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Marine biodiversity in the dark: an ecological assessment of a unique submerged cave system in the eastern Mediterranean Sea

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Abstract

Submerged caves in the Mediterranean Sea are unique ecosystems, sustaining diverse benthic communities shaped by environmental gradients. This study focused on the Jubilee Shoals cave system in the eastern Mediterranean (Cyprus), providing a quantitative analysis of benthic community composition, spatial distribution across cave zones, and the influence of environmental factors. The data were collected using closed-circuit rebreathers; 341 photo quadrats were analysed with specialised software to assess biodiversity within this cave system. Overall, 59 sessile taxa were identified at different rank, with sponges comprising 33% of richness. Phototrophic species dominated the entrance zone, while sponges and other low-light-adapted organisms prevailed in the darker areas. Light availability significantly influenced community structure. A progressive decline in biotic coverage from entrance to dark zones and an increase in non-living substrates highlighted the environmental limitations of more remote cave sections. Detailed 2-D and 3-D mapping enhanced our understanding of the structural complexity of the cave and species distribution. These findings emphasise the ecological vulnerability of sea caves and highlight the need for focused conservation efforts. This study establishes a foundation for future ecological studies in deep marine cave systems.

Keywords: Dark habitats; Benthic communities; Scientific diving; Marine biodiversity; Hard substrates.

Introduction

Submerged caves in the Mediterranean Sea are exceptional reservoirs of ecological information, show-casing distinct ecological features. They have long intrigued scientists with early researchers laying the groundwork for our current understanding of their ecological significance (Riedl, 1966). The Mediterranean Sea hosts in excess of 3,000 documented sea caves, with more than 260 located in the eastern Mediterranean (Giakoumi *et al.*, 2013). Scientific studies have consistently highlighted the rich biodiversity within submarine caves (Bussotti *et al.*, 2006; Gerovasileiou *et al.*, 2015; Gerovasileiou & Bianchi, 2021). Recognised as crucial and vulnerable ecosystems, sea caves receive protection under Annex I of the Habitats Directive (92/43/EEC) and are classified under habitat code 8330 as “Submerged or partially submerged sea caves” (European Commission, 2013).

The biodiversity within submarine caves is stratified across various sections (zones), each hosting distinct species assemblages shaped by gradients of biotic and abiotic factors. For example, the light gradient along the exterior-interior axis of marine caves creates distinct ecological niches, promoting the establishment of diverse assemblages (Harmelin, 1985). Typically, taxonomic richness and coverage decrease from the entrance towards the innermost parts of the caves (Gili *et al.*, 1986; Gerovasileiou & Bianchi, 2021).

Marine caves are typically divided into three zones based on light penetration: (a) the entrance (E), (b) the semi-dark (SD), and (c) the dark zone (D). This zonation was first introduced by Pérès & Picard in the mid-20th century, was further elaborated by researchers such as Harmelin in the 1980s and is still widely accepted until today (Pérès & Picard, 1964; Harmelin, 1985; Martí *et al.*, 2004; Gerovasileiou & Bianchi, 2021). Marine caves exhibit a variety of geomorphological features and submersion levels. The depth, dimensions, and orientation of their entrances significantly influence light penetration and water exchange with the open environment.

The depth, dimensions, and orientation of cave entrances can influence light penetration and water exchange with the open environment, which in turn may affect internal conditions such as food availability and oxygen renewal. Shallow entrances allow more light to penetrate, supporting photosynthetic organisms, whereas deeper or narrower entrances limit light and reduce primary producers. The orientation of the entrance may also play a role; for instance, entrances facing prevailing currents may facilitate greater water exchange, enhancing nutrient influx and oxygen renewal, whereas sheltered orientations might result in more limited exchange. The orientation of the entrance also plays a role; for instance, entrances facing prevailing currents may facilitate greater water exchange, enhancing nutrient influx and oxygen renewal, whereas sheltered orientations might result in more stagnant conditions. These factors collectively shape the unique ecological dynamics within marine caves. These include fully or partially submerged blind caves, tunnels, and pits, each varying in topographical complexity (Bianchi *et al.*, 1986; Dimarchopoulou *et al.*, 2018; Gerovasileiou & Bianchi, 2021).

Submarine blind caves represent cryptic habitats in the littoral zone, characterised by a light gradient, progressively diminishing from the entrance to the cave's innermost sections. These caves have limited water circulation and are typically oligotrophic (Harmelin, 1985). Organisms inhabiting cave walls and ceilings either prefer lit conditions (photophilic) or avoid light (sciaphilic). The absence of light leads to an oligotrophic environment, characterised by depleted benthic communities. Species in these zones are adapted to scarce food and varying levels of competition and predation (Harmelin, 1985; Martí *et al.*, 2004; Bussotti *et al.*, 2006; Gerovasileiou & Voultsiadou, 2016; Gerovasileiou *et al.*, 2017). Sea caves often serve as habitats for numerous protected and rare species, some of which may be endemic or associated with deep-sea ecosystems (Harmelin, 1985; Harmelin & Vacelet, 1997; Rosso *et al.,* 2013; Gerovasileiou & Voultsiadou, 2012; Gerovasileiou *et al.*, 2015). The latter is mainly because of the absence of ambient light and the increased oligotrophy in the dark zones of the caves, creating conditions analogous to those at much greater depths (Harmelin *et al.*, 1985; Gerovasileiou & Bianchi, 2021).

Until now, little was known about the marine cave habitats in Cyprus as there is only one published case study of a small blind cave in the Marine Protected Area of Kakoskali by Jimenez *et al.* (2019). The cave hosts a unique ecosystem of calcifying organisms and microbial communities that build "pseudostalactites" or "biostalactites" (Guido *et al*., 2017; Jimenez *et al*., 2019; Di Franco *et al*., 2021). Unfortunately, the cave and its bioconstructions are being affected by human activities, non-indigenous species, and climate change faster than our capacity to generate knowledge about their associated biodiversity (Jimenez *et al*., 2019). Beyond the regional knowledge gap, the present work provides one of the very few quantitative assessments of a "deep" circalittoral submerged marine cave at the Mediterranean scale. Since shallow marine caves are generally more strongly affected by anthropogenic pressures and climate-driven disturbances, investigating deeper cave systems can provide valuable baseline information on cave ecosystems that are comparatively less impacted. At the same time, deeper caves may have particular ecological importance, as they can support higher taxonomic and functional diversity, harbour unique taxa, and potentially act as refugia from surface perturbations and warming (Gonzalez-Marrero *et al.*, 2026).

To address the local knowledge gap in Cyprus while also contributing rare quantitative data from a deep circalittoral marine cave at the Mediterranean scale, the current study aimed to: (1) provide a quantitative description of the sessile benthic communities within the particular cave system at Jubilee Shoals, on the southern side of the island; (2) investigate the spatial distribution of organisms inside this cave; (3) evaluate the effects of abiotic factors on cave ecosystem biodiversity; and (4) delineate community clusters and characterise their distribution patterns.

Methods

Study site

The study was carried out in a submerged cave system (34.609472, 32.790083), locally known as the 'Jubilee Shoals' or 'Petra tis Avdhimou', situated off the southwest coast of the Republic of Cyprus (Fig. 1). Field surveys were conducted from May to November 2023. Jubilee Shoals consists of a submerged rocky reef encompassing a complex ecosystem. The top of the shoal is situated at a depth of 17–21 meters below the sea surface, while its base extends to approximately 45–55 meters, depending on the surrounding seabed morphology. The reef includes three habitats of the EU Habitats Directive: *Posidonia oceanica* beds (habitat code 1120) on the reef top, 'reefs' (habitat code 1170) also featuring coralligenous assemblages along the cliff walls, and the herein studied cave (habitat code 8330). The Jubilee cave has three entrances that are located at depths between 40 and 45 m, and an average depth of 38 m, with galleries and bifurcations branching into blind ends, forming a unique ecosystem. The shoals and cave system are popular destinations for both recreational diving and fishing activities.

