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First record of the giant anemone, *Relicanthus daphneae*, at active hydrothermal vent fields in the Indian Ocean

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Abstract

While the giant anemone, *Relicanthus daphneae*, has been described as a characteristic inhabitant of the East Pacific Ocean since 1991, there are relatively few published occurrences worldwide. Here, we present the discovery and molecular verification of *R. daphneae* along the southern Central Indian Ridge, at the Rodriguez Triple Junction, and along the northern Southeast Indian Ridge within the BGR contract area for the exploration of marine massive sulphide deposits in the Indian Ocean. Individuals were solitary and attached exclusively to basalt hard substrates on the periphery of hydrothermal vent fields, at distances from active vents between 66 and 710 m. We report megafauna observed in close proximity to *R. daphneae* and, in one case, polychaetes on its tentacles and oral disc. For the first time, the giant anemone was observed capturing prey, a shrimp of the species *Rimicaris kairei*. Beyond this remark on the diet of these anemones, we also report other behavioural aspects for this species.

Introduction

Sea anemones are ecologically important inhabitants of shallow-water and deep-sea habitats globally, and exhibit high taxonomic and morphological diversity (Daly, 2006; Xiao *et al.*, 2019; McFadden *et al.*, 2021). They are considered 'habitat providers' (Briones-Fourzán *et al.*, 2012) and often establish associations with other organisms such as endosymbiotic algae, sponges, crustaceans and fish (Rodríguez *et al.*, 2014; Xiao *et al.*, 2019; McFadden *et al.*, 2021). Generally, anemones are suspension feeders, and can utilize a large range of nutritional sources, from detritus to small vertebrates (Xiao *et al.*, 2019).

The giant anemone, *Relicanthus daphneae* (Daly, 2006), initially described as *Boloceroides daphneae*, is known for its remarkable size and long tentacles. Column diameters up to 1 m (Daly, 2006) and individual tentacles approximately 2 m in length (Cantwell and Newman, 2016) have been estimated from *in situ* observations. The tentacles are strongly tapered with trailing threadlike tips that are often curled (Daly, 2006; Xiao *et al.*, 2019). The tentacles bear primarily adhesive functioning spirocysts that are thought to enable *R. daphneae* to capture large prey (Daly, 2006). Living organisms are pale pink to purplish-red in colour, with darker coloration towards the mouth parts.

Relicanthus daphneae has been observed in various habitats across the Pacific and Southern Oceans (Figure 1). It was initially observed and sampled on the East Pacific Rise (EPR) (Gage and Tyler, 1991; Daly, 2006), but has been observed since throughout the Pacific Ocean including the Galapagos Islands (Xiao *et al.*, 2019) (observation excluded from Figure 1 as no coordinates were available), the Clarion Clipperton Zone (CCZ) (Amon *et al.*, 2016) and the Mariana trench (Glickson *et al.*, 2017). Additionally, it was observed on the East Scotia Ridge (ESR) in the Southern Ocean (Magalhães *et al.*, 2017; Xiao *et al.*, 2019), and in the South China Sea (Song *et al.*, 2021). On the EPR and ESR, *R. daphneae* occurred on the periphery of active hydrothermal vent fields (Daly, 2006; Magalhães *et al.*, 2017; Xiao *et al.*, 2019), within nodule fields in the CCZ (Amon *et al.*, 2016), and around mud volcanoes and cold seeps at the Mariana trench (Glickson *et al.*, 2017). In the South China Sea, juveniles of *R. daphneae* were settled on plastic debris (Song *et al.*, 2021).

In this study, we extend the known global distribution of *R. daphneae* presenting the first observations and DNA barcoding results in the Indian Ocean. We provide 16S, 18S and COI sequences, and present physiological and environmental data associated with the documented individuals. In addition, we report megafauna observed in proximity to *R. daphneae* and describe, for the first time, a feeding behaviour exhibited during an expedition in 2022.

