Eurythenes sigmiferus and *Eurythenes andhakarae* (Crustacea: Amphipoda) are sympatric at the abyssal Agulhas Fracture Zone, South Atlantic Ocean, and notes on their distributions

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ABSTRACT

Cryptic species in the deep ocean are rapidly being identified with molecular evidence and as a result, new species are being described. Consequently, our understanding of distributions among the revised landscape of species needs to be reassessed. A model example is the large scavenging amphipod, *Eurythenes gryllus* (Lichtenstein in Mandt, 1882), which historically was thought to have a eurybathic and cosmopolitan distribution. Molecular evidence has since led to the separation of *E. gryllus* into ten named species and truncating its range to bi-polar bathyal depths. This study focuses on two species; *Eurythenes sigmiferus* and *Eurythenes andhakarae* d'Udekem d'Acoz and Havermans, 2015, and presents new records of both species from 5,493 m in the previously unsampled Agulhas Fracture Zone, South Atlantic Ocean (42.77°S, 10.05°E). We paired morphology with DNA barcoding at two mitochondrial regions to achieve robust identification and assessed their wider geographic range by reassessing historical records. Their overlapping presence at the Agulhas Fracture Zone expands their known ranges to the non-polar South Atlantic Ocean. Specifically, for *E. sigmiferus,* the data suggests this species has a multi-ocean tropical to temperate distribution from abyssal to shallow hadal depths (3,410–6,097 m). *Eurythenes andhakarae* is not restricted to the Southern Ocean but is distributed across the Antarctic Polar Front to the temperate South Atlantic Ocean between abyssal and hadal depths (3,069–7,099 m), with a presence at bathyal depths requiring molecular confirmation. This study highlights that pairing new expeditions with a re-inspection of rich historical collections exploration can fill in data gaps across species ranges and, ultimately, biogeography.

1. Introduction

The growth of DNA barcoding has led to the detection of genetically divergent lineages among morphologically similar species, or cryptic species, and new species descriptions (e.g., Bickford et al., 2007; Pfenninger and Schwenk, 2007; Vrijenhoek, 2009; Brasier et al., 2016; González-Wevar et al., 2022; Maroni et al., 2022). The discovery of cryptic species often requires a reassessment of species distributions across the genus (Darling and Carlton, 2018). The need for reassessment is becoming increasingly common in the deep ocean. A model example of the phenomenon of cryptic diversity in the deep ocean is the amphipod genus *Eurythenes* S. I. Smith in Scudder, 1882). Due to their large size and ease of capture with baited traps, *Eurythenes* has been the focus of many biological studies, with most presuming to study the species *Eurythenes gryllus* (Lichtenstein in Mandt, 1822) (e.g., Baldwin and Smith, 1987; Ingram and Hessler, 1987; Hargrave et al., 1994; Thurston et al., 2002; Perrone et al., 2003; Eustace et al., 2016). However, molecular phylogenetics indicated that *E. gryllus* was not a single

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species but a complex of species (France and Kocher, 1996; Havermans et al., 2013). Havermans et al. (2013) identified morphological differentiations and subsequent bodies of work have expanded the genus from four to ten nominal species with several undescribed lineages (d'Udekem d'Acoz and Havermans, 2015; Horton et al., 2020; Narahara-Nakano et al., 2018; Weston et al., 2020; Weston et al., 2021).

The division of *E. gryllus* into an array of cryptic species has resulted in complex geographic and bathymetric patterns across the genus (Havermans, 2016). Before the discovery of the species complex, *E. gryllus* was considered to have a cosmopolitan distribution and an extremely eurybathic range, spanning depths between 50 and 8,000 m (Ainley et al., 1986; Fujii et al., 2013). Currently, *E. gryllus sensu stricto* has a more limited range, being reduced to bipolar distribution at bathyal depths between 750 and 3,000 m (Havermans, 2016). All species within this complex have differing distributions, spanning from multiple oceans to single records, only known from a single oceanic feature, which was not an apparent trend when they were all considered *E. gryllus*. Further, ranges of *Eurythenes* can overlap, with documented instances of sympatry with multiple species recovered in the same trap (Bribiesca-Contreras et al., 2021; Eustace et al., 2016; Havermans, 2016; Horton et al., 2020).

