/10.1007/s10682-024-10291-7>
- van den Berg, C. P., Endler, J. A., & Cheney, K. L. (2023). Signal detectability and boldness are not the same: The function of defensive coloration in nudibranchs is distance-dependent. *Proceedings of the Royal Society B: Biological Sciences*, 290(2003), 20231160. <https://doi.org/10.1098/rspb.2023.1160>
- van den Berg, C. P., Endler, J. A., Papinczak, D. E. J., & Cheney, K. L. (2022). Using colour pattern edge contrast statistics to predict detection speed and success in triggerfish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology*, 225(23), jeb244677. <https://doi.org/10.1242/jeb.244677>
- van den Berg, C. P., Hassler, G., Blomberg, S. P., Wilson, N. G., Suchard, M. A., & Cheney, K. L. (2024). Data for 'Diel activity correlates with colour pattern morphology of heterobranch sea slugs'. *UQ ESpace*. <https://doi.org/10.48610/a36c277>
- van den Berg, C. P., Hollenkamp, M., Mitchell, L. J., Watson, E. J., Green, N. F., Marshall, N. J., & Cheney, K. L. (2020). More than noise: Context-dependant luminance contrast discrimination in a coral reef fish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology*, 223(21), jeb.232090. <https://doi.org/10.1242/jeb.232090>
- van den Berg, C. P., Santon, M., Endler, J. A., & Cheney, K. L. (2024). Highly defended nudibranchs "escape" to visually distinct background habitats. *Behavioral Ecology*, 35(5). <https://doi.org/10.1093/beheco/arae053>
- van den Berg, C. P., Santon, M., Endler, J. A., Drummond, L., Dawson, B. R., Santiago, C., Weber, N., & Cheney, K. L. (2024). Chemical defences indicate bold colour patterns with reduced variability in aposematic nudibranchs. *Proceedings of the Royal Society B: Biological Sciences*, 291(2027), 20240953. <https://doi.org/10.1098/rspb.2024.0953>
- van den Berg, C. P., Troscianko, J., Endler, J. A., Marshall, N. J., & Cheney, K. L. (2020). Quantitative colour pattern analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods in Ecology and Evolution*, 11(2), 316–332. <https://doi.org/10.1111/2041-210X.13328>
- Vorobyev, M., & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 265(1394), 351–358. <https://doi.org/10.1098/rspb.1998.0302>
- Wägele, H. (2004). Potential key characters in Opisthobranchia (Gastropoda, Mollusca) enhancing adaptive radiation. *Organisms, Diversity and Evolution*, 4, 175–188. <https://doi.org/10.1016/j.ode.2004.03.002>
- Wägele, H., Ballesteros, M., & Avila, C. (2006). Defensive glandular structures in opisthobranch molluscs—From histology to ecology. *Oceanography and Marine Biology*, 44, 197–276. <https://doi.org/10.1201/9781420006391.ch5>
- Wägele, H., & Willan, R. (2000). Phylogeny of the Nudibranchia. *Zoological Journal of the Linnean*, 130, 83–181. <https://doi.org/10.1006/zjls>
- White, T. E., & Umbers, K. D. L. (2021). Meta-analytic evidence for quantitative honesty in aposematic signals. *Proceedings of the Royal Society B: Biological Sciences*, 288(1949), 20210679. <https://doi.org/10.1098/rspb.2021.0679>
- Winters, A. E., Chan, W., White, A. M., van den Berg, C. P., Garson, M. J., & Cheney, K. L. (2022). Weapons or deterrents? Nudibranch molluscs use distinct ecological modes of chemical defence against predators. *Journal of Animal Ecology*, 91(4), 831–844. <https://doi.org/10.1111/1365-2656.13643>
- Winters, A. E., White, A. M., Dewi, A. S., Mudianta, I. W., Wilson, N. G., Forster, L. C., Garson, M. J., & Cheney, K. L. (2018). Distribution of defensive metabolites in nudibranch molluscs. *Journal of Chemical Ecology*, 44(4), 384–396. <https://doi.org/10.1007/s10886-018-0941-5>
- Winters, A. E., Wilson, N. G., van den Berg, C. P., How, M. J., Endler, J. A., Marshall, N. J., White, A. M., Garson, M. J., & Cheney, K. L. (2018). Toxicity and taste: Unequal chemical defences in a mimicry ring. *Proceedings of the Royal Society B: Biological Sciences*, 285(1880), 20180457. <https://doi.org/10.1098/rspb.2018.0457>
- Wollenberg, K. C., & John Measey, G. (2009). Why colour in subterranean vertebrates? Exploring the evolution of colour patterns in caecilian amphibians. *Journal of Evolutionary Biology*, 22(5), 1046–1056. <https://doi.org/10.1111/j.1420-9101.2009.01717.x>
- Yong, K. W. L., Mudianta, I. W., Cheney, K. L., Mollo, E., Blanchfield, J. T., & Garson, M. J. (2015). Isolation of norsesterterpenes and spongian diterpenes from *Dorisprismatica* (=Glossodoris) atomarginata. *Journal of Natural Products*, 78(3), 421–430. <https://doi.org/10.1021/np500797b>
- Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T. Y. (2017). Ggtree: An R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, 8(1), 28–36. <https://doi.org/10.1111/2041-210X.12628>

SUPPORTING INFORMATION

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

Table S1: Detailed summary of species sampling collection and daytime activity.

Table S2: Toxicity and unpalatability values based on mantle extract data from Winters et al. (2022).

Table S3: Taxonomic classification of species.

Table S4: List of all 158 parameters in the initial analysis before the removal of auto-correlated parameters.

Table S5: List of all QCPA colour pattern metrics of the colour adjacency (CAA, yellow), visual contrast (VCA, blue) and boundary strength analysis (BSA, red), with added listing of local edge analysis (LEIA, green) parameters.

Table S6: Factor values for each species.

Figure S1: COI and 16S tree with *Tayuva lilacina* included as a representative of *Discodoris* sp.

Figure S2: Taxonomic tree using the character matrix from Table S3.

Figure S3: The resulting Supertree.

Figure S4: To select an appropriate number of factors for PFA, we ran our analysis under several different factor dimensions $k=1, \dots, 6$.

Figure S5: Receiver operating characteristic (ROC) curve of the ability of factor 1 to predict daytime activity using logistic regression. Residual autocorrelation among parameters in the 55 retained colour pattern descriptors.

Figure S7: Highest and lowest representative images for highlighted parameters in Figure 1.

Figure S8: Artistic arrangement of species images, subjectively sorted by colour.

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