Materials and methods

Study area and sample and imagery collection

Annual expeditions have been surveying the Federal Institute for Geosciences and Natural Resources (BGR) contract area for massive polymetallic sulphide deposits since 2013 as part

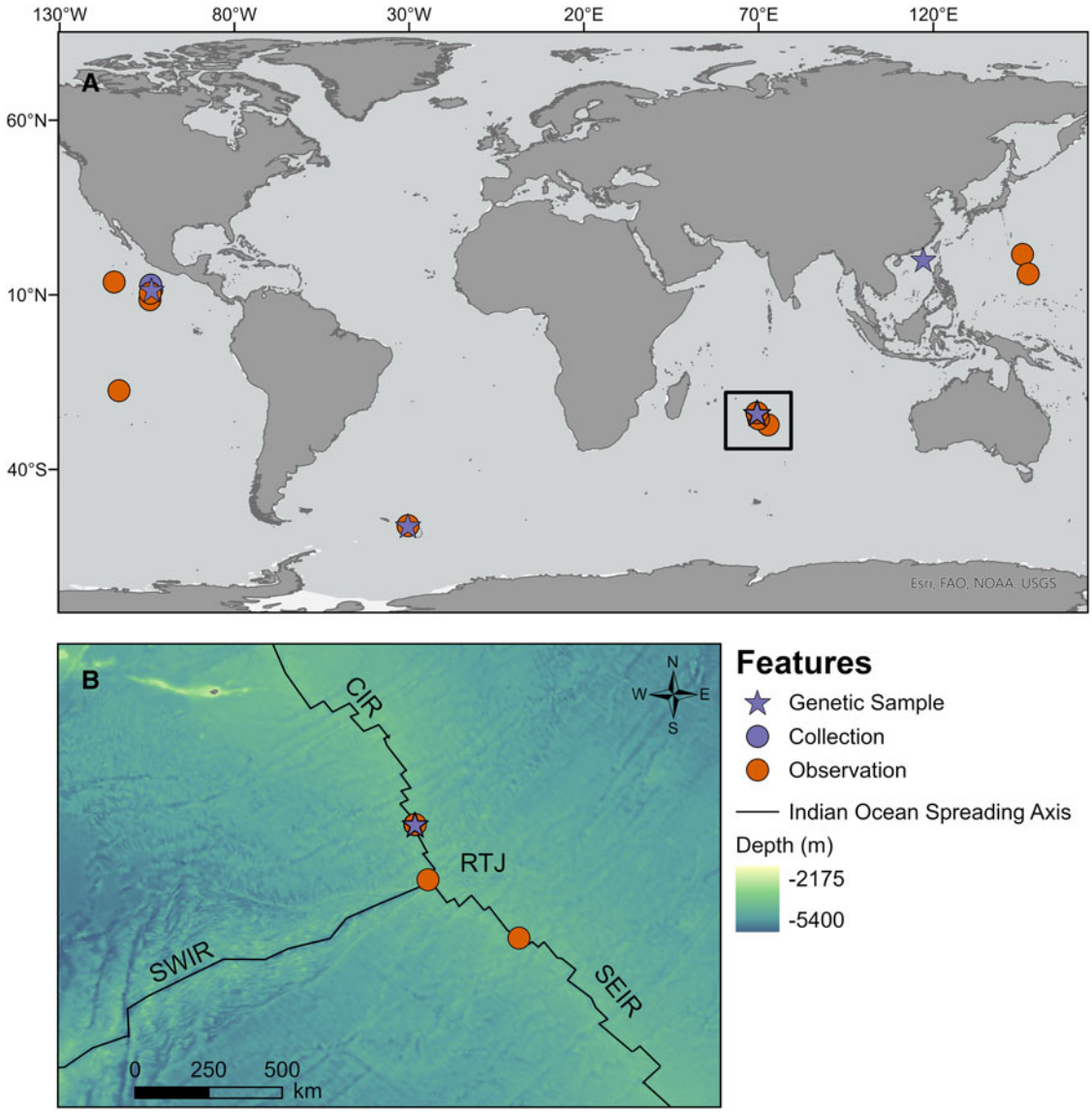

Figure 1. (a) Overview of global locations where *Relicanthus daphneae* has been observed (orange) or collected (purple). Stars represent where molecular analysis has been conducted. The black box outlines the data presented in this study. (b) Three sites where *R. daphneae* was observed within the BGR contract area in the Indian Ocean. SWIR, Southwest Indian Ridge; CIR, Central Indian Ridge; RTJ, Rodriguez Triple Junction; SEIR, Southeast Indian Ridge. Bathymetry map provided by: GEBCO Compilation Group (2020) GEBCO 2020 Grid (doi:10.5285/a29c5465-b138-234d-e053-6c86abc040b9).

of the Indian Ocean Exploration (INDEX) Project. The BGR contract area includes the Rodriguez Triple Junction (RTJ), and portions of the Central Indian Ridge (CIR) and the Southeast Indian Ridge (SEIR). Photographs of *R. daphneae* were obtained using a remotely operated vehicle (ROV) and video sledge aboard the R/V Sonne (2017) and R/V Pelagia (2021, 2022) (Table 1).

The multi-functional video sledge STROMER (German abbr. for 'Simpler TauchROboter, Multifunktional ERweiterbar'), developed by the BGR, recorded continuous HD-video imagery and photographs using a downward looking Canon PowerShot G15 (4000 × 3000 pixels, 180 dpi) or SONY ILCE-9 (6000 × 4000 pixels, 350 dpi) camera, illuminated by a LED lamp, high-intensity discharge lamp and flashlights. Two lasers spaced 30 cm apart were projected onto the seafloor in the field of view of the camera for scaling. The ROV ROPOS (Remotely Operated Platform for Ocean Science; Canadian Scientific Submersible Facilitation) recorded continuous HD-video imagery and manually captured high-resolution DSC photographs using a SONY ILCE-9 (6000 × 4000 pixels, 350 dpi) digital still camera. Two lasers spaced 10 cm apart were projected into the field of view for scaling.