Data collection

Data were collected using closed-circuit rebreathers that recycle the diver's exhaled air, preventing bubble formation and their impact by reducing the amount of silt dislodged from cave ceilings and walls and eliminating the formation of pockets of gas on the cave's ceiling. Additionally, rebreathers enable extended bottom time and reduced decompression requirements (Resaikos *et al.*, 2024). To prepare for the surveys, three exploratory

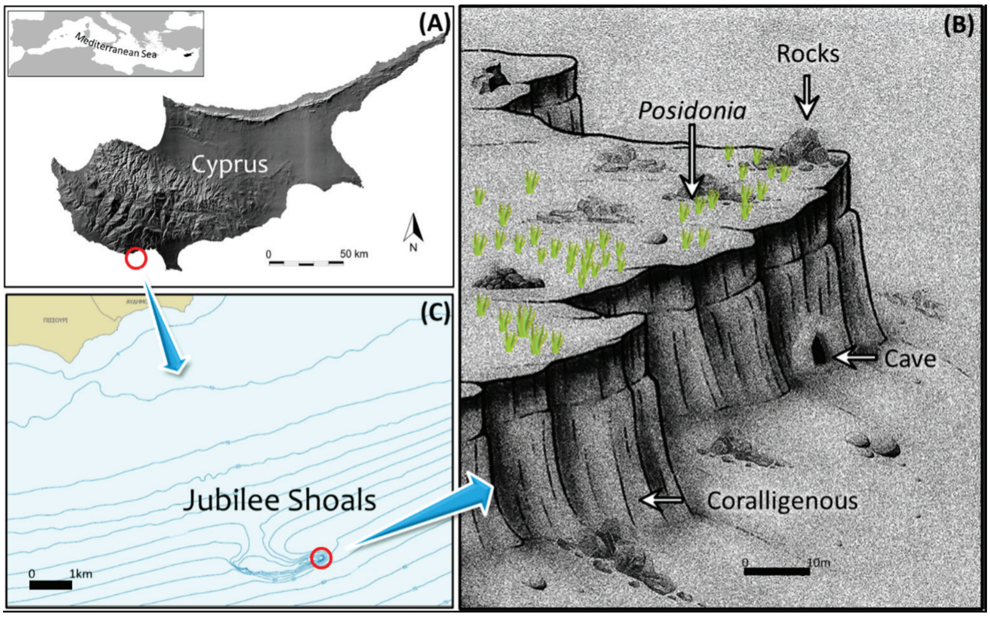

Fig. 1: Location of Cyprus Island in the Mediterranean (A), bathymetry (isobaths 5 m increments) of the Jubilee Shoals (B) and scheme of its eastern end with entrance to the submerged cave system (C).

technical dives were conducted between 2020 and 2021 at Jubilee Shoals and within the cave. These initial dives provided essential insights into the cave's morphology and environmental conditions, considered for the design of the sampling. Based on light availability, the cave system was divided into three ecological zones – entrance (E), semi-dark (SD), and dark (D) – following the bionomic model developed by Pérès & Picard (1964). Given that the cave system has three distinct entrances, it was essential to divide it into ten sections for a more detailed analysis. We examined the three entrances, three semi-dark sections, and four dark areas (Fig. 2).

Surveys and sampling were conducted carefully to minimise disturbance. Photo-quadrats (19 cm × 13 cm in size) were taken along the cave walls and ceiling across all investigated zones (Fig. 3) using an Olympus TG-6 underwater camera equipped with a WEEFINE Smart Focus 5000 video light to document the hard substrate epibenthic communities. Photographs were collected throughout each zone, from the lowest to the highest points relative to the seafloor, to capture the complete vertical range. In most cases, the walls were vertical, which determined the orientation of sampling. Within each section, photo-quadrats were distributed along the horizontal extent of the zone, including areas close to transitional boundaries and more interior points, to ensure spatial representativeness. Sampling was therefore not restricted to a single "central" point per zone but covered the within-zone variability across sections. High-resolution macro-photos and limited targeted samples were also collected to aid in the identification

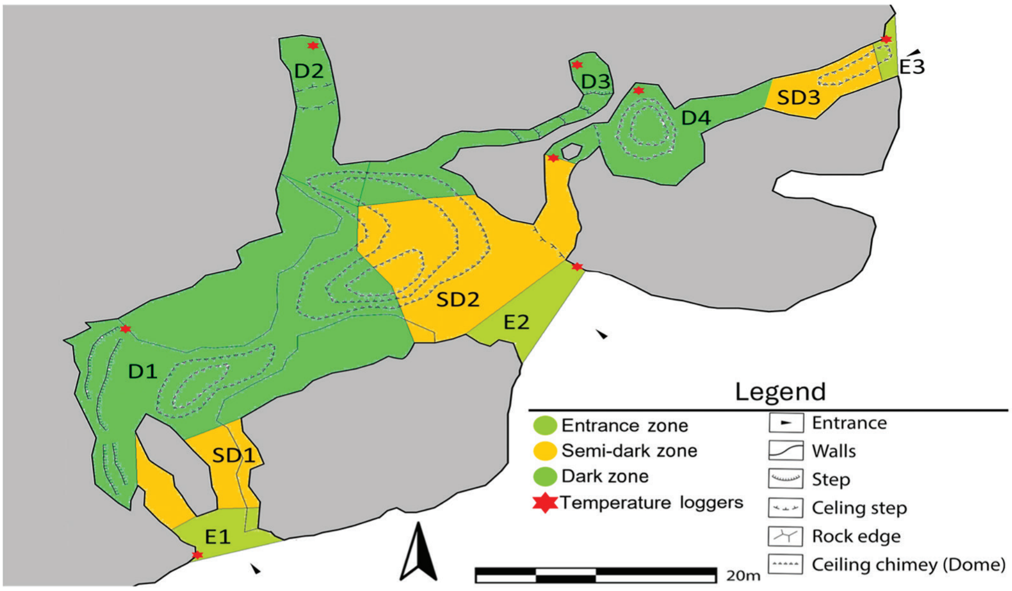

Fig. 2: Plan view of the cave system geomorphology with the studied sections – zones (according to light penetration), and the location of temperature loggers. E: entrance; SD: semi-dark; D: dark. **Software:** Visual Topo (Szukalski, 1998); Therion (Budaj, 2008).

