Zoological Journal of the Linnean Society, 2024, **201**, zlae102
https://doi.org/10.1093/zoolinnean/zlae102
Advance access publication 19 August 2024
Original Article

OXFORD

Original Article

Biogeography and phylogeny of the scavenging amphipod genus *Valettietta* (Amphipoda: Alicelloidea), with descriptions of two new species from the abyssal Pacific Ocean

Eva C.D. Stewart[1,2,*], Guadalupe Bribiesca-Contreras[2], Johanna N.J. Weston[3], Adrian G. Glover[2], Tammy Horton[4]

[1]School of Ocean and Earth Sciences, University of Southampton, Southampton, SO14 3ZH, United Kingdom
[2]Life Sciences Department, Natural History Museum, London, Cromwell Road, South Kensington, SW7 5BD, United Kingdom
[3]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, United States
[4]National Oceanography Centre, Southampton, SO14 3ZH, United Kingdom

[*]Corresponding author. School of Ocean and Earth Sciences, University of Southampton, Southampton, SO14 3ZH, United Kingdom. E-mail: ecds1n21@soton.ac.uk

ABSTRACT

Valettietta Lincoln & Thurston, 1983 (Amphipoda: Alicelloidea) is an infrequently sampled genus of scavenging amphipod, with a known bathymetric range from 17–5467 m encompassing a variety of habitats from anchialine caves to abyssal plains. Molecular systematics studies have uncovered cryptic speciation in specimens collected from the abyssal Pacific, highlighting uncertainty in the description of *Valettietta anacantha* (Birstein & Vinogradov, 1963). Here, we apply an integrative taxonomic approach and describe two new species, *Valettietta trottarum* sp. nov. and *Valettietta synchlys* sp. nov., collected at abyssal depths in the Clarion-Clipperton Zone, Pacific Ocean. Both species can be distinguished by characters of the gnathopods, uropod 3, and the inner plate of the maxilliped. Further, molecular phylogenetic analyses of two mitochondrial (16S rDNA and *COI*) and two nuclear (Histone 3 and 28S rRNA) regions found both new species to form well-supported clades and allowed us to re-identify previously published records based on genetic species delimitation. The biogeography of *Valettietta* is discussed in light of these re-evaluated records, and a new taxonomic key to the genus is provided. These new taxa highlight the strength of applying an integrated taxonomic approach to uncover biodiversity, which is critical in regions being explored for potential industrial purposes.

Keywords: cryptic species; integrative taxonomy; DNA barcoding; Clarion-Clipperton Zone; deep-sea mining; biodiversity

INTRODUCTION

Traditionally the deep ocean (> 200 m depth) has been regarded as a largely homogenous environment with a lack of obvious isolating barriers to gene flow (Sanders 1968). This homogeneity was thought to result in cosmopolitan distributions of taxa (Madsen 1961), with patterns of diversity largely controlled by biological interactions such as competition and predation (McClain and Hardy 2010). This notion of a lack of barriers to gene flow has now been challenged, as increased deep-sea exploration has revealed complex topographical and hydrological features such as mid-ocean ridges, trenches, polymetallic nodule fields, and hydrothermal vents (Lonsdale 1977, Paull *et al.* 1984, Danovaro *et al.* 2014, Riehl *et al.* 2020), as well as high levels of biodiversity and endemism (Grassle and Maciolek 1992, Glover *et al.* 2002, Brandt *et al.* 2007, Stewart *et al.* 2023). Increased research effort has demonstrated that the deep sea, and particularly the abyssal plains (3000–6000 m), is a heterogenous environment, wherein biological communities are influenced by complex interactions between physical (e.g. hydrography), biological (e.g. competition), and historic factors (e.g. palaeoceanographic cooling) (McClain and Hardy 2010). The interplay between these factors can lead to species having complicated biogeographic ranges, with potential dispersal capability not necessarily reflecting actual geographic and bathymetric distributions (Lester *et al.* 2007).

Received 5 March 2024; revised 20 May 2024; accepted 10 July 2024
[Version of Record, first published online 19 August 2024, with fixed content and layout in compliance with Art. 8.1.3.2 ICZN. urn:lsid:zoobank.org:pub:E64C1409-DD34-4196-ACDB-B72DCE516D4A]

The use of molecular methods combined with higher resolution sampling efforts have allowed for some of these assumed cosmopolitan distributions to be untangled, revealing cases of endemism (Downey *et al.* 2018, Weston *et al.* 2021a), species with wide ranges but spatially fragmented populations (Brandt *et al.* 2007, Havermans 2016, Weston *et al.* 2022), and cases of bipolar distributions (Havermans *et al.* 2013, Georgieva *et al.* 2015). In addition, molecular analyses have led to a rapid increase in the identification of morphologically similar, yet genetically distinct, 'cryptic' species (Vrijenhoek 2009, Havermans *et al.* 2013, Brasier *et al.* 2016). The identification of cryptic species subsequently requires the reassessment of biogeographic patterns across the genera in which they are found (Havermans 2016).