In total, eight specimens were observed (Table 1), but only six could be confidently identified (Figure 2). The other two specimens could not be confidently identified due to high vehicle altitude and low image resolution and, therefore, were not used for any measurements (Supplementary Figure S1). Four of the six identified individuals were seen on the southern CIR (on transects I22-071ROV, I22-073ROV), one on the RTJ (transect I21-093STR) and the other on the SEIR (transect I17-94STR) (Table 1).

Morphological measurements and geological distances

The column diameter and maximum tentacle length of each *R. daphneae* was measured from the imagery in ImageJ (Schneider *et al.*, 2012). Distance from each specimen to the nearest active venting chimney complex and to the nearest inactive sulphide occurrence, as observed during INDEX expeditions (vent field names and coordinates not published), was measured in QGIS3.10 using navigation data collected by STROMER and ROPOS during deployments.

Sample acquisition and genetic analysis

A portion of a tentacle from one individual was sampled with the manipulator arm of ROV ROPOS. On board, the sample was

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Table 1. Metadata of eight *Relicanthus daphneae* individuals recorded in this study, including imagery tool (Platform), transect name, depth, substrate to which specimen was attached and distances to nearest active and inactive hydrothermal site

Area	Year	Date	Platform	Transect	Specimen in Figure 2	Column width (cm)	Tentacle length (cm)	Depth (m)	Substrate	Distance to	
										−Active (m)	−Inactive (m)
SEIR	2017	27 September	STR	I17-94STR	A	14	197	3005	Altered Basalt	66	226
RTJ	2021	16 December	STR	I21-93STR	F	22	143	2993	Basalt	90	127
sCIR	2022	29 November	STR	I22-70STR	–	–	–	3047	Basalt	335	115
sCIR	2022	29 November	STR	I22-70STR	–	–	–	3052	Basalt	258	222
sCIR	2022	30 November	ROV	I22-71ROV	B	9	84	3012	Basalt	685	432
sCIR	2022	30 November	ROV	I22-71ROV	C	4	56	3008	Basalt	640	409
sCIR	2022	01 December	ROV	I22-73ROV	D	14	91	3088	Basalt	172	354
sCIR	2022	01 December	ROV	I22-73ROV	E	12	97	3063	Basalt	710	360