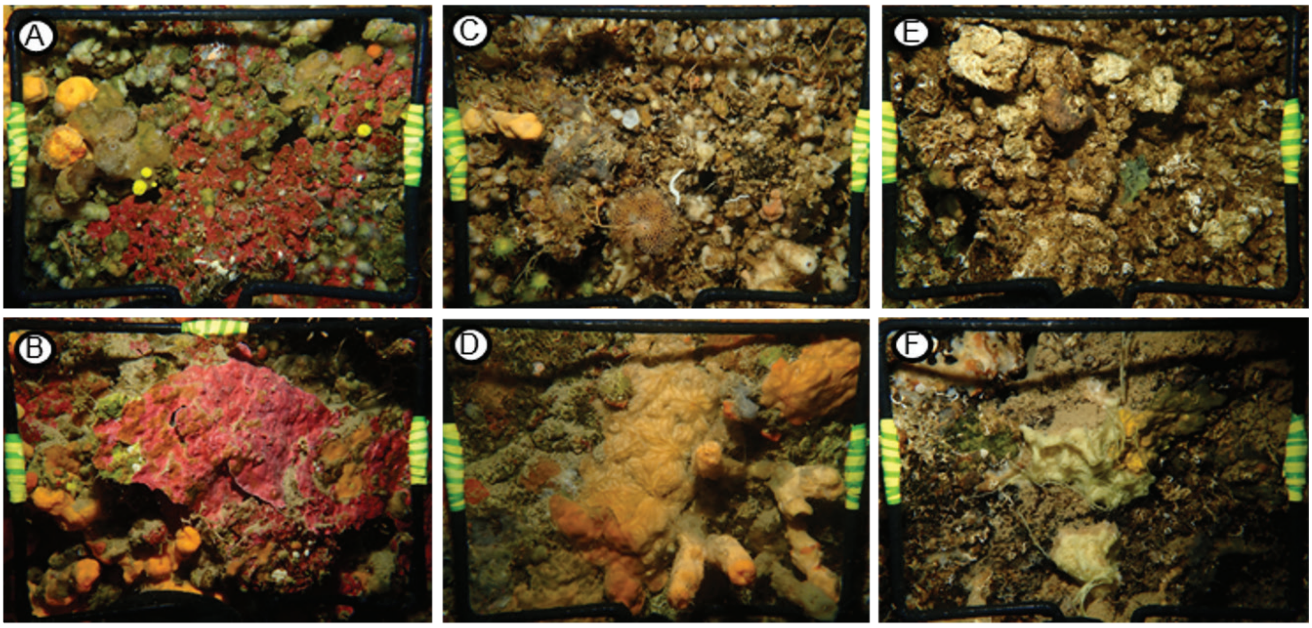

Fig. 3: Photo-quadrats collected from different cave zones: (A) entrance ceiling; (B) entrance wall; (C) semi-dark ceiling; (D) semi-dark wall; (E) dark ceiling; and (F) dark wall. Photo credit: Vasilis Resaikos.

of organisms to the lowest possible taxonomic level. Within each zone, photo-quadrats were taken from the ceiling and wall, hereafter referred to as 'positions'. The percent coverage of sessile organisms was quantified using PhotoQuad, an image processing software developed for marine ecological studies (Trygonis & Sini, 2012). On each photo-quadrat, 100 uniformly spaced points were laid and each point was assigned to a sessile taxon, a morpho-functional category, or to abiotic substrate.

To determine the optimal number of quadrats for accurately capturing biodiversity across all zone/position combinations, rarefaction analyses were performed (Fig. S1, Supplementary Material). The optimal number of quadrats was determined when the rarefaction curve stabilised or the rate of increase of species richness was less than 10% with the addition of five extra photos. Based on these analyses, the optimal number of photo-quadrats required were: 25 for the entrance ceiling, 18 for the entrance wall, 18 for the semi-dark ceiling, 14 for the semi-dark wall, 11 for the dark ceiling, and 18 for the dark wall. Photo credit: Vasilis Resaikos. For this study, the optimal number of photo-quadrats was applied to cover all ten sections of the cave system: three entrance ceilings (3×25) and walls (3×18), three semi-dark ceilings (3×18) and walls (3×14), and four dark ceilings (4×11) and walls (4×18) (Fig. 2), resulting in a total of 341 photos analysed. Most taxa were identified to the species level, but in cases where visual identification was challenging, broader morphological groups such as "turf algae" were used.

A traditional but detailed mapping protocol (Iliffe *et al.,* 2001) was followed to create both two- (2-D) and three-dimensional (3-D) models of the cave system. Briefly, the method involved the systematic placing of a main line circularly from wall to wall, with each tying point designated as a topographic station. At each station, azimuth measurements were recorded to the subsequent tying point, and depth readings were taken using a dive computer. The distance between two tying points along the line was measured using knotted sections of the line. A total of 650 m of caving line was used. *In situ* sketches and extensive photographic documentation were produced at each station to cross-check the models. Software, such as Visual Topo (Szukalski, 1998), Therion (Budaj, 2008), and Blender (Hess, 2013), were subsequently used to generate a 3-D model and corresponding map.

To investigate temperature differences within the cave system and their potential effect on biological communities, a total of eight temperature data loggers (Electric Blue, Portugal) were placed throughout the cave system (Fig. 2). The loggers were set to record once every hour and were left in place for one annual cycle from 24 June 2023 to 24 June 2024.

Data analyses

Biotic coverage (%), taxonomic richness (S), Shannon-Wiener diversity index (H'), Pielou's species evenness index (J') and coverage (%) of non-living substrates (NLS) were calculated as univariate community indicators. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated by plotting residuals against fitted values, revealing that the data were not normally distributed. To compare differences of community indicators in relation to the cave zones, the Kruskal-Wallis test with Dunn's post-hoc multiple comparisons test were conducted. To investigate the impact of quadrat position (ceiling, wall) on community indicators, the non-parametric Mann-Whitney U test was conducted.

Multivariate analyses were conducted to assess the impact of the various numerical and categorical variables on species composition and coverage. Prior to calculating dissimilarity metrics using the Bray-Curtis dissimilarity index, biotic coverage data were square root transformed to balance the influence of both high and low coverage values. A hierarchical cluster analysis was then applied to the Bray-Curtis dissimilarity matrix using Ward's minimum variance method, which aims to form distinct groups by minimising the within-cluster sum of squares (Ward, 1963). The spatial pattern of community structure was visualised using non-metric multidimensional scaling (nMDS) (Mead, 1992). To find indicator taxa within each community cluster, the Indicator Value Index (IndVal) was applied on the fourth-root transformed coverage data, using the 'indicspecies' R package (De Cáceres, 2023). IndVal is a method used to identify taxa strongly associated with a particular group, calculated based on specificity (relative abundance) and fidelity (relative frequency) of each species within that group (Dufrêne & Legendre, 1997; Cáceres & Legendre, 2009). The calculated specificity and fidelity values are multiplied to produce the test statistic, the Indicator Value. The statistical significance of indicator taxa was tested by random permutation of stations (9999 permutations) and only significant indicator taxa (p-value < 0.05) were selected and discussed per community cluster.

A statistical significance threshold of $\alpha = 0.05$ was used for all statistical tests. Standard deviation (SD) was calculated for all mean values. All statistical analyses were conducted using R software. The 'vegan', 'MASS', 'BIOMASS' R packages were used for statistical analyses and 'ggplot2' and 'ggpubr' R packages were used for data visualisation.

Results

Geomorphological description

From a geomorphological perspective, the Jubilee Shoal cave system appears to be partly collapsed. Identifying the rock types is challenging due to thick bioconstructions, and the organogenic sediment covering the cave's bedrock (Fig. 4). Within the system, three narrow passages leading to greater depths (45–50 m) have been mapped and designated as passages A, B, and C, with the latter being the deepest cave entrance. Additionally, three blind domes or cupolas with chimneys were observed in the cave's ceiling, labelled as features D, E, and F. A 3-D model was constructed to better illustrate the structural complexity of the cave system (Fig. 5).

Composition of benthic communities

A total of 59 taxa, belonging to nine higher taxonomic groups (HTGs), were identified to the lowest possible taxonomic level: 24 Porifera, 8 Cnidaria, 6 Bryozoa, 3 Polychaeta, 7 Rhodophyta, 4 Chlorophyta, 4 Brachiopoda, 3 Ascidiacea, and 1 Foraminifera (Table 1). Additionally, five types of non-living substrate (NLS) were identified. The percentage cover of each HTGs and NLS substantially differed by cave zone (Fig. 6).

Porifera and NLS together accounted for more than 30% of the total assessed photo-quadrat area across the cave system (Table S2, Supplementary Materials). NLS increased progressively from the entrance to the dark zones of the cave, from 14.8% average percentage cover at the entrance (E) to 34.4% in the semi-dark zone (SD), reaching 49.6% in the dark zone (D). Porifera showed significant variation in coverage across zones, with the highest coverage of 37.6% at the entrance, followed by 31.9% in the dark zone, and 28.4% in the semi-dark zone. Two other HTGs, Bryozoa and Rhodophyta, exceeded 10% coverage each in the cave system. Bryozoa were most abundant in the semi-dark zone (19.2%), followed by the dark zone (11.3%), with considerably lower coverage at the entrance (4.4%). In contrast, Rhodophyta were predominantly found at the entrance, with a coverage of 25.5%, compared to significantly lower cover in the semi-dark (2.5%) and dark zones (0). Cnidaria accounted for 5.4% of the total cave coverage, with a decreasing trend from the entrance (8.3%) to the semi-dark (5.7%) and dark zones (2.3%). The remaining HTGs contributed less than 3% each to the overall cave coverage (Table S2, Supplementary Materials).