Of the two species considered here, *Eurythenes andhakarae* d'Udekem d'Acoz and Havermans, 2015 was first molecularly identified and described from ~330 individuals that were collected from five stations across abyssal depths (3,070–4,693 m) of the Weddell Sea (Antarctica) (d'Udekem d'Acoz and Havermans, 2015; Havermans et al., 2013; Havermans, 2016). Jamieson et al. (2021b) documented three individuals by morphology-only from hadal depths (6,044–7,099 m) of the South Sandwich Trench (Antarctic to sub-Antarctic). Within this genus, *E. andhakarae* is considered visually cryptic with fine differences in the relative shape of the pereopod 6–7 merus and basis, the anterior lobe of the head, and the number of nodular spines on the inner plate of the maxilliped (d'Udekem d'Acoz and Havermans, 2015). In visual contrast, *Eurythenes sigmiferus* d'Udekem d'Acoz and Havermans, 2015 is the largest species and possesses a distinctive crested dorsal ridge. The only certain record of *E. sigmiferus* is based on a single specimen from the type locality, the Brazil Basin (tropical Atlantic Ocean) at 4,480 m (d'Udekem d'Acoz and Havermans, 2015). Yet, 16S sequences and published photographs showing the distinctive ridge suggest a possible wider distribution into the Gulf of Mexico, ~5,000 m off South Africa in the Indian Ocean, and the shallow-hadal depths of the Kermadec Trench in the Pacific Ocean (Barnard, 1961; d'Udekem d'Acoz and Havermans, 2015; Escobar-Briones et al., 2010; Horton et al., 2020). Together, the rarity of records, revisions to taxonomy, and shared habitats make characterising distributions complicated with substantive data gaps.

While knowledge of geographic and bathymetric distributions within *Eurythenes* has improved, sampling the deep ocean is logistically and technically difficult, and thus records are limited. Expeditions to unsampled localities and re-inspection of historical collections provide the opportunity to reassess the distribution of many *Eurythenes* species. In this study, we focused on presenting new records of *E. andhakarae* and *E. sigmiferus* from the previously unsampled Agulhas Fracture Zone, South Atlantic Ocean as part of the Five Deeps Expedition in February 2019 (Jamieson et al., 2021b). Morphological examination of specimens was paired with DNA barcoding of two mitochondrial markers, 16S ribosomal DNA (16S) and cytochrome *c* oxidase I (COI), to achieve robust identifications. We then placed these new records from the Agulhas Fracture Zone in the context of the wider geographic range of these species. To provide an updated assessment, we supplemented the genetic dataset with new data for (1) *E. andhakarae* from hadal depths of the South Sandwich Trench (Jamieson et al., 2021b), (2) an individual published as *E. gryllus* from the South Fiji Basin, Pacific Ocean but with re-inspection, its morphologically appears to be consistent with *E. sigmiferus* (Lacey et al., 2016), and (3) *E.* c.f. *sigmiferus* material from the Gulf of Mexico that has historically been sequenced for 16S (Escobar-Briones et al., 2010). The presence of *E. sigmiferus* and *E. andhakarae*

at the Agulhas Fracture Zone expands their known ranges to the non-polar South Atlantic Ocean and increases our knowledge about the biogeography of *Eurythenes* across the deep ocean.

2. Methods & materials

2.1. Study area

The Agulhas Fracture Zone is a long, linear bathymetric feature formed broadly perpendicular to the Mid-Atlantic Spreading Ridge and extends eastward to offshore the coastal city of Durban, south-east South Africa (Jokat and Hagen, 2017). Parallel to the Agulhas Fracture Zone is the pronounced Agulhas Ridge (Fig. 1A) that forms a 3,400 m high feature from the ridge crest to the deepest point of the trough (Fig. 1B) located ~1,220 km south-west of South Africa. Another comparably high ridge runs parallel to the Agulhas Ridge and forms the southeast boundary of the intervening fracture zone trough mapped during this study (Fig. 1A).

2.2. Physical mapping and biological sampling

The Agulhas Fracture Zone was concurrently mapped and sampled from 16th to 19th February 2019 from the DSSV *Pressure Drop* (Fig. 1A). The study was part of the South Sandwich Trench Expedition and the larger circumglobal Five Deeps Expedition (Jamieson, 2020; Jamieson et al., 2021b). The fracture zone axis was mapped, along a single line traveling from south to northeast and one short cross line perpendicular to the axis of the fracture zone at the sample site, with an EM124 hull-mounted multibeam echosounder (MBES; see Bongiovanni et al. (2022) for specifics on MBES data acquisition and processing).

One free-fall lander, *Skaff*, was deployed on 17th February 2019 to the station AFZ_SK1_5400, at coordinates 42.7669°S 10.0519°E to a depth of 5,493 m, the deepest point of the fracture zone (Fig. 1B). The lander was equipped with a baited HD video camera (IP Multi SeaCam 3105; Deep Sea Power and Light, San Diego, CA) that continuously filmed for the duration of the deployment, a baited invertebrate trap (15 cm × 100 cm funnel tubes), and a conductivity, temperature, and depth (CTD) profiler (SBE 49 FastCAT; SeaBird Electronics, Bellevue, WA). The bait was whole mackerel (*Scomber* sp.). Three mackerel (~100 g per fish) were attached to the bait arm, and one mackerel was cut up and placed into two mesh bags in the trap. The lander video data was assessed to provide a visual interpretation of the seafloor habitat substrate and local biodiversity.