sCIR, southern Central Indian Ridge; RTJ, Rodriguez Triple Junction; SEIR, Southeast Indian Ridge; STR, STROMER (video sledge); ROV, remotely operated vehicle.
Minimum distance to active and inactive hydrothermal site was measured in QGis 3.10 to the centre of the nearest known active chimney complex of an active hydrothermal vent site and to the nearest inactive hydrothermal deposit.

assigned a unique reference number (voucher specimen code: I22_0227), the tissue was fixated in 96% ethanol and stored in −20 °C. Total genomic DNA was extracted at the Senckenberg am Meer, German Centre for Marine Biodiversity Research – DZMB, and sequenced by Macrogen in Amsterdam, the Netherlands. The DNA extraction was carried out using 30 µl Chelex (InstaGene Matrix, Bio-Rad) according to the manufacturer's protocol. Approximately 1800 bp of 18S, 750 bp of 16S and 630 bp of cytochrome c oxidase subunit I (COI) were amplified using primers listed in Table 2. PCR reactions were conducted using an Eppendorf Mastercycler Pro S (Eppendorf, Hamburg, Germany) and each PCR reaction contained: 13.0 µl of AccuStart II PCR ToughMix (2X) (QuantaBio), 9.0 µl of H_2O, 0.5 µl of each primer (10 pmol/µl) and 2 µl of DNA template in a mixture of total 25 µl. The PCR amplification profile for each marker is listed in Table 3. PCR products were visualized after electrophoresis on 1% agarose gels and the reactions which produced bands were sent to be sequenced in both directions using either the primers themselves or the M13 sequence tails (Table 2).

Double stranded sequences were assembled and checked with Geneious version 2023.1.1 (Kearse *et al.*, 2012). When possible, sequences were translated into amino acids prior to analysis to ensure that no gaps or stop codons were present in the alignment. Species identification was performed using the BLAST search from Geneious version 2023.1.1 (Kearse *et al.*, 2012) and BOLD system (Ratnasingham and Hebert, 2007) tree-based identification.

Results and discussion

Occurrence and habitat properties

Prior to this study, there were only 19 published records of *R. daphneae*. Here, we contribute six new observations (Figure 2), expanding the database by more than 30%, totalling 25 records, and extend the global species distribution to the Indian Ocean. These are the only records of *R. daphneae* in the BGR contract area since the launch of the INDEX program (up to and including the 2022 field season). Compared to the depth covered by INDEX expeditions since 2013 (~1600–3700 m), *R. daphneae* was observed over a relatively narrow depth range of 2993–3088 m (Table 1) which is within the globally observed depth range for this species (2400–4400 m) (Daly, 2006; Amon *et al.*, 2016; Magalhães *et al.*, 2017). On the EPR (Pacific Ocean) and East Scotia Ridge (Southern Ocean), *R. daphneae* occurs at a shallower depth (2400–2650 m) (Daly, 2006; Magalhães *et al.*, 2017) while on the Mariana Trench and the CCZ (Pacific Ocean), it occurs at a greater depth (3300–4400 m) (Amon *et al.*, 2016; Glickson *et al.*, 2017). In the South China Sea, juveniles were found on plastic debris that was collected at 1890 m depth (Song *et al.*, 2021). Although this is the shallowest record of *R. daphneae*, it is possible that the debris was transported post settlement and does not truly represent the depth at which the species occurs.