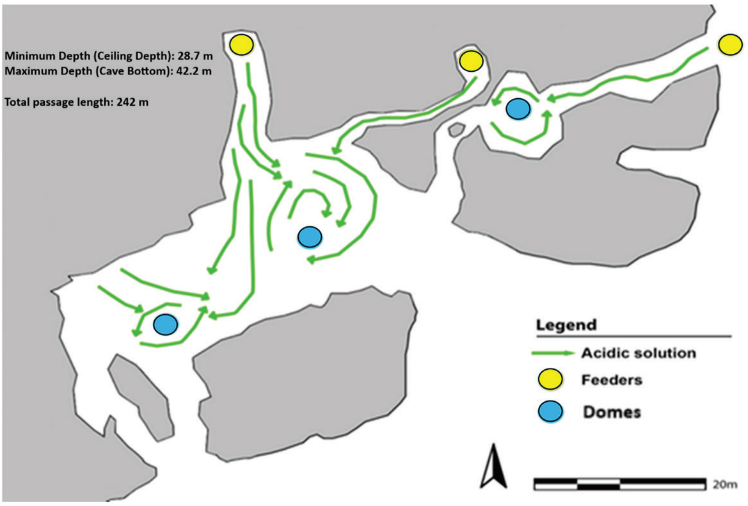

Fig. 4: Geomorphological characterisation of the Jubilee Shoals cave system. **Software:** Visual Topo (Szukalski, 1998); Therion (Budaj, 2008).

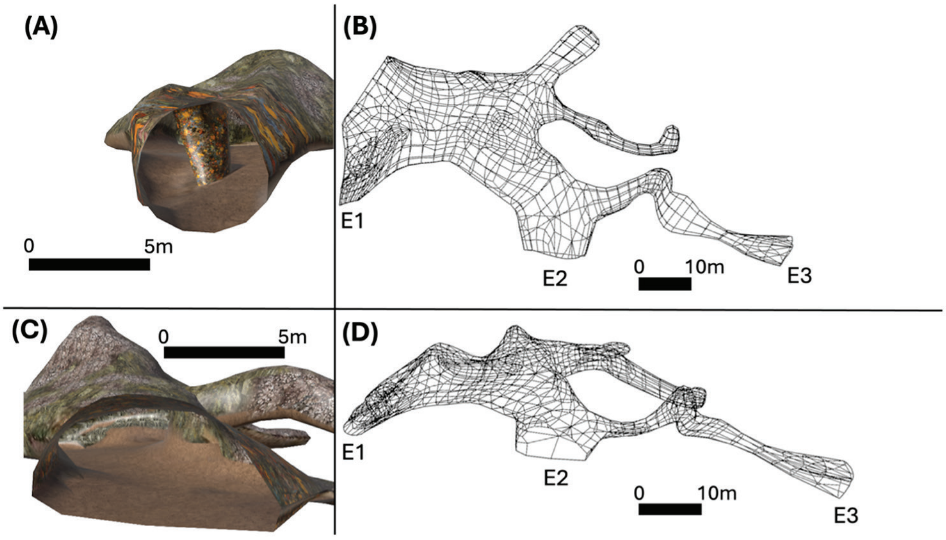

Fig. 5: (A) 3-D representation of the Entrance 1; (B) Top view of the cave system; (C) 3-D representation of the Entrance 2; (D) Side view of the cave system. **Software:** Visual Topo (Szukalski, 1998); Therion (Budaj, 2008); Blender (Hess, 2013).

Table 1. Checklist of identified taxa and corresponding percentage cover across the three cave zones. HTG: Higher taxonomic group, TBC: Total biotic cover, TC: Total cover, BCE: Biotic cover at the entrance zone, BCSD: Biotic cover at the semi-dark zone, BCD: Biotic cover at the dark zone, NLS: Non-living substrate.

Taxon	HTG	TBC (%)	BCE (%)	BCSD (%)	BCD (%)
Miniacina miniacea (Pallas, 1766)	Foraminifera	1.76	4.87	0	0
Chlorophyta sp.1	Chlorophyta	1.14	0.32	2.63	0.76
Turf Algae	Chlorophyta	0.99	0.68	2.59	0.01
Chlorophyta sp.2	Chlorophyta	0.7	1.75	0.23	0
Palmophyllum crassum (Naccari) Rabenhorst, 1868	Chlorophyta	0.07	0	0.07	0.15
Calcareous Algae sp.1	Rhodophyta	7.77	20.82	0.86	0
Calcareous Algae sp.2	Rhodophyta	0.45	0.41	0.81	0
Calcareous Algae sp.3	Rhodophyta	0.64	1.77	0	0
Calcareous Algae sp.4	Rhodophyta	0.48	0.69	0.79	0
Calcareous Algae sp.5	Rhodophyta	0.37	1.02	0	0
Calcareous Algae sp.6	Rhodophyta	0.19	0.53	0	0
Calcareous Algae sp.7	Rhodophyta	0.07	0.19	0	0
Agelas oroides (Schmidt, 1864)	Porifera	8.05	13.66	8.06	2.25
Timea unistellata (Topsent, 1892)	Porifera	4.42	3.22	4.44	5.64
Diplastrella bistellata (Schmidt, 1862)	Porifera	4.3	5.58	4.24	3.02
Dendroxea lenis (Topsent, 1892)	Porifera	3.97	0.23	3.74	8.03
Phorbas tenacior (Topsent, 1925)	Porifera	2.4	6.63	0	0
Haliclona (Soestella) mucosa (Griessinger, 1971)	Porifera	2.2	0.09	1.59	4.89
Porifera sp.1	Porifera	1.29	1.76	1.76	0.23
Clathrina sp.	Porifera	0.96	0.09	0.4	2.33
Merlia sp.	Porifera	0.67	0.12	0	1.79
Porifera sp.2	Porifera	0.58	1.06	0.49	0.17
Hexadella pruvoti Topsent, 1896	Porifera	0.55	0.39	1.03	0.33
Axinella sp.	Porifera	0.52	0.94	0.33	0.25
Porifera sp.3	Porifera	0.5	0.69	0.13	0.6
Porifera sp.4	Porifera	0.48	0.15	0.52	0.37
Petrosia (Petrosia) ficiformis (Poiret, 1789)	Porifera	0.41	1.13	0	0
Cacospongia mollior Schmidt, 1862	Porifera	0.38	0.24	0	0.85