2.3. Species identification with paired morphology and DNA barcoding

Upon recovery and initial sorting on deck, all *Eurythenes* spp. individuals were photographed with a Canon EOS 750D DSLR camera, Tamron SP 90 mm f/2.8 VC USD Macro 1:1 VC Lens with a polarising filter, and Falcon Eyes CS-730 copy stand, and then preserved in 70% ethanol. Photographs were processed with Helicon Focus and Helicon Remote software (Helicon Soft). Post-cruise, all *Eurythenes* spp. were sorted into morphotypes and preliminarily identified to the lowest rank possible following d'Ukdekem d'Acoz and Havermans (2015) and Weston et al. (2021) with a stereomicroscope (Wild Heerbrugg M8). Particular attention was given presence and shape of dorsal ridging, the shape of the anterior lobe of the head, the number of nodular spines on the inner plate of the maxilliped, the palm profile and dactylus length of gnathopod 1 and 2, the shape of the pereopod 7 basis and merus, and the presence of a tooth on epimeron plate 3. To standardise measurements, all *Eurythenes* spp. were straightened and total body length, from the tip of the rostrum to the end of the telson, was measured with digital callipers. Specimens are housed at the University of Western Australia.

Two partial mitochondrial markers, 16S and COI, were analyzed to validate all morphological identifications documented in this study. From the two morphologically identified *Eurythenes* morphotypes from
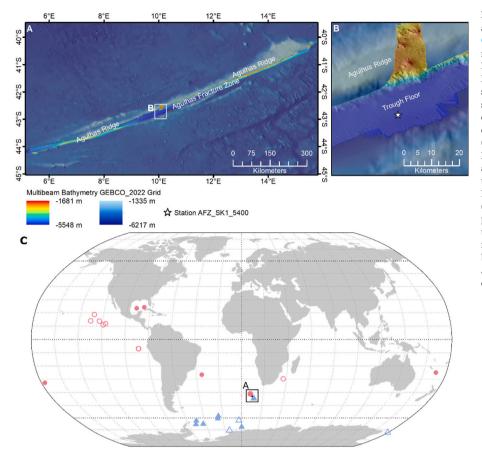
Fig. 1. (A) Bathymetry of the Agulhas Fracture Zone and Agulhas Ridge. Regional bathymetric data from GEBCO Bathymetric Compilation Group (2022), multibeam bathymetry data acquired during the Five Deeps Expedition (Unpublished, Caladan Oceanic LLC, see Bongiovanni et al. (2022) for technical specifications). (B) Inset map showing the location of station AFZ_SK1_5400 (42.7669°S 10.0519°E) at the deepest point of the fracture zone. (C) Geographic distribution of the amphipods *Eurythenes sigmiferus* (red circle) and *Eurythenes andhakarae* (blue triangle). New records from the Agulhas Fracture Zone (box A) are from the same location, denoted by the dot, but the symbols have been separated for visual clarity. Records with corresponding DNA barcoding support (16S, COI, and/or 18S) and higher confidence of true presence are indicated by filled-in symbols. Records that are based on morphology only, thus lower confidence of true presence, are shown by empty symbols. The black box denotes the location of the Agulhas Fracture Zone. References for historical records are available in Supplementary Table 2.

the Agulhas Fracture Zone, four individuals from each type, spanning the size ranges, were selected for molecular taxonomy. In addition, we supplemented the molecular dataset with three *E. andhakarae* individuals recovered from depths of 6,044, 6,640, and 7,099 m in the South Sandwich Trench (sampling details in Jamieson et al., 2021a), one *E. sigmiferus* morphotype from 4,102 m in the South Fiji Basin that was published as *E. gryllus* (Lacey et al., 2016), and five *E. sigmiferus* morphotypes from 3,410 in the Gulf of Mexico, also published as *E. gryllus* (Escobar-Briones et al., 2010).

Total genomic DNA was extracted from pleopod 1 using either a magnetic bead-based protocol (Oberacker et al., 2019) or a DNeasy Blood and Tissue Kit (Qiagen). As described in Ritchie et al. (2015), the 16S region (~260 bp) was amplified with the primer sets AMPH1 (France and Kocher, 1996) and 'Drosophila-type' 16SBr (Palumbi et al., 1991), and the COI region (~658 bp) was amplified with LCO1490 and HCO12198 (Folmer et al., 1994). PCR products were purified either enzymatically or with a Millipore Multiscreen 96-well PCR Purification System. Products were bidirectionally sequenced, using the same primers as in the PCR reactions, with an ABI 3730XL sequencer either by Eurofins Genomics, The Natural History Museum Sequencing Facility, London, or the Australian Genome Research Facility.

Electropherograms were manually trimmed in MEGA X (Kumar et al., 2018). Any ambiguous base calls were denoted with N. COI sequences were translated to assess for stop codon presence. Sequences were initially compared with species diagnostic barcodes using the NCBI BLASTn website.