All individuals observed on the Indian Ridge were seen less than 1 km away from both active and inactive hydrothermal deposits (Table 1). We do not believe that the occurrence of *R. daphneae* is limited to the periphery of hydrothermal vent fields exclusively, but rather that enhanced food availability is likely beneficial. We measured the closest reported distance of this species at 66 m from an active hydrothermal vent, shorter than the previously known distance of >100 m (Daly, 2006; Xiao *et al.*, 2019). Four of the six identified specimens occurred around a single active hydrothermal vent field, while the remaining two specimens were observed at two different active vent fields.

Despite the wide range of substrate types identified along imagery transects (sediments, basalts [distinguishable as ridges,

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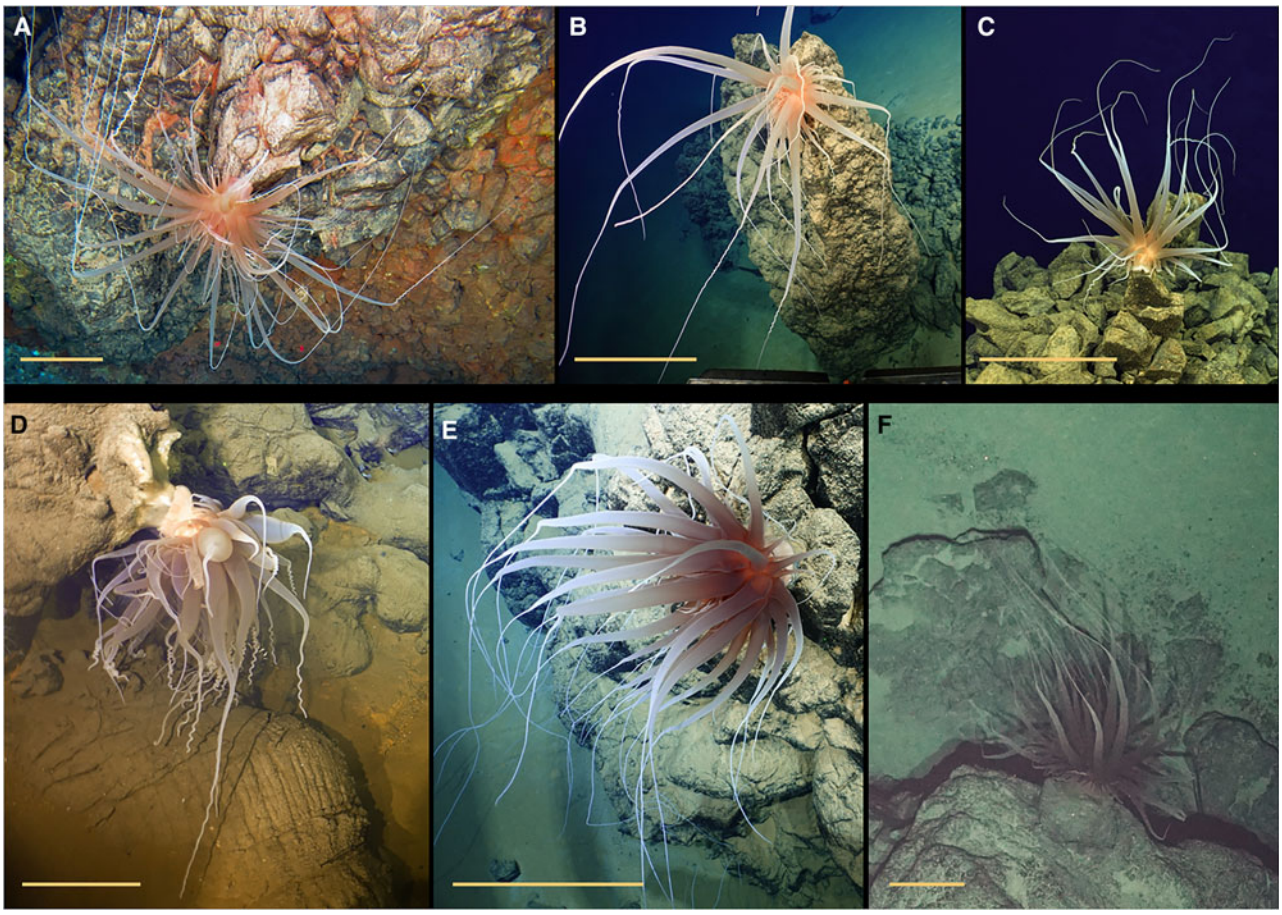