There have been many examples of this work in studies of deep-sea scavenging amphipods (Crustacea: Peracarida), particularly within the genus *Eurythenes* S.I. Smith in Scudder, 1882. Combinations of morphological and molecular studies have expanded the genus from three, to now 10 formally described species within the past decade (d'Udekem d'Acoz and Havermans 2015, Narahara-Nakano *et al.* 2018, Weston *et al.* 2020a, 2021a). Each species has unique patterns of distribution, ranging from endemism (*Eurythenes atacamensis* Weston & Espinosa-Leal in Weston, Espinosa-Leal, Wainwright, Stewart, González, Linley, Reid, Hidalgo, Oliva, Ulloa, Wenzhöfer, Glud, Escribano, Jamieson, 2021; Weston *et al.* 2021a), bi-polar, cosmopolitanism [*Eurythenes gryllus s.s.* (Lichtenstein in Mandt, 1822); Havermans, 2016], presence across multiple oceans (*Eurythenes maldoror* d'Udekem d'Acoz & Havermans, 2015; Havermans, 2016), as well as documented instances of sympatry (Eustace *et al.* 2016, Horton *et al.* 2020, Bribiesca-Contreras *et al.* 2021, Weston *et al.* 2023). As the deep sea becomes increasingly explored by humans for potential resources (Hein *et al.* 2013), characterizing species ranges, population connectivity, and life history strategies is essential for understanding the resistance and resilience of both species and whole ecosystems to anthropogenic impact (Palumbi 2003, Stewart *et al.* 2023). However, the evaluation of cryptic speciation in other scavenging genera beyond *Eurythenes* and *Paralicella* Chevreux, 1908 is limited (Havermans 2016, Jażdżewska *et al.* 2021a). This bias is driven, in part, by a low number of records and material distributed over a wide geographic range.

Herein, we explore cryptic speciation within the infrequently recorded genus of *Valettietta* Lincoln & Thurston, 1983 (family Valettiopsidae Lowry & De Broyer, 2008). *Valettietta* was erected by Lincoln and Thurston (1983), as a sister taxon to *Valettiopsis* Holmes, 1908. While originally placed in the family Lysianassidae Dana, 1849, this placement was questioned by Thurston (1989) based on the presence of a toothed incisor process on the mandibles, eventually leading to the establishment of the family Valettiopsidae by Lowry and De Broyer (2008). *Valettietta* is currently represented by five species: *Valettietta lobata* Lincoln & Thurston, 1983 and *Valettietta gracilis* Lincoln & Thurston, 1983 both from the abyssal Atlantic Ocean, *Valettietta anacantha* (Birstein & Vinogradov, 1963) described from the abyssal Philippine Trench, *Valettietta punctata* Bellan-Santini, 1985 from bathyal depths in the Mediterranean Sea, and *Valettietta cavernicola* Stock & Iliffe, 1990 from a shallow anchialine cave in the Galapagos Islands (see Supporting information, Table S1 for all available records). Diagnostic characters of the genus include a large coxal plate 1, subequal to the coxa of gnathopod 2, and a urosome lacking a strong acute tooth (Lincoln and Thurston 1983, Lowry and De Broyer 2008). A new species of *Valettietta* was reported from submarine canyons of the Western Iberian Peninsula by Duffy *et al.* (2012). However, this has not since been described formally and has no available molecular data for comparison.

Increased sampling in the central Pacific has led to an uptick in recorded observations of the species *V. gracilis* and *V. anacantha* (Jamieson *et al.*, 2011; Ritchie *et al.*, 2015; Lacey *et al.*, 2016; Patel *et al.*, 2020; Bribiesca-Contreras *et al.*, 2021; Mohrbeck *et al.*, 2021; see Supporting information, Table S1 for full list of published records). This includes records of *V. gracilis* in the New Hebrides Trench (Pacific Ocean), approximately 16,500 km from its type locality (Ritchie *et al.* 2015). While it is not unknown for scavenging amphipod species to have such broad distributions (Havermans 2016), without comparative molecular data from the type locality these records cannot be confirmed. Further, *V. anacantha* has often been tentatively identified in published records due to uncertainties with the original description as a result of an incomplete set of illustrations. Recent molecular work on specimens identified as *V.* cf. *anacantha* has identified two distinct genetic lineages found in the Clarion-Clipperton Zone (CCZ), an expansive area of the Pacific abyssal seafloor under investigation for polymetallic nodule mining, highlighting potential cryptic diversity within the species and genus (Bribiesca-Contreras *et al.* 2021, Mohrbeck *et al.* 2021).

Considering the morphological uncertainty within *Valettietta*, we applied an integrative taxonomic approach to identify these two cryptic lineages within the CCZ, and formally describe them as: *Valettietta trottarum* sp. nov. and *Valettietta synchlys* sp. nov., also providing an updated key to the genus. We used mitochondrial (16S and *COI*) and nuclear (28S and *H3*) DNA sequences for these new species, and for *V. gracilis* from the type locality to investigate the phylogenetic placement of the genus *Valettietta*. Previous records of *Valettietta* are re-evaluated, and the biogeography of the genus discussed.

MATERIAL AND METHODS

Specimen collection

The material examined consisted of 42 individuals collected across four expeditions (see Table 1 for location data, and Supporting information, Table S2 for all examined material including accession numbers). Of these, 36 were sampled from the Clarion-Clipperton Zone (CCZ), central Pacific Ocean, five from the Porcupine Abyssal Plain (PAP), north Atlantic Ocean, and one from the Wallaby-Zenith Fracture Zone (WZFZ), east Indian Ocean (Fig. 1).

Specimens from the CCZ were collected from the UK-1 contract area in April–May 2015 on the MIDAS (Managing Impacts of Deep-seA reSource exploitation) JC120 expedition on board the RRS *James Cook* (detailed in Jones 2015), and from the NORI-D contract area in November–December 2022 on board the MV *Island Pride* on Campaign C7B.