As some lineages are only represented by 16S sequences, two datasets, one for each marker, were assembled to resolve identifications linked with either type material, high-confident identifications, or defined but known unnamed lineages (Supplemental Table 1) (d'Udekem d'Acoz and Havermans, 2015; Escobar-Briones et al., 2010; France and Kocher, 1996; Havermans, 2016; Havermans et al., 2013; Horton

et al., 2020; Narahara-Nakano et al., 2018; Weston et al., 2021, 2020). All previously published sequences of *E. andhakarae, E. sigmiferus,* and *E.* c.f. *sigmiferus* that were available by May 2022 on GenBank and the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert, 2007) were included. *Alicella gigantea* Chevreux (1899), *Hirondellea dubia* Dahl, 1959, and *Bathycallisoma schellenbergi* (Birstein and Vinogradov, 1958) were selected as outgroups, as these deep-sea scavenging amphipods are in either separate superfamilies or families and sufficiently divergent from *Eurythenes* spp. (Ritchie et al., 2015). Alignments for both loci were created using the MAFFT v7 (Katoh et al., 2019) webserver with the FFT–NS–1 strategy and trimmed to equal length. Due to the comparatively short length of *E. andhakarae* JX887112, we tested and chose to not include it in the final COI alignment to increase the dataset by 169 bp. For individuals of *E. andhakarae* represented by identical accession numbers (i.e., JX887065 and JX887116), we selected one individual to represent that sequence. The two aligned datasets were: (1) 16S with 47 individuals (138 bp), and (2) COI with 55 individuals (470 bp) (Supplemental Table 1).

We applied three species delimitation methods to support specimen identifications within *Eurythenes*, two distance-based methods and one tree-based method. The first distance-based method was the Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021), which was conducted on the ASAP webserver with the JC69 Jukes-Cantor parameter. The second distance-based method, the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2011), was executed with default settings on the ABGD webserver. The tree-based method was the Bayesian implementation of the Poisson Tree Processes (bPTP) (Zhang et al., 2013). The input tree for bPTP was inferred using the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) software package v1.10.4 (Suchard et al., 2018). For both datasets, the HKY $+$ I $+$ G model (Hasegawa et al., 1985) and an uncorrelated relaxed clock were selected. Two independent runs were executed for 40M

generations, sampling every 10,000 generations, for each gene and approach. Outputs were reviewed with Tracer v1.7 to ensure convergence (effective sample size >200) and merged in LogCombiner v1.8.4. The maximum clade credibility tree was determined by TreeAnnotator v1.8.4, after burning the first 10% of states. The unrooted tree was analyzed on the bPTP webserver for 200,000 generations, thinning of 100, and a burn-in of 25%. In addition, inter- and intraspecific pairwise p-distances (transition and transversion substitution included and complete deletion) were calculated using MEGA X.

For *E. andhakarae,* the relationship between the COI haplotypes and the geographic distribution of 25 individuals were investigated by building a haplotype network from a 524 bp alignment using the statistical parsimony method (Templeton et al., 1992) in PopART v1.7 (Leigh and Bryant, 2015) (Supplemental Table 1). The graphical presentation of the gene trees and haplotype network was annotated in Inkscape v0.92.2 (The Inkscape Team, 2017).

Presence records for *E. sigmiferus* and *E. andhakarae* are collated from two sources — publications and records available from the Ocean Biodiversity Information System (OBIS) (Supplemental Table 2) (Barnard, 1961; d'Udekem d'Acoz and Havermans, 2015; Escobar-Briones et al., 2010; Havermans, 2016; Havermans et al., 2013; Jamieson et al., 2021b; Lacey et al., 2016; Meurisse and Semal, 2020; NIWA, 2018; Patel et al., 2020). We ranked the identification confidence of each record. Higher confidence was given to records with DNA barcoding support. Lower confidence was given to morphology-only identifications, particularly records only present on OBIS. A global map of presence records (Fig. 1C) was created using R Statistical Software (v4.2.1; R Core Team, 2022) with the packages *rgdal, sf, ggmap,* and *ggplot2* (Bivand et al., 2022; Kahle and Wickham, 2013; Pebesma, 2018; Wickham, 2016).
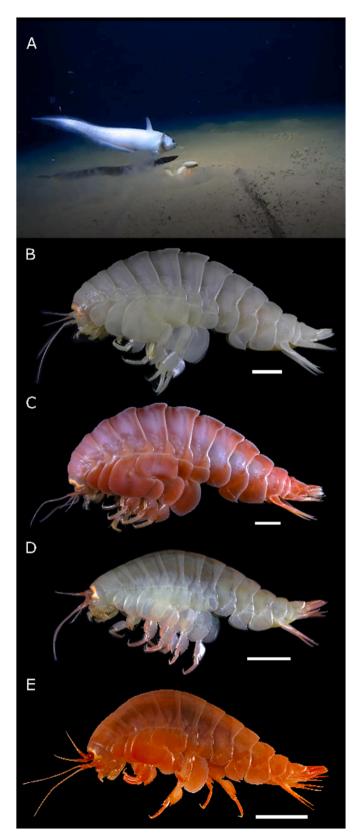
3. Results