Figure 2. *Relicanthus daphneae* individuals observed within the BGR contract area in the Indian Ocean during three INDEX expeditions between 2017 and 2022. *Relicanthus daphneae* was recorded on the Southeast Indian Ridge (SEIR) (a), the southern Central Indian Ridge (sCIR) (b–e), and the Rodriguez Triple Junction (f). The specimen in panel (d) was photographed after sampling a tentacle for molecular analysis and shows the other tentacles retracted and curled at the distal end. Scale bars are 30 cm length (photograph copyright BGR).

pillows, fault scarps, sheet flow or talus], blocky gabbro or sulphide deposits), all observed specimens were attached to exposed basalt or hydrothermally altered basalt at the edge of a vertical fault scarp or on elevated pillows (Figure 2; Table 1). On the EPR, *R. daphneae* also colonized large basalt outcrops, cliffs and boulders and were oriented with tentacles into the current (Daly, 2006). It is possible that giant anemones position themselves to maximize food availability in the current.

Table 2. List of primers and tails used for amplification and sequencing, including related literature

Primer	Primer sequence	Reference
16S SHA	ACGGAATGAACTCAAATCATGT	(Cunningham and Buss, 1993)
16S SHB	TCGACTGTTTACCAAAAACATA	(Cunningham and Buss, 1993)
18SE	CTGGTTGATCCTGCCAGT	(Hillis and Dixon, 1991)
18S-R2	AGCTGGAATTACCGCGGCTGCT	(Laakmann *et al.*, 2013)
18S-CF2	GAAACTTAAAGGAATTGACGGAA	(Laakmann *et al.*, 2013)
18S-F1	AGCAGCCGCGGTAATTCCAGCT	(Laakmann *et al.*, 2013)
18S-CR1	CCTTCCGTCAATTCCTTTAAGT	(Laakmann *et al.*, 2013)
18SL	CACCTACGGAAACCTTGTTACGACTT	(Hamby and Zimmer, 1988)
jgLCO1490	TITCIACIAAYCAYAARGAYATTGG	(Geller *et al.*, 2013)
jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA	(Geller *et al.*, 2013)
M13FP	TGTAAAACGACGGCCAGT	(Schuelke, 2000)
M13R-pUc	CAGGAAACAGCTATGAC	(Messing, 1983)

Morphological and behavioural observations

Consistent with existing descriptions of *R. daphneae* (Daly, 2006), all specimens in this study were solitary with distances of >50 m between individuals. Epimegafauna in the immediate vicinity of *R. daphneae* included other anemones (Actiniaria fam. indet. [DZMB_2021_0020, Gerdes *et al.*, 2021]), a hydrozoan (*Candelabrum* sp. indet.) and stalked poriferans. Three scale worms, likely of the family Polynoidae, were observed moving on the tentacles and disc of a specimen of *R. daphneae* (Supplementary Video S1, Neufeld *et al.*, 2024). These scale worms are similar to others (Polynoidae gen. indet.) that have been observed on Actiniarian anemones (Actinostolidae gen. indet.) within the INDEX area (Gerdes *et al.*, 2021).

Column width ranged from 4 to 22 cm ($n = 6$ specimens) (Table 1) which was within the lower range of previously reported *in situ* measurements (2–100 cm) (Daly, 2006), while the maximum tentacle length ranged from 56 to 197 cm although it was difficult to obtain accurate measurements. In most cases, tentacles were partially cut out of the image or retracted, resulting in an underestimation of the length.