Table 1. Collection information for *Valettietta* spp. from expeditions to the Porcupine Abyssal Plain and the Clarion-Clipperton Zone. For full specimen data see Supporting information, Table S2

Expedition	Station	Latitude (°)	Longitude (°)	Depth (m)	Date
JC120	039	17.321	-122.833	4230	29/04/2015
JC120	008	16.891	-123.004	4313	22/04/2015
C7B	AT-03	10.330	-117.17	4290	13/12/2022
DY077	083	49.007	16.419	4850	28/04/2017

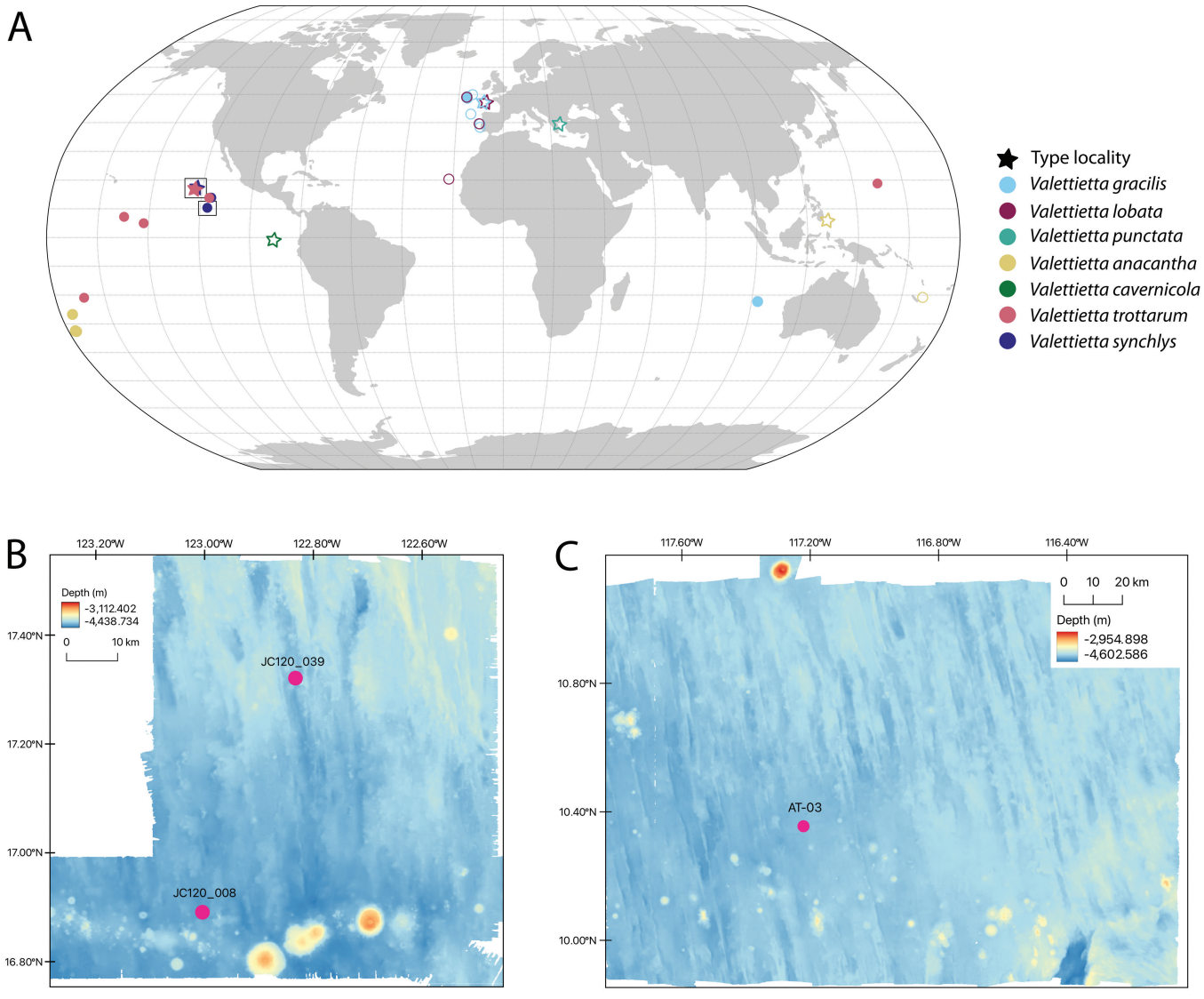

Figure 1. Distribution of *Valettietta* Lincoln & Thurston, 1983 species. A, all published records of *Valettietta* species. Stars represent type localities and circles all other records. Filled shapes indicate specimens identified by morphology and DNA, and empty shapes indicate specimens identified by morphology only. Individuals only tentatively identified by morphology (e.g. *V.* cf. *gracilis*), or only identified to genus level are not shown. Black boxes indicate location of map sections B and C. B, type locality for *V. trottarum* and *V. synchlys* within the Clarion-Clipperton Zone. C, paratype locality for *V. synchlys* within the Clarion-Clipperton Zone. See Supporting information, Table S2 for full specimen collection details.

Specimens from the PAP were collected on the RRS *Discovery* cruise DY077 in April–May 2017 (Lampitt 2017). Specimens were collected using DE-rated Mark And Recapture (DEMAR) amphipod traps (see Horton *et al.* 2020) baited with Scombridae fish. Collection information for the specimen from the WZFZ can be found in Weston *et al.* (2020b).