3.1. Geology, habitat, and in situ community

The bottom temperature at station AFZ_SK1_5400 was 0.95 °C, with the coldest temperature (0.88 °C) recorded at 4,542 m during the descent in the water column. Visual interpretation of seafloor composition was made from the lander video data revealing the seafloor at 5,493 m to consist of very soft, fine-grained sediment likely representative of a comparatively thick sediment blanket located on the floor of the fracture zone axis, where the bait arm was completely buried in the sediment (Fig. 2A). While unmeasured, a current was noticeable in the video data with particles being transported in the water column.

Across the 7:49 h of bottom-time, the in-situ community comprised two genera morphotypes of scavenging amphipods, large *Eurythenes* spp. and small *Paralicella* spp. individuals. Quantifying the number of individuals was not possible, due to their size and movement and the partially submerged bait arm. Amphipods were interacting with the bait, with some presumed to be directly feeding on the bait as only mackerel bones returned to the surface, and appeared to swim in and out of the soft sediment (Fig. 2A). In addition to amphipods, two fishes interacted with the bait or the amphipods on the bait, and they were previously identified as *Coryphaenoides armatus* and *Bassozetus* sp. inc. 5-AFZ (Jamieson et al., 2021a). Moving in the camera's field of view but not interacting with the bait arm, 165 larvaceans and nine trachymdusae were observed (Jamieson and Linley, 2021). No other faunal groups, such as isopods or holothurians, were observed.

3.2. Eurythenes DNA barcoding and species delimitation

The paired morphological assessment and species delimitation supported that two species of *Eurythenes* were present in the trap, *E. sigmiferus* (Fig. 2B and C) and *E. andhakarae* (Fig. 2D and E). In both species, the diagnostic characters presented in d'Udekem d'Acoz and Havermans (2015) were seen in the Agulhas Fracture Zone material. In
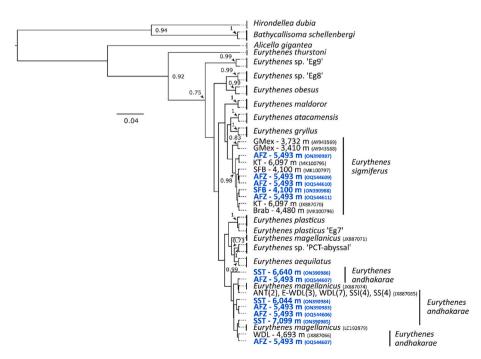

Fig. 2. (A) In situ still at 4h:25min after landing of the bait arm buried in the sediment and bait-attending fauna, including *Coryphaenoides armatus* and amphipods, from 5,493 m in the Agulhas Fracture Zone, South Atlantic Ocean. Pre-ethanol preservation of (B–C) *Eurythenes sigmiferus* and (D–E) *Eurythenes andhakarae,* where the white scale bars represent 1 cm.

total, there were 27 *E. andhakarae* individuals, ranging from 3.1 to 13.4 cm in total length, with an average of 5.8 cm. There were 32 *E. sigmiferus* individuals, ranging from 3.6 to 11.3 cm in total length and averaging 7.3 cm. Before ethanol preservation, individuals of both species varied in body colour from white to crimson red. In addition to the 59 *Eurythenes* spp., seven *Paralicella* spp. individuals were recovered.

The DNA barcoding dataset consisted of a total of 24 sequences for seven *E. andhakarae* and ten *E. sigmiferus* which were deposited in GenBank (Supplementary Table 1). Amplification of COI from the single *E. sigmiferus* from the South Fiji Basin, three *E. sigmiferus* from the Agulhas Fracture Zone, and one *E. andhakarae* from the Agulhas Fracture Zone was not successful.

The 16S phylogeny nested the four Agulhas Fracture Zone *E. sigmiferus* individuals with other *E. sigmiferus*, which included those from the Gulf of Mexico (Escobar-Briones et al., 2010), Brazil Basin (d'Udekem d'Acoz and Havermans, 2015), Kermadec Trench (public on GenBank), and South Fiji Basin (this study), to form a single, monophyletic *E. sigmiferus* clade (Fig. 3). The new seven *E. andhakarae* individuals from the Agulhas Fracture Zone and South Sandwich Trench were polyphyletic within a larger clade that also consisted of the comparative *E. andhakarae* material (Havermans et al., 2013), and two individuals with published identifications as *Eurythenes magellanicus* (H. Milne Edwards, 1848) (Havermans et al., 2013; Narahara-Nakano et al., 2018). However, the result of *E. magellanicus* being polyphyletic was an artifact of the short alignment with a low number of parsimony-informative sites (41 out of 138 overall), where testing of longer alignments placed these individuals into a separate, monophyletic clade.