During the sampling procedure of the tentacle for molecular analysis, no autotomy, either of the sampled or of any other tentacles, could be observed as reported by Daly (2006). However, we

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Table 3. PCR amplification profiles used in this study

Primer	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
16S SHA/16S SHB	94 °C/3 min	94 °C/40 s	47 °C/1 min	72 °C/1 min	72 °C/6 min	40
18SE/18SL	94 °C/3 min	94 °C/30 s	52 °C/45 s	72 °C/2 min	72 °C/3 min	40
jgLCO1490/jgHCO2198	94 °C/3 min	94 °C/30 s	45 °C/45 s	72 °C/45 s	72 °C/2 min	40

only sampled a single tentacle, and the observed autotomy was reported when sampling entire *R. daphneae* specimens. The disturbance caused by sampling provoked a retraction behaviour of almost all tentacles, causing the tips to curl and widening of the tentacle base (Figure 2D).

We observed for the first time, *R. daphneae* capturing live prey (Supplementary Video S2, Neufeld *et al.*, 2024), the shrimp *Rimicaris kairei* Watabe and Hashimoto, 2002. A single live *R. kairei* was stuck to the side of a tentacle of *R. daphneae*, moving its antennae, pleopods and legs. The movement of the partially entangled shrimp seemed to provoke *R. daphneae* to wrap its tentacle around the captured specimen, presumably to prevent it from escaping. It has been previously suggested that the unusually large size of the tentacles and spirocysts (organelles specialized for adhesion) enable these anemones to capture large free-swimming fauna (Daly, 2006). However, specimens have never been observed with prey, either captured or partially digested.

Molecular results

Three sequences were obtained from the analysed material and fragment lengths ranged from 639 to 1786 bp. All sequences were barcode compliant and placed in BIN BOLD: AEI2630. Comparing our COI barcoding result with GenBank and BOLD databases, we obtained 100% similarity score with: Cnidaria, Anthozoa, Actiniaria, Relicanthidae, *Relicanthus*, *R. daphneae* – GenBank MN055612 (South China Sea; Song *et al.*, 2021) and MK947129 (Pacific and Southern Oceans; Xiao *et al.*, 2019).

In addition, genetic analysis also revealed a high degree of similarity (≥99.9%) between the sequenced 16S and 18S genes and existing sequences in the GenBank database. The 16S showed a 99.9% similarity score to MK947129 (Pacific and Southern Oceans; Xiao *et al.*, 2019). Similarly, the 18S exhibited 100% similarity to KJ483028 (Pacific Ocean; Rodrígues *et al.*, 2014) and 99.9% similarity to MN059669 (South China Sea; Song *et al.*, 2021).

While molecular data suggest a widespread distribution of *R. daphneae* across the Pacific, Southern and Indian Oceans (Figure 1a), it is important to consider these findings in conjunction with morphological observations and a larger sample size. Although the analysed specimens exhibited high genetic similarity to *R. daphneae* sequences from various locations, subtle morphological variations may exist between populations. Therefore, further investigation, incorporating both morphological examinations and a more extensive molecular dataset is needed to confirm the species identification and fully understand the biogeographic patterns of *R. daphneae* with potential regional variations.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315424001127.

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Data availability. The photographs analysed during the current study and the generated tables containing aerial estimation and annotated occurrence data are available from the corresponding author on reasonable request. Sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers: COI – PP264562, 16S – PP264564 and 18S – PP264563. Additional relevant specimen information, taxonomic classifications, photos, DNA barcodes, used primer pairs and trace files were uploaded to the Barcode of Life Data Systems (BOLD; www.boldsystems.org; Ratnasingham and Hebert, 2007), project "Indian Ocean Hydrothermal Vent Fauna – INDEX", subproject "Indian Ocean Hydrothermal Vent Megafauna" in the public dataset "INDEX_Megafauna_2024_2" with dataset code: DS-INMEG242 and DOI: dx.doi.org/10.5883/DS-INMEG242. Supplement videos are available on PANGAEA (https://doi.org/10.1594/PANGAEA.967443).

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Competing interest. None.

Ethical standards. All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable. The study is compliant with CBD and Nagoya protocols.

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