Continued

Table 1 continued

Taxon	HTG	TBC (%)	BCE (%)	BCSD (%)	BCD (%)
Spirastrella sp.	Porifera	0.28	0.59	0.23	0
Dictyoceratida	Porifera	0.18	0.34	0.21	0
Dictyonella sp.	Porifera	0.17	0	0.53	0.05
Thymosiopsis cuticulatus Vacelet & Pérez, 1998	Porifera	0.13	0	0	0.37
Acanthella acuta Schmidt, 1862	Porifera	0.13	0.3	0.07	0
Ircinia sp.	Porifera	0.06	0.04	0.15	0
Ircinia oros (Schmidt, 1864)	Porifera	0.02	0	0	0.05
Leptopsammia pruvoti Lacaze-Duthiers, 1897	Cnidaria	2.11	2.34	3.74	0.52
Madracis pharensis (Heller, 1868)	Cnidaria	1.92	4.88	0.55	0
Polycyathus muellerae (Abel, 1959)	Cnidaria	0.64	0.38	0.59	0.95
Polycyathus sp.	Cnidaria	0.26	0.29	0.02	0.44
Caryophyllia sp.	Cnidaria	0.23	0.12	0.25	0.31
Aglaopheniidae	Cnidaria	0.18	0.08	0.52	0
Eudendrium sp.	Cnidaria	0.04	0.11	0	0.01
Hydrozoa	Cnidaria	0.04	0.07	0.03	0.03
Serpulidae sp.2	Polychaeta	0.93	0.19	0.35	2.18
Serpulidae sp.1	Polychaeta	0.86	0.61	2.05	0.15
Serpulidae sp.3	Polychaeta	0.03	0	0	0.08
Novocrania anomala (Müller, 1776)	Brachiopoda	0.33	0.42	0.6	0.01
Joania cordata (Risso, 1826)	Brachiopoda	0.04	0.01	0.04	0.08
Brachiopoda sp.1	Brachiopoda	0.03	0.05	0.02	0.01
Brachiopoda sp.2	Brachiopoda	0	0	0	0.01
Smittina cervicornis (Pallas, 1766)	Bryozoa	5.51	0.22	6.24	10.37
Beania mediterranea Souto, Nascimento, Reverter-Gil & Vieira, 2019	Bryozoa	2.18	0.2	6.32	0.82
Adeonella pallasii (Heller, 1867)	Bryozoa	2.1	2.93	3.63	0
Hippellozoon sp.	Bryozoa	0.91	0.38	2.62	0.03
Cheilostomatida	Bryozoa	0.22	0.46	0.17	0.02
Frondipora verrucosa (Lamouroux, 1821)	Bryozoa	0.15	0.17	0.26	0.03
Rhopalaea neapolitana Philippi, 1843	Ascidiacea	0.5	0.46	1.08	0.05
Halocynthia papillosa (Linnaeus, 1767)	Ascidiacea	0.09	0.15	0.11	0
Lissoclinum perforatum (Giard, 1872)	Ascidiacea	0.03	0.05	0.01	0.03
NLS	**Type**	**TC (%)**	**CE (%)**	**CSD (%)**	**CD (%)**
Sediments	Abiotic	13.09	12.08	8.37	18.02
Deposits of Mn	Abiotic	7.58	0.36	8.85	13.99
Marine snow	Biotic	5.75	1.76	13.67	3.34
Empty serpulid tubes	Biotic	3.3	0	2.11	7.68
Other biogenic origin	Biotic	3.22	0.58	8.02	6.53

Biological indicators and analysed factors

The results of the non-parametric Kruskal-Wallis test indicated that the cave zone had a significant effect on biotic coverage, non-living substrates (NLS), and species richness. The coverage of benthic communities decreased progressively from the entrance to the dark zone, with mean biotic percent coverage of 86.4% ± 12.7%, 78.6% ± 7.1%, and 51.6% ± 5.1% in the entrance, semi-dark, and dark zones, respectively. There were significant differences both between the entrance and dark zones (post-hoc $p < 0.01$) and between the semi-dark and dark zones (post-hoc $p < 0.01$). In contrast, the coverage of NLS significantly increased from the entrance to the dark zone (Fig. 7).

The mean taxonomic richness in the entrance, semi-dark, and dark zones was 31.3 ± 8.7, 28.3 ± 7.7, and 17.1 ± 5.6 taxa, respectively. Post-hoc analysis indicated that the dark zone had significantly lower richness compared to the entrance ($p < 0.01$) and semi-dark zone ($p = 0.05$)

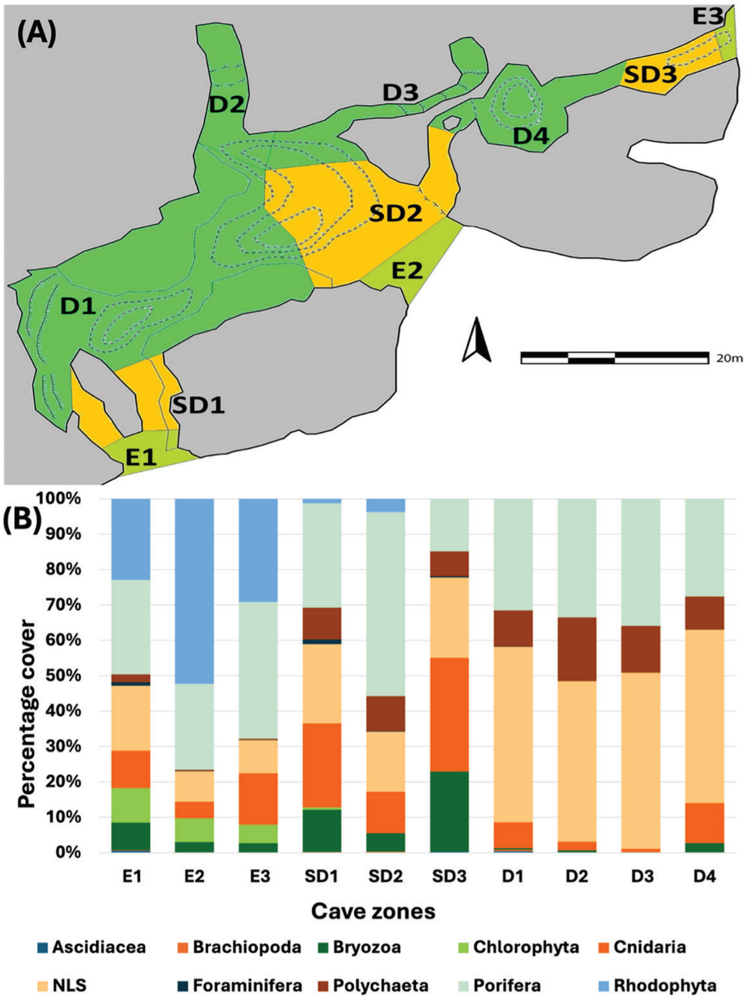

Fig. 6: (A) Plan view of the cave system; colours indicate the different zones (E: entrance zone, SD: semi-dark zone, D: dark zone) corresponding to panel (B). **(B)** Percentage cover of each taxa and non-living substrate (NLS) types across the cave zones. **Software:** Visual Topo (Szukalski, 1998); Therion (Budaj, 2008); Blender (Hess, 2013).

(Fig. 7). The mean values of H' diversity in the entrance, semi-dark, and dark zone were 2.5 ± 0.3, 2.5 ± 0.3, and 2.1 ± 0.4, respectively (Fig. 7), with no evident significant differences. The mean Pielou's species evenness in the entrance, semi-dark, and dark zones was 0.7 ± 0.02, 0.8 ± 0.1, and 0.8 ± 0.1, with no evident significant differences; all values were equal to or above 0.7, suggesting an even species distribution within zones.

The Mann-Whitney U test revealed that only Pielou's species evenness significantly differed ($p = 0.02$) between the two positions (wall and ceiling).

The mean annual temperature of all combined cave entrances, semi-dark zones, and dark zones was 19.73 ± 0.13 (n=3 loggers), 19.71 ± 0.09 (n=1), and 19.94 ± 0.11 (n=4), respectively (Table S1 and Fig. S2 in Supplementary Materials). Although the mean annual temperature did not vary much between the different zones and is thus of low biological significance, the Kruskal-Wallis' test revealed that there is a statistically significant difference between them ($p < 0.001$). Specifically, there is significant difference in mean temperature between the dark zones and the entrance ($p = 0.002$) and semi-dark ($p = 0.001$) zones but not between the entrance and semi-dark ($p = 0.845$).