From the COI data, the three delimitation approaches identified 15 or 16 distinct species lineages (Fig. 4A). *Eurythenes andhakarae* was consistently delineated as a well-supported clade (BPP: 0.89), consisting of individuals from the greater Weddell and Scotia Seas (Havermans et al., 2013; Havermans 2016), South Sandwich Trench (this study), and Agulhas Fracture Zone (this study). The pairwise p-distances within the *E. andhakarae* ranged from 0 to 1.9% and 8.5–16.8% among the other *Eurythenes* lineages. For *E. sigmiferus*, the distance-based methods lumped the new Agulhas Fracture Zone and the new Gulf of Mexico individuals as one lineage. However, the bPTP analysis split the two groups (BPP: 1). The pairwise p-distances ranged from 0 to 2.1% between the two populations and 8.7–17.0% among the other species. The

published *E. c.f. sigmiferus* sequences from the Clarion-Clipperton Zone (Mohrobeck et al., 2015) were placed in a separate group with *Eurythenes maldoror* d'Udekem d'Acoz and Havermans, 2015.

The *E. andhakarae* COI haplotype network has seven haplotypes across the 25 individuals (Fig. 4B). The network pattern was star-shaped around one dominant haplotype. One Agulhas Fracture Zone and one South Sandwich individual shared the dominant haplotype. The two Agulhas Fracture Zone individuals and the 6640 and 7099 m from the South Sandwich Trench ranged from three to six mutational steps removed from the dominant haplotype.

4. Discussion

Eurythenes is an iconic genus of deep-ocean-dwelling amphipods. Despite being well-studied, molecular evidence has revealed high levels of cryptic diversity within the genus thus, species need to be re-examined to understand present-day distributions. Here, we sampled the Agulhas Fracture Zone at 5,493 m and paired morphological assessments with DNA barcoding to support the identification of, *E. sigmiferus* and *E. andhakarae,* present in sympatry. Their presence at the Agulhas Fracture Zone extends the known range of both species to the South Atlantic Ocean. Further, these new records were placed in the context of the wider geographic range of these species by re-evaluating historical records in other basins, like the Gulf of Mexico and Southwest Pacific Ocean. By linking these new records with a re-assessment of historical records, we gained biogeographic insights from the expanded exploration of the deep ocean.

4.1. Eurythenes sigmiferus - tropical to temperate abyssal distribution

Eurythenes sigmiferus, with its keeled dorsal ridge, is one of the most distinctive species within the genus, but it is also among the rarest, with the only confirmed record being the holotype from abyssal depths of the Brazil Basin (d'Udekem d'Acoz and Havermans, 2015). With the discovery of a comparatively large population (n = 32) at the Agulhas Fracture Zone, key insights into *E. sigmiferus*'s size, geographic, and vertical distribution can be gleaned. The holotype is 5.3 cm, which is smaller than the average size (7.3 cm) of the individuals collected here. Moreso than presented by the holotype, *E. sigmiferus* strongly exhibits gigantism, with four individuals measured at total lengths over 10 cm.

Fig. 3. Bayesian inferred tree based on a 16S rRNA (146 bp) showing the relationship of new sequences of *Eurythenes sigmiferus* and *Eurythenes andhakarae* (shown as bold, blue) within *Eurythenes*. Branch nodes have Bayesian posterior probabilities support values greater than 0.7. For *E. sigmiferus* and *E. andhakarae,* the sampling location and depth (m) are provided, if known. Locations are abbreviated as AFZ: Agulhas Fracture Zone, ANT: East Antarctic Peninsula, Brab: Brazil Basin, E-WDL: Eastern Weddell Sea, GMex: Gulf of Mexico, SFB: South Fiji Basin, SS: Scotia Sea, SSI: South Sandwich Islands, SST: South Sandwich Trench, WDL: Weddell Sea. References for comparative sequences are in Supplemental Table 1.
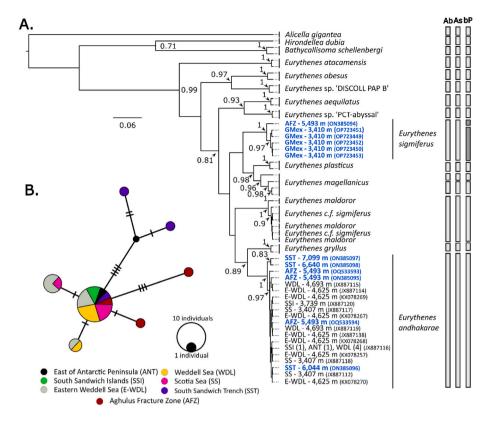
Fig. 4. (A) Bayesian inferred tree showing species boundaries and relationship of new sequences of *Eurythenes sigmiferus* and *Eurythenes andhakarae* (shown as bold, blue) based on COI (470 bp). Branch nodes have Bayesian posterior probabilities support values greater than 0.7. Results from the three molecular species delimitation methods, AS: ASAP, AB: ABGD, and bP: bPTP, are shown with vertical bars, with disagreements (dark grey). Locations are abbreviated as AFZ: Agulhas Fracture Zone, ANT: East Antarctic Peninsula, Brab: Brazil Basin, E-WDL: Eastern Weddell Sea, GMex: Gulf of Mexico, SFB: South Fiji Basin, SS: Scotia Sea, SSI: South Sandwich Islands, SST: South Sandwich Trench, WDL: Weddell Sea. (B) Statistical parsimony haplotype network of 25 individuals of *E. andhakarae* based on COI (524 bp). Colors represent a location. The circle size is proportional to the number of individuals with a given haplotype. The lines between haplotypes denote the number of base pair differences between haplotypes. The small black dots represent predicted but not directly sampled haplotypes. References for comparative sequences are in Supplemental Table 1.