The holotypes were selected from among DNA barcoded specimens to minimize the potential for future taxonomic and nomenclatural issues. Type material and slides are deposited at the Natural History Museum, London (NHMUK). GenSeq nomenclature is applied to the type material following Chakrabarty *et al.* (2013).

Table 2. Primers and PCR programs used for DNA amplification

Gene	Primer		Sequence (5' – 3')	PCR program	Reference
COI	LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG	1 × (5 min at 95 °C), 35 × (30 s at 95 °C, 30 s at 49 °C, 1 min at 74 °C), 1 × (5 min at 74 °C)	Folmer *et al.* 1994
	HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA		Folmer *et al.* 1994
16S	16SFt_amp	Forward	GCRGTATIYTRACYGTGCTAAGG	1 × (2 min at 95 °C), 35 × (30 s at 95 °C, 30 s at 50 °C, 45 s at 72 °C), 1 × (5 min at 72 °C)	Lörz *et al.* 2018
	16SRt_amp	Reverse	CTGGCTTAAACCGRTYTGAACTC		Lörz *et al.* 2018
28S	28Sftw	Forward	AGAAACTAACMAGGATTCCYYTAGTA	1 × (2 min at 95 °C), 35 × (40 s at 94 °C, 40 s at 50 °C, 40 s at 72 °C), 1 × (10 min at 72 °C)	Corrigan *et al.* 2014
	28Srtw	Reverse	ACTTTCCCTCAYGGTACTTGT		Corrigan *et al.* 2014
H3	HisH3f	Forward	AAATAGCYCGTACYAAGCAGAC	1 × (2 min at 95 °C), 35 × (40 s at 94 °C, 40 s at 45 °C, 40 s at 72 °C), 1 × (10 min at 72 °C)	Corrigan *et al.* 2014
	HisH3r	Reverse	ATTGAATRTCYTTGGGCATGAT		Corrigan *et al.* 2014

Morphological assessment and illustration

Dissected parts were mounted in either polyvinyl-lactophenol stained with lignin pink or Aquatex. Illustrations were prepared using Wild M5, Leica™ MZ 7.5, and Leica™ DMR stereomicroscopes with an attached camera lucida. Illustrations were scanned and digitally inked using Adobe Illustrator (Coleman 2003).

A loan of the type material of *Valettietta anacantha,* which is held at the Zoological Museum of Moscow University, was not possible; however, photographs of the prepared slide (accession number: Mb-1109) were made available by the curators and compared with our specimens. Examination of the slide found the illustrations of Birstein & Vinogradov (1963) to be accurate to the material.

The following abbreviations have been used: A, antenna; E, epimeron; Ep, epistome; GN, gnathopod; LL, lower lip; Md, mandible; MX, maxilla; MXP, maxilliped; P, pereopod; T, telson; U, uropod; UL, upper lip; l, left; r, right. Setal and mouthpart classifications follow Lowry and Stoddart (1993, 1995). Specimens with no identifiable secondary sexual characteristics are noted as 'immature'.

DNA extraction, amplification, and sequencing

Two mitochondrial [16S rRNA (16S), and cytochrome oxidase subunit I (*COI*)] and two nuclear [28S rRNA (28S) and early stage histone 3 (*H3*)] genetic markers were used to assess the phylogenetic position of the genus *Valettietta*. Various combinations of these markers have proved useful for the reconstruction of phylogenetic relationships within the scavenging Amphipoda at ordinal or familial levels (Corrigan *et al.* 2014, Ritchie *et al.* 2015). To increase the robustness of phylogenetic analyses, additional amplifications were undertaken on specimens from the families Alicellidae Lowry & De Broyer, 2008, Cyclocaridae Lowry & Stoddart, 2011, Hirondelleidae Lowry & Stoddart, 2010, Scopelocheiridae Lowry & Stoddart, 1997, and Uristidae Hurley, 1963 from specimens originally published in Bribiesca-Contreras *et al.* (2021), as well as newly collected specimens of *Eurythenes maldoror* (see Supporting information, Table S3 for full detail of comparative material).

DNA was extracted from a pair of pleopods using QuickExtract™ DNA extraction solution (Lucigen), following manufacturer guidelines, and adapted for a digestion time of 45 min. Regions of 16S, *COI*, 28S, and *H3* were amplified with published primer sets (Folmer *et al.* 1994, Corrigan *et al.* 2014, Lörz *et al.* 2018). The PCR mix for each reaction contained 10.5 µl of Red Taq DNA Polymerase 1.1× MasterMix (VWR), 0.5 µl of each primer (10 µM), and 1 µl of DNA template. Primers and PCR conditions are detailed in Table 2. We also attempted to amplify a fragment of 18S rDNA using the following primer pairs: 18SA (AYCTGGTTGATCCTGCCAGT; Medlin *et al.* 1988) and 18SB (ACCTTGTTACGACTTTTACTTCCTC; Nygren and Sundberg 2003), and 18SA1F (CCTACTTCTGGTTGATTCCTTGCCAGT) and 1800R (TAATGATCCTTCCGCAGGTT; both Steiner and Dreyer 2003). However, the two primer sets failed to produce sufficient PCR products for sequencing.

PCR products were purified using a Millipore Multiscreen 96-well PCR Purification System and sequenced using the same primers as used for amplification, using an ABI 3730XL DNA Analyzer (Applied Biosystems) at the Natural History Museum Sequencing Facilities. Using Geneious 7.0.6 (Kearse *et al.* 2012), for each gene fragment contigs were assembled by aligning both forward and reverse sequences, chromatograms were visually inspected, and ambiguous base calls were corrected manually.