Community clusters and distribution patterns

The Ward clustering analysis identified three well-defined community clusters (Fig. 8). A 'label' was attributed to each community cluster based on the cave zone (entrance, semi-dark, dark) and the position (ceiling, wall). The term 'entrance' was attributed to the community cluster including all the positions from the entrance zone. The label 'semi-dark/dark ceiling' was given to the community cluster characterised by sampling stations from the ceiling in both the semi-dark and dark zones. Similarly, the 'semi-dark/dark wall' label was applied to the community cluster consisting of wall sampling stations from both the semi-dark and dark zones. The only sampling station that differed from the other stations in the defined clusters was 'E1_W' (Fig. 8), which refers to the wall position in the entrance zone 1. 'E1_W' exhibited this differentiation primarily because the walls in entrance 1 contained a higher percentage of NLS and a lower percentage of biotic coverage compared to entrances 2 and 3. At entrance 1 this was related to a significant accumulates accumulation of sediment, which led to its grouping within the 'semi-dark/dark wall' community cluster.

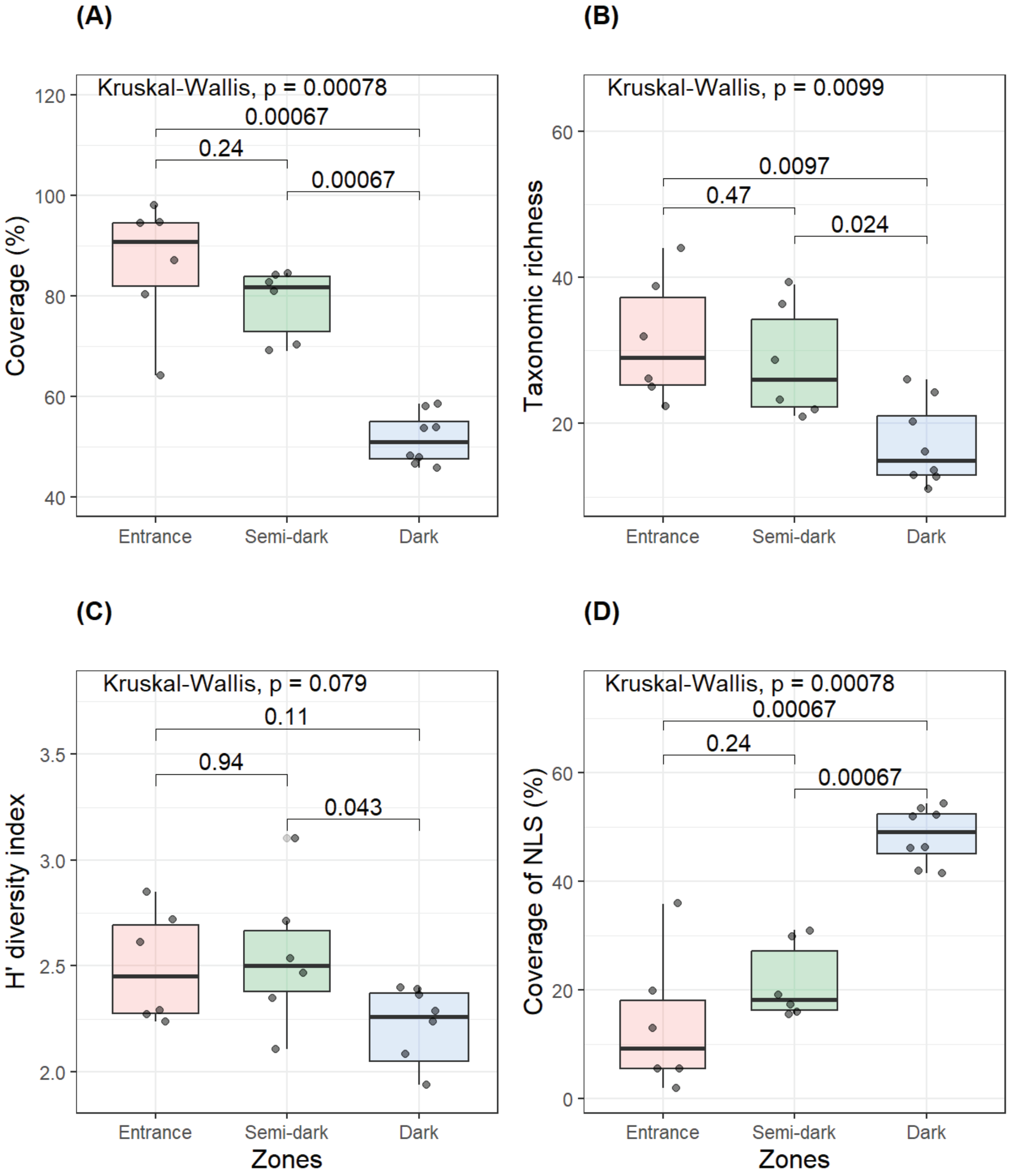

Fig. 7: Differences between cave zones and (A) percent coverage of living organisms, (B) taxonomic richness, (C) Shannon-Wiener diversity index, and (D) percentage cover of non-living substrate. Numbers on bars indicate the p-value of Dunn's post-hoc test.

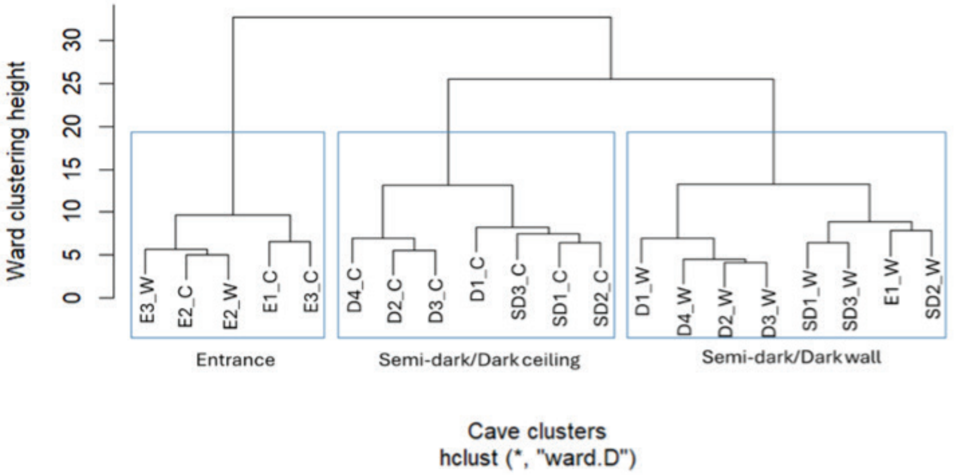

Fig. 8: Community cluster partition. Ward's minimum variance cluster analysis based on Bray-Curtis dissimilarity matrix using fourth-root transformed biotic coverage data from 20 sampling stations.

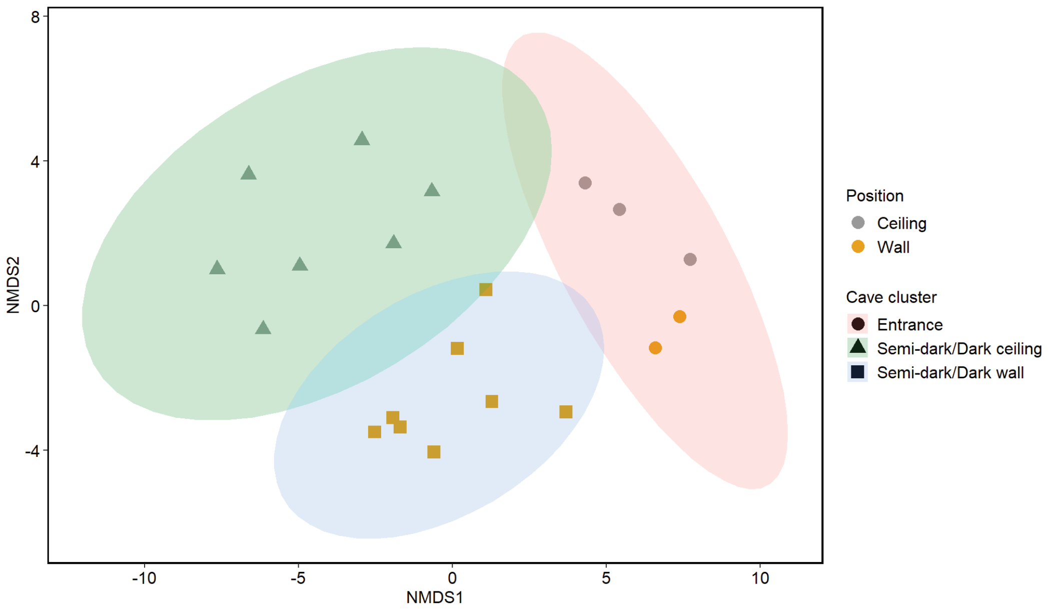

Fig. 9: Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity distances of biotic coverage across the cave system. Each point represents the biotic coverage of each sampling station. Ellipses indicate 95% confidence intervals of multivariate t-distribution around centroids of the groupings with zone as factor. Stress level: 0.045.