Yet, these large individuals remain smaller than the largest recorded *Eurythenes* at 15.4 cm (Baldwin and Smith, 1987).

Finding this large population suggests that *E. sigmiferus* might not be functionally rare but rather has a multi-ocean distribution and is perceived as rare due to under-sampling of the deep ocean (McClain, 2021). The 16S data provides strong evidence that the specimen from the Agulhas Fracture Zone is the same species as the holotype from the Brazil Basin and is also present in the Gulf of Mexico, Kermadec Trench, and South Fiji Basin (Fig. 3). While records of *E. sigmiferus* are based on morphology and we recognize the resolution limitations associated with <300 bp 16S fragments (e.g., the placement of *E. magellanicus* in the 16S phylogeny; Jażdżewska et al., 2021), we can be confident that *E. sigmiferus* has a tropical to temperate distribution in the Pacific and Atlantic Oceans between depths of 3,410–6,097 m (Fig. 1). More physical and genetic evidence is needed to verify its presence in the Indian Ocean. Curiously, it remains unconfirmed whether *E. sigmiferus* is present in the Clarion-Clipperton Zone and Peru Basin. Patel et al. (2020) identified it in collections by morphology-only and Mohrbeck et al. (2021) nominally identified different samples from the same region as *E. c.f. sigmiferus*. Yet, the delimitation results strongly suggested that *E. c.f. sigmiferus* are in fact *E. maldoror* (Fig. 4). This identification highlights the importance of pairing morphological information with DNA barcode data to strengthen confidence in identifications, particularly in the deep ocean where specimens can be rare and recovered and preserved in unfavourable conditions.

Although the sample sizes are small, both phylogenies showed an intriguing hint of geographic structuring among the Gulf of Mexico and other locations. Specifically, in the COI phylogeny, there was discordance among the delineation results either lumping or splitting the Agulhas Fracture Zone individual (depth 5,493 m) from the Gulf of Mexico (3,140 m; Fig. 4a). The bPTP method, however, is prone to oversplitting taxa, including amphipods (e.g., Luo et al., 2018; Jażdżewska et al., 2021), and the p-distances were below the commonly applied 3% divergence threshold for crustaceans (Hebert et al., 2003). Together, these points more towards evidence of genetic isolation between two

populations rather than two cryptic species. While outside this scope and limited by the mitochondrial dataset, this pattern may be explained in part by the semi-oceanographic isolation of the western Gulf of Mexico with the Sigsbee Abyssal Gyre and Florida Straits, a relatively shallow geologic sill (max 1,800 m) that separates the Gulf of Mexico and Atlantic basins (Pérez-Brunius et al., 2018). Across multiple taxa, including octopus (Timm et al., 2020), coral (Studivan and Voss, 2018), and tanaid (Drumm and Kreiser, 2012), the Florida Straits and Gulf of Mexico circulation patterns are shown to drive genetic isolation. Resolving the level of gene flow and degree of isolation among *E. sigmiferus* populations is merited for future work.

4.2. Eurythenes andhakarae – Crossing the Antarctic Polar Front from abyssal to hadal depths

Eurythenes andhakarae is a visually cryptic species within the genus, and unlike sister species, it has been well sampled across the wider Weddell Seas at abyssal depths (d'Udekem d'Acoz and Havermans, 2015). Here, similarities were documented to historical collections in the size of the catch, the variation of body colour from white to bright red, and the range of sizes of individuals. Before the Five Deeps Expedition, *E. andhakarae* was recovered during the ANDEEP II and III expeditions from seven stations with catches ranging from 1 to 120 specimens (d'Udekem d'Acoz and Havermans, 2015), which makes the catches at the Agulhas Fracture Zone (n = 27) and South Sandwich Trench (n = 1 per station) not outliers. From the ANDEEP expeditions, the documented size in type material ranged from the 3.4 cm immature holotype to a 9.5 cm paratype male (d'Udekem d'Acoz and Havermans, 2015). The Agulhas Fracture Zone catch extends both the known minimum and maximum sizes. Notably, only two individuals were larger than 7.2 cm, at 8.9 and 13.4 cm, and the other 25 individuals were smaller. The skewed size distribution could be indicative of segregation between size classes. This suggestion was originally posited in the description and further highlights that much work is needed on the life history of *Eurythenes*.