Phylogenetics

To construct phylogenetic trees, comparative species sequences were retrieved from GenBank for available species from the families Alicellidae, Valettiopsidae, Cyclocaridae, Uristidae, Scopelocheiridae, Eurytheneidae, and Hirondelleidae (Havermans *et al.* 2013, Corrigan *et al.* 2014, Ritchie *et al.* 2015, Jażdżewska and Mamos 2019, Weston *et al.* 2020b, 2021a, Bribiesca-Contreras *et al.* 2021, Jażdżewska *et al.* 2021a, Mohrbeck *et al.* 2021, Kniesz *et al.* 2022; Supporting information,

Tables S1, S3). The species *Epimeria* (*Pseudepimeria*) *grandirostris* (Chevreux, 1912) was used as an outgroup in all phylogenies.

Sequences for *COI* and *H3* were aligned using MUSCLE (Edgar 2004) in MEGA X (Kumar *et al.* 2018); with nucleotides translated into amino acids to identify pseudogenes based on the presence of stop codons. Sequences for 16S and 28S were aligned using MAFFT v.7 (Katoh and Standley 2013) with the iterative refinement method FFT-NS-i. Un-alignable regions were filtered using the Gblocks server (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks), allowing gap positions in final blocks and less strict flanking positions.

These alignments were used in four different datasets: (1) *COI* alignment of all publicly available *Valettietta COI* sequences, plus sequences from comparative amphipod families; (2) 16S alignment of all publicly available *Valettietta* 16S sequences, plus sequences from comparative amphipod families; (3) concatenated *COI* and 16S alignment from specimens with sequence data for both genes, excluding sequences with low percentage identity (< 70%) to other *Valettietta* sequences (mtDNA); and (4) concatenated *COI*, 16S, 28S, and *H3* alignment of a reduced dataset of only specimens with sequences for all four genes (mtDNA + nuclear). Two sequences from Ritchie *et al.* (2015) (accession numbers KP713950 and KP456094) were excluded from analyses as Jażdżewska *et al.* (2021b) determined them to be misidentifications. Single gene phylogenies (*COI* and 16S) were estimated to allow for molecular re-identification of previously published *Valettietta* sequences. Accession numbers for all comparative sequences used can be found in Supporting information, Tables S1 and S3. Individual gene alignments were concatenated in Geneious. The best substitution models for each partition (each marker, and each codon position for *COI* and *H3*) were determined using PartitionFinder 2 (Lanfear *et al.* 2017).

Phylogenetic trees were estimated using both Bayesian inference (BEAST v.2.4.7; Bouckaert *et al.* 2014) and a maximum-likelihood approach implemented with IQ-TREE v.2.2.6. (Minh *et al.* 2020). The best inferred substitution model for each partition was estimated using ModelFinder (Kalyaanamoorthy *et al.* 2017). Node support in IQ-TREE was estimated using 1000 ultrafast bootstraps (Hoang *et al.* 2018). BEAST analyses were performed with trees and clock models linked, a Yule tree model, and a relaxed clock log normal. Two independent runs of a maximum of 70 million steps were combined after discarding 10% as burn-in. Runs were checked for convergence (Effective Sample Size (ESS) > 200) and a median consensus tree was estimated from the combined post-burn-in samples.

Sequence divergences were compared within and between the *Valettietta* species using the Kimura two-parameter (K2P) distance model (Kimura 1980) on 53 *COI* sequences (14 of *V. trottarum*; six of *V. gracilis*; 33 of *V. synchlys*), undertaken with MEGA X. K2P was chosen as it is used as a standard model for *COI* barcoding studies and allows direct comparison with other studies (e.g. Havermans *et al.* 2013). Relationships among haplotypes were explored for the *COI*, 16S, *H3*, and 28S genetic markers using the software PopART v.1.7. (Leigh and Bryant 2015) and applying the minimum-spanning algorithm.

Species delimitation was done using two methods, one distance-based and one tree-based. The tree-based method was a Bayesian Poisson Tree Process (bPTP) model (Zhang *et al.* 2013) conducted on the web interface (https://species.h-its.org/ptp/). The bPTP model infers species boundaries using speciation or branching events based on substitution rates. This was conducted on all four phylogenetic trees. The distance-based method used was Assemble Species by Automatic Partitioning (ASAP; Puillandre *et al.* 2021) undertaken on the web interface (https://bioinfo.mnhn.fr/abi/public/asap/) using the Kimura (K80) substitution model with a gap-width of 1.5. ASAP uses pairwise genetic distances to define groups and a scoring system to estimate species partitions. The ASAP method was applied to the two concatenated alignments (mtDNA and mtDNA + nuclear).

RESULTS

Molecular phylogenetics and genetic divergence

Within *Valettietta*, 41 specimens were successfully sequenced for a total of 83 gene amplicons: 36 for *COI* (~650 bp), 33 for 16S (~400 bp), seven for *H3* (~300 bp), and seven for 28S (~315 bp). An additional 22 specimens from the families Alicellidae, Cyclocaridae, Hirondelleidae, Eurytheneidae, Scopelocheiridae, and Uristidae were successfully sequenced for a total of 56 gene amplicons: two for *COI* (~650 bp), 10 for 16S (~400 bp), 22 for *H3* (~300 bp), and 22 for 28S (~315 bp). GenBank accession numbers for all new sequences are provided in Supporting information, Tables S2 and S3. Subsequently, we present phylogenies for two single gene datasets (16S, Supporting information, Fig. S1; *COI*, Supporting information, Fig. S2), a wider concatenated dataset of 16S and *COI* (mtDNA; Fig. 2), and a smaller concatenated dataset of 16S, *COI*, *H3*, and 28S (mtDNA + nuclear; Fig. 3).