The nMDS plot effectively distributed the sampling stations into three main groups (Fig. 8). The low stress level (0.05) indicates good ordination, minimising the risk of drawing false inferences (Clarke, 1993).

Indicator species of community clusters

Of the 59 taxa identified, only 14 were significantly associated with the community clusters. From these, nine were significantly associated with the ‘Entrance’ cluster (3 Rhodophyta, 3 Chlorophyta, 2 Porifera, and o1ne Bryozoa), two with the ‘semi-dark/dark ceiling’ cluster (1 each from Cnidaria and Polychaeta), and three with the ‘semi-dark/dark wall’ cluster (all Porifera) (Table 2). These findings highlight the differences in biological diversity across the community clusters. The ‘entrance’ cluster included the highest number of species, followed by the ‘semi-dark/dark wall’, and ‘semi-dark/dark ceiling’ clusters. In terms of biotic cover, the ‘entrance’ cluster had the highest values (90.9 ± 7.2), followed by the ‘semi-dark/dark ceiling’ (65.4 ± 17.6) and ‘semi-dark/dark wall’ (61.3 ± 12.1) clusters. The NLS was highest in the ‘semi-dark/dark wall’ cluster (38.7 ± 12.1), followed by the ‘semi-dark/dark ceiling’ (34.6 ± 17.6) and ‘entrance’ (9.1 ± 7.2) clusters.

The results revealed significant differences in biotic coverage, Pielou’s species evenness and coverage of NLS ($p < 0.01$) among the community clusters. According to Dunn’s post-hoc test, biotic cover and NLS differed significantly between the ‘entrance’ and ‘semi-dark/dark ceiling’ clusters and between the ‘entrance’ and ‘semi-dark/dark wall’ clusters. For Pielou’s evenness, significant differences were found between the ‘entrance’ and ‘semi-dark/dark wall’ clusters and between the ‘semi-dark/dark ceiling’ and ‘semi-dark/dark wall’ clusters.

Discussion

The Jubilee Shoals cave system represents an ecologically important habitat whose biodiversity had not been previously documented. Most Mediterranean marine caves investigated to date are shallow or semi-submerged (often not exceeding 15 m), while information on deeper caves remains limited and only a small number reach the 30–40 m range (Gerovasileiou & Voultsiadou, 2012; Gerovasileiou & Bianchi, 2021). The study of deeper marine caves, such as the Jubilee Shoals (40–45 m depth), is particularly a challenge due to their confined conditions and technical diving limitations. This study unveiled a structurally complex and biologically diverse ecosystem with clear zonation patterns and distinct sessile assemblages.

The marked decline in biotic coverage from the entrance (86.4%) to the dark zone (51.6%) is consistent with established ecological models of marine caves, where diminishing light and restricted circulation with distance from the entrance reduce photosynthetic activity and food. This zonation pattern, also observed in other Mediterranean caves such as Agios Vasilios cave in Lesvos (Gerovasileiou *et al*., 2017), one of the few Mediterranean cave studies conducted at relatively large depths using a comparable quantitative photoquadrat approach, and other caves in the basin (Harmelin 1997; Bianchi & Morri, 2000; Benedetti-Cecchi *et al*., 1996; Gerovasileiou *et al*., 2017; Guido *et al*., 2019; Digenis *et al*., 2022) confirms light attenuation as a major driver of benthic community structure. Although light penetration at 40–45 m is typically low in the western Mediterranean, the clearer waters of the eastern basin may permit greater light penetration, potentially influencing benthic assemblages near cave entrances.

Temperature measurements revealed negligible variation (< 0.25°C) among zones. Given the minimal thermal gradients, temperature is unlikely to structure community

Table 2. Cave clusters and their respective total taxonomic richness, mean H’ diversity and the proportion of zones in each cluster. The statistically significant dominant species (p-value < 0.05) in terms of their coverage in each cluster are shown with their respective contribution and p-value. SD: standard deviation.

Cave cluster	Zone proportion (Entrance/Semi-dark/Dark)	Total taxonomic richness (min-max)	H´diversity (+SD)	Indicator taxon	Higher taxonomic group	Specificity (%)	Fidelity (%)	IndVal (%)
Entrance	5/0/0	57 (22–44)	2.4 (0.2)	Calcareous Algae sp.1	Rhodophyta	98	100	99
				Turf Algae	Chlorophyta	90	100	95
				Calcareous Algae sp.2	Rhodophyta	98	100	94
				Calcareous Algae sp.3	Rhodophyta	100	80	89
				Palmophyllum crassum	Chlorophyta	100	80	89
				Petrosia ficiformis	Porifera	100	60	78
				Cheilostomatida	Bryozoa	60	100	77
				Macroalgae	Chlorophyta	62	80	70
				Agelas oroides	Porifera	62	80	70
Semi-dark/Dark ceiling	0/3/4	48 (11–36)	2.2 (0.5)	*Madracis pharensis*	Cnidaria	61	100	78
				Serpulidae sp.2	Polychaeta	58	88	76
Semi-dark/Dark wall	1/3/4	53 (13–39)	2.5 (0.3)	*Merlia* sp.	Porifera	62	100	79
				Clathrina sp.	Porifera	66	88	76
				Thymosiopsis cuticulatus	Porifera	55	100	74

composition, as most submerged caves maintain stable conditions through exchange with open seawater facilitated by the cave's orientation and morphology (Bianchi & Morri, 1994; Gerovasileiou & Voultsiadou, 2016; Gerovasileiou & Bianchi, 2021). Instead, reduced water circulation, limited food availability, and light attenuation act as the main constraints, excluding photophilic species and favouring sciaphilic and heterotrophic taxa that dominate the darker zones. Overall, distance from the entrance emerges as the primary environmental gradient shaping light availability, hydrodynamics, and benthic community structure.

While mean temperatures were homogeneous among zones, the highest values occurred during October and November across loggers, including in the dark sections (Fig. S2). This seasonal signal is most likely driven by regional water-mass warming and deepening of the thermocline, rather than by processes internal to the cave. In the area of the Jubilee Shoals and at the eastern coast of Cyprus, temperature loggers at 50 m depth in coralligenous habitats have recorded 24–28°C for a few days during significantly warm periods, such as Marine Heat Waves (Jimenez, unpublished data). Although not extreme, high temperature values have been recorded in summer in caves from Sardinia (Romano *et al*., 2020), Catalonia-Spain (Martí *et al*., 2004), and the Aegean Sea (Gerovasileiou & Bianchi, 2021). Importantly, the absence of persistent intra-cave thermal gradients supports the interpretation of effective water exchange rather than thermal stratification or actual stagnation as observed in a singular cave in Marseilles, France, where bottom topography created the entrapment of water of higher density in the inner chamber (Harmelin 1997). This interpretation is consistent with previous cave studies where seasonal temperature differences between cave and external waters were used as an indicator of relative water renewal (Harmelin, 1969).