The integrative taxonomic results have provided compelling evidence to suggest that its range extends from the abyssal Weddell Sea to hadal depths of the South Sandwich Trench and across the Antarctic Polar Front and Mid-Atlantic Ridge to the Agulhas Fracture Zone (Figs. 3 and 4). While Agulhas Fracture Zone presence is well-supported, it remains unconfirmed whether *E. andhakarae* has a circumpolar distribution, with a morphology-only record at 1,990 m in the Ross Sea (NIWA, 2018) (Fig. 1). As *E. andhakarae* is morphologically similar to *E. gryllus s. s.* (d'Udekem d'Acoz and Havermans, 2015), confirming presence with DNA barcoding on the opposite side of Antarctica and at bathyal depths is warranted. Until then, *E. andhakarae*'s distribution can conservatively be described as 3069–7099 m from the Southern to the South Atlantic Ocean.

The presence of *E. andhakarae* north of the Antarctic Polar Front is a pertinent finding. Investigations of connectivity across the Antarctic Polar Front have revealed that contemporary movement in and out of the Southern Ocean is rare with studies detecting species-level breaks associated with this oceanographic barrier (ribbon worm: Thornhill et al., 2008; Gentoo penguins: Vianna et al., 2017; sea snails: González-Wevar et al., 2021; fish: Arkhipkin et al., 2022). This body of work, however, has largely been focused on epipelagic and sessile benthic taxa, leaving deep-ocean benthopelagic brooding taxa largely untested. Here, the *E. andhakarae* haplotype network revealed preliminary patterns that merit further investigation to test the role of the Antarctic Polar Front in shaping the populations of abyssal species. At these depths, this oceanic system could be less of a restrictive barrier, with oceanographic connectivity via the Weddell Sea Deep and Bottom Waters (de Carvalho Ferreira and Kerr, 2017), and the availability of habitats with similar <1 °C temperatures, substrate, and food scarcity (Jörger et al., 2014; Galaska et al., 2017). In particular, the shared dominant haplotype with individuals from the Weddell Sea indicates some historical connectivity (Fig. 4B), and curiously the deeper-dwelling South Sandwich Trench individuals had the most divergent haplotypes. One hypothesis is that the South Sandwich Trench and Aguhlus Fracture Zone are peripheral populations, located at the edge of *E. andhakarae*'s vertical and geographic range, and less connected abyssal populations south of the Antarctic Polar Front (Alleume-Benharira et al., 2006; Pironon et al., 2017). While this study is limited by the COI marker, the initial phylogeographic patterns provide valuable direction to future work to pair *E. andhakarae* with population genomic approach, such as RAD-seq, to finely investigate the degree of population connectivity in and across the Antarctic Polar Front for abyssal benthic taxa.

5. Conclusion

Overall, this work demonstrates that with an increase in sampling, the reassessment of historical data, and the integration of both molecular and morphological identification data, new species descriptions and species-level histories are beginning to be realised. By improving our sampling scope at an unsampled deep-ocean fracture zone, we were able to extend the known geographic range of these sympatric *Eurythenes* species and begin to generate informed phylogeographic hypotheses concerning historic demography and potential levels of populations connectivity. This work falls within a growing body of literature focusing on deep-ocean Amphipoda and highlights the differing evolutionary and demographic trajectories across features, oceans, genera, and species (e.g., Bribiesca-Contreras et al., 2021; Jażdżewska et al., 2021; Weston and Jamieson, 2022; Weston et al., 2022). Gaining insight into contemporary, as well as historic phylogeographic structures, across the deep ocean helps support future marine spatial planning or protection efforts. Together, this work should act as a framework to pair new expeditions with a re-inspection of rich historical collections to expand knowledge of distributions and biogeographic patterns.

CRediT (Contributor roles taxonomy) author statement

Johanna Weston: Conceptualization, Investigation, Data curation, Writing, Visualization; **Eva Stewart**: Investigation, Visualization, Writing; **Paige Maroni**: Investigation, Writing; **Heather Stewart**: Investigation, Data curation, Writing, Funding acquisition; **Alan Jamieson**: Conceptualization, Investigation, Writing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The genetic sequences generated by this study have been deposited on GenBank and the two supplemental tables specified the compiled datasets.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr.2023.104050.

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