The placement of *V. trottarum* and *V. synchlys* within the *Valettietta* genus varied depending on the sequence type. In the *COI* phylogeny *V. gracilis* was sister to *V. synchlys*, forming a clade which was reciprocally monophyletic to *V. trottarum* (Supporting information, Fig. S2). In comparison, in the 16S phylogeny, *V. gracilis* was basal, and *V. synchlys* was a sister taxa to *V. trottarum* (Supporting information, Fig. S1). Based on these single gene phylogenies, a number of previously published sequences identified as species of *Valettietta* can be re-identified. A full summary of these changes in identifications can be found in Supporting information, Table S1. Four specimens identified as *V. anacantha* in Ritchie *et al.* (2015) and Ritchie *et al.* (2017) [16S accession numbers: KP456092, KP456093, KP456095, KX034322] have 16S sequences bearing 82–85% similarity to sequences of *V. gracilis*, *V. trottarum*, and *V. synchlys*. Without examining the morphology of the specimens it is not possible to absolutely ascertain their identity. However, we suggest the possibility that these sequences do represent the true *V. anacantha*. Within the 16S phylogeny, these *V. anacantha* sequences formed a clade that was basal to all other species of *Valettietta* and delimited as a separate species by bPTP analysis (Supporting information, Fig. S1).

Both the maximum-likelihood and Bayesian mtDNA phylogenies (Fig. 2) grouped the three *Valettietta* species together, to form a single monophyletic *Valettietta* clade with high posterior probability and bootstrap support. This clade was sister to *Valettiopsis*, which together formed a monophyletic Valettiopsidae clade. Within *Valettietta*, *V. gracilis* formed a clade which was sister to *V. synchlys*, and reciprocally monophyletic to