This study documented 59 sessile taxa, highlighting its ecological significance and contributing valuable baseline data to regional cave biodiversity assessments. Among recorded taxa, 33% were sponges. This dominance of sponges across all zones is consistent with observations from other Mediterranean marine caves, where sponges often prevail due to the stable environmental conditions and limited light penetration (Gerovasileiou & Voultsiadou, 2012, 2016; Gerovasileiou *et al*., 2016, 2017; Guido *et al.,* 2019; Digenis *et al.,* 2022; Derrien *et al*., 2024). The taxonomic richness recorded at Jubilee Shoals is comparable to that reported for other eastern Mediterranean cave systems, including the Agios Vasilios cave in Lesvos (Gerovasileiou *et al*., 2017) and the Kakoskali cave in Cyprus (Jimenez *et al*., 2019), and contributes valuable baseline data to regional cave biodiversity assessments (Gerovasileiou & Voultsiadou, 2012, 2016).

Multivariate analysis largely confirmed the distinct zonation observed during exploratory surveys. The first cluster represents the cave entrances, characterised by distinct biodiversity. The other two clusters included both the semi-dark and dark zones, where biological communities grouped based on position (ceiling or wall) rather than zone. This grouping reflects the similar species composition and biotic coverage in the 'Semi-dark/Dark ceiling' (mean = 65.4% ± 17.6) and 'semi-dark/dark wall' (mean = 61.3% ± 12.1) clusters, compared to the 'entrance' (90.9% ± 7.2) cluster. The grouping of semi-dark and dark samples by substrate orientation (ceiling vs wall) likely reflects microhabitat differences within the cave that can be comparable in magnitude to broad zonation effects once light is strongly reduced. Similar approaches that explicitly consider wall versus ceiling surfaces have been applied in Mediterranean cave studies using quantitative benthic surveys, including sponge-focused and photoquadrat-based assessments (Gerovasileiou & Voultsiadou, 2016; Gerovasileiou *et al*., 2017). While many cave studies primarily emphasised the entrance-to-interior gradient, fine-scale heterogeneity related to surface orientation is increasingly recognised as an important component of community structuring. In our case, this may help explain why semi-dark and dark samples did not separate strictly by zone, but rather by position, highlighting the role of within-zone variability in deep submerged cave habitats.

The IndVal analysis identified characteristic taxa for each community cluster (Table 2). At the entrance, Rhodophyta, especially Calcareous Algae sp. 1 (most propably *Mesophyllum* spp.), dominated, consistent with their adaptation to habitats sheltered from direct, high irradiance levels as well as low-light conditions (Gibson *et al*., 2006). Their decline in the semi-dark and dark zones highlights light's controlling role in macroalgal distribution. The occurrence of Chlorophyta beyond the entrance, including records in the dark zone, is unusual for submerged cave systems and may reflect localised microhabitat conditions, seasonal variability, or occasional advection of propagules into inner sections. However, given the lack of direct light measurements and seasonal replication, we interpret this pattern cautiously and treat it as an observation that requires targeted investigation in future surveys. Noteworthy is the find that Chlorophyta exhibited slightly higher coverage in the semi-dark zone compared to the entrance. This pattern may reflect random or seasonal variability, as well as localised differences in substrate stability or sediment accumulation that facilitate colonisation in specific semi-dark sections. However, given the absence of light measurements and seasonal replication, this interpretation also remains tentative. In the Semi-dark/Dark ceiling cluster, the scleractinian coral *Madracis pharensis* was prominent. This species is a common dweller of eastern Mediterranean caves (Gerovasileiou *et al*., 2015) and it is the only stony coral in the Mediterranean that can survive both with and without zooxanthellae, a behaviour allowing it to thrive in both well-lit and low-light environments. Its high ecological plasticity explains its sporadic presence also in the 'entrance' cluster. *Caryophyllia* sp. was observed across all cave zones, with cover increasing from the entrance (0.12%) to the semi-dark (0.25%) and dark zones (0.31%), reflecting their adaptation to low-light environments and their structural role in aphotic communities (Gerovasileiou *et al.,* 2015). In the 'Semi-dark/Dark wall' cluster, Porifera was the predominant group, particularly *Thymosiopsis cuticulatus* auct, date and species of the genera *Merlia* and *Clathrina*, typical of sciaphilic assemblages (Gerovasileiou & Voultsiadou, 2012; Grenier *et al*., 2018; Guido *et al.,* 2019).

Despite the unique environment of the Jubilee Shoals cave system and its exceptional depth (> 40 m), its zonation patterns resemble those described in shallower marine caves in the Eastern Mediterranean Sea (e.g., Gerovasileiou *et al*., 2017; Digenis *et al*., 2022). Notably, the Agios Vasilios cave in Lesvos (24–40 m depth), provides a close analogue with similar photo-quadrat-based analyses demonstrating zonation driven by light and water movement (Gerovasileiou *et al*., 2017).

Although the entrances occur at considerable depth (> 40 m), our results suggest that horizontal distance from the entrance can remain a primary structuring gradient in fully submerged caves, potentially overriding absolute depth. In this context, cave geometry and hydrodynamics (e.g., wide tunnel-like passages facilitating exchange) may play a stronger role than depth alone in shaping community composition. Therefore, Jubilee Shoals provides an informative example of how deep cave systems can still exhibit zonation patterns comparable to shallower caves, while maintaining subtle differences linked to its morphology and circulation.

This study is unique in its examination of a lengthy as well as deep Mediterranean cave system at depths exceeding 40 m. Although other studies in the Mediterranean exist at depths of 34–39 m, most involve shorter caves (~ 10 m) that do not require extensive penetration (Costantini *et al.,* 2018). Studies beyond 30 m depth are uncommon, as divers on open-circuit equipment face significant time constraints and must have advanced experience and training to enter deep caves. This challenge was addressed in our study by technical diving approach following decompression procedures but most of all by using closed-circuit rebreathers, which extend bottom time, increase gas availability, and minimise decompression time, making them essential for research in deep cave systems (Resaikos *et al.,* 2024). Our study sheds light on the biodiversity and distribution of organisms in Mediterranean caves deeper than 40 m, addressing a substantial knowledge gap about these unique ecosystems. To the best of our knowledge, this is the first quantitative attempt in the Mediterranean to document sessile benthic communities at such depths using photoquadrat analysis, providing a reference framework for future research in deep submerged cave ecosystems. In addition, the study provides valuable baseline information to assess the role of deep cave habitats as potential refugia for climate change and anthropogenic disturbances.

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Supplementary Data

The following supplementary information is available online for the article:

Fig. S1*:* Rarefaction curves to estimate the expected number of species (taxonomic richness) according to the number of photo-quadrats from different sections of the Jubilee Shoals cave: (A) Entrance zone ceiling; (B) Entrance zone wall; (C) Semi-dark zone ceiling; (D) Semi-dark zone wall; (E) Dark zone ceiling; (F) Dark zone wall. The optimal number of photo quadrats necessary for a proper representation are shown with red arrows.

Fig. S2: Temperature distribution in the different parts of the cave system.

Table S1. Measured temperature at different zones of the Jubilee Shoals cave system.

Table S2. Decreasing percentages of Higher taxonomic groups (HTG) cover and Non-living substrate (NLS) across the three cave zones. BCE: Biotic cover at the entrance zone, BCSD: Biotic cover at the semi-dark zone, BCD: Biotic cover at the dark zone, TBC: Total biotic cover.

Table S3. Cave sections and Positions of the taxa found in the studied cave. HTG: Higher taxonomic group, NLS: Non-living substrate; Cave sections: (see Figure 2); Position: Ceiling (C), Wall (W).