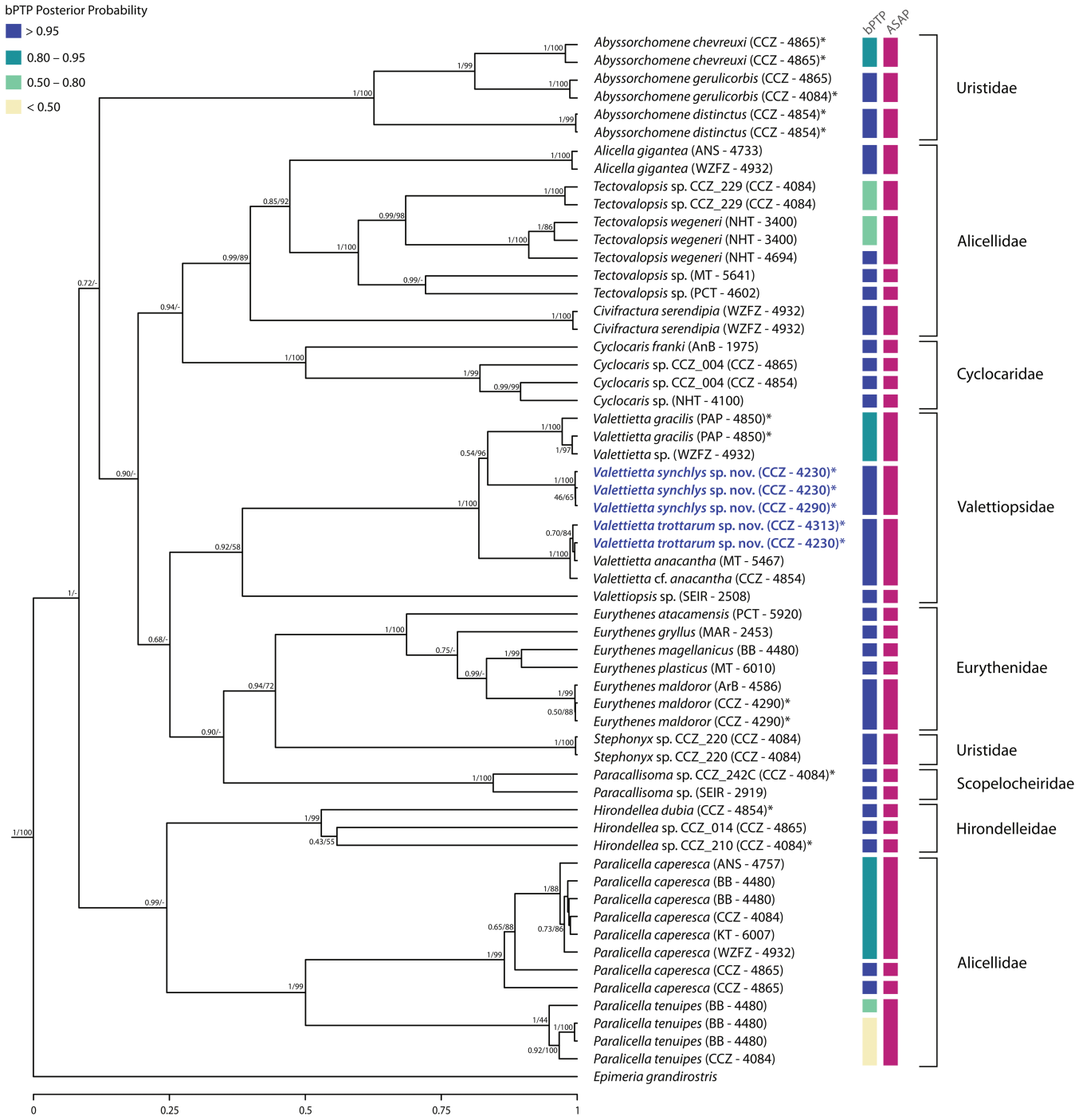

Figure 2. Bayesian tree showing the relationships between amphipod species based on a concatenated dataset of 16S and *COI* sequence data. Bayesian posterior probability values greater than 0.5 (before /) and maximum-likelihood bootstrap values (after /) are shown on branch nodes. Branches are labelled by species name, with collection location and depth (m) in brackets. Asterisks indicate sequences added by this study. Species delimitation results and family are denoted on the right. Scale indicates time relative to root age. Location abbreviations are as follows, CCZ—Clarion-Clipperton Zone; ANS—Afanasy Nikitin Seamount; WZFZ—Wallaby Zenith Fracture Zone; NHT—New Hebrides Trench; MT—Mariana Trench; PAP—Porcupine Abyssal Plain; PCT—Peru-Chile Trench; AnB—Angola Basin; ArB—Argentine Basin; BB—Brazil Basin; KT—Kermadec Trench; SEIR—South East Indian Ridge; MAR—Mid-Atlantic Ridge. For complete references and accession numbers for sequences see Supporting information, Tables S2, S3.

V. trottarum. However, node support for these clades was mixed (posterior probability = 0.54; bootstrap = 96) and so this relationship cannot be fully resolved. The specimen identified as *Valettietta* sp. from the WZFZ clustered closely with specimens of *V. gracilis* from the PAP.

This topology was mirrored within the maximum-likelihood and Bayesian mtDNA + nuclear phylogenies (Fig. 3), with *V. gracilis* forming a clade that was sister to *V. synchlys* and reciprocally monophyletic to *V. trottarum*. However, there was also low posterior probability support for the relationship between

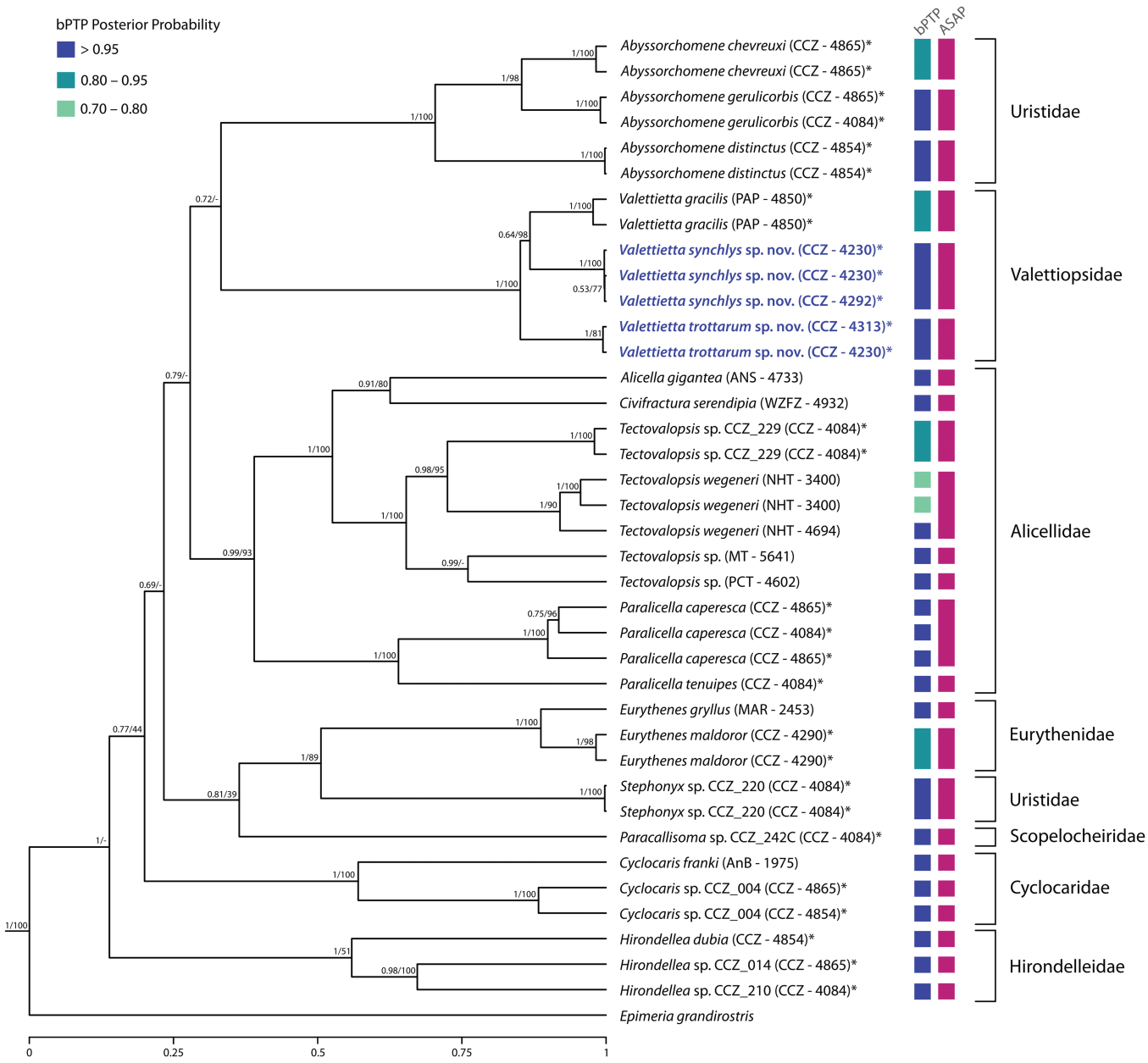

Figure 3. Bayesian tree showing the relationships between amphipod species based on a concatenated dataset of 16S, *COI*, *H3*, and 28S sequence data. Bayesian posterior probability values above 0.5 (before /) and maximum-likelihood bootstrap values (after /) are shown on branch nodes. Branches are labelled by species name, with collection location and depth (m) in brackets. Asterisks indicate sequences added by this study. Species delimitation results and family are denoted on the right. Scale indicates time relative to root age. Location abbreviations are as follows, CCZ—Clarion-Clipperton Zone; ANS—Afanasy Nikitin Seamount; WZFZ—Wallaby Zenith Fracture Zone; NHT—New Hebrides Trench; MT—Mariana Trench; PAP—Porcupine Abyssal Plain; PCT—Peru-Chile Trench; AnB—Angola Basin; MAR—Mid-Atlantic Ridge. For complete references and accession numbers for sequences see Supporting information, Tables S2, S3.

V. gracilis and *V. synchlys*. Hence, the relationships between these clades cannot be fully resolved. While in the mtDNA phylogeny the Valettiopsidae formed a sister clade to a nested clade of Eurytheneidae, Uristidae (*Stephonyx* sp.), and Scopelocheiridae, in the Bayesian mtDNA + nuclear phylogeny, *Valettietta* formed a reciprocally monophyletic clade with *Abyssorchomene* (Uristidae). This pattern was not found in the maximum-likelihood mtDNA + nuclear phylogeny, instead Valettiopsidae was reciprocally monophyletic with Alicellidae. Neither the Bayesian nor maximum-likelihood methods on either of the concatenated datasets were able to confidently resolve the relationships between families at higher levels.

Valettietta gracilis, *V. trottarum*, and *V. synchlys* were delimited as well supported clades by bPTP analysis across all phylogenies, except for the 16S phylogeny for which support values for all delimited species were low (Figs 2–3; Supporting information, Figs S1S2). An additional species delimitation method (ASAP) performed on the concatenated mtDNA and mtDNA + nuclear alignments also produced the same pattern of delimitation, consistent with our morphological identifications. The

level of *COI* sequence divergence between the three barcoded species of *Valettietta* was high, ranging from 8–10% (Table 3). Furthermore, a clear barcoding gap between the highest intraspecific (0.6%) and lowest interspecific (8%) divergences could be observed. The specimen from the WZFZ identified as *Valettietta* sp. [GenBank accession number: MN262182] had between 0–0.0068% genetic divergence with specimens of *V. gracilis*. Minimum-spanning haplotype networks of the 16S and *COI* genes found distinct clusters corresponding to each sequenced *Valettietta* species (Supporting information, Fig. S3). In comparison, all species were found to share the same 28S haplotype, while the *H3* haplotypes differed by only one mutational step.

Systematics

Order Amphipoda Latreille, 1816

Superfamily Alicelloidea Lowry & De Broyer, 2008

Family Valettiopsidae Lowry & De Broyer, 2008

Genus *Valettietta* Lincoln & Thurston, 1983

Type species: *Valettietta lobata* Lincoln & Thurston, 1983, original designation.

Diagnosis (after Lincoln and Thurston 1983): Body robust, compressed; pleosome well developed; urosome segment 1 with weak dorsal process. Segment 3 broad and flattened dorsally with lateral margins raised. Antenna 1 and 2 elongate, slender, about equal length; peduncle articles 2–3 of antenna 1 compressed, flagellum article 1 conjoint, accessory flagellum well developed, multi-articulate. Upper lip weakly notched. Lower lip without inner lobes, mandibular lobes prominent. Mandible with robustly dentate incisor, spine row strong, interspersed with plumose setae, molar large and triturative, palp attached level with molar, article 2 elongate with proximal and distal margin setose. Maxilla 1 inner plate densely setose along entire inner margin, palp robust, 2-articulate. Maxilla 2 inner and outer plates subequal length, inner plate with dense mediodistal and facial setae. Maxilliped basic; outer plate with short stout inner marginal spines grading distally to robust elongate spines. Coxal plates 1–4 forming continuous series; plate 4 with deep posterior emargination. Coxal plate 5 anterior lobe not deeper than posterior lobe. Gnathopod 1 subchelate; palm oblique; gnathopod 2 subchelate or simple. Pereopods 5–7 basis expanded with prolonged rounded posterodistal lobe. Uropods biramous, lanceolate, spinose. Telson triangular, deeply cleft. Branchial lobes with small accessory lobe close to base.

Included species: *Valettietta lobata* Lincoln & Thurston, 1983; *Valettietta gracilis* Lincoln & Thurston, 1983; *Valettietta punctata* Bellan-Santini, 1985; *Valettietta anacantha* (Birstein & Vinogradov, 1963); *Valettietta cavernicola* Stock & Iliffe, 1990; *Valettietta synchlys*; *Valettietta trottarum*.

***Valettietta synchlys* sp. nov.**

Figs 4–7

Valettietta cf. *anacantha* CCZ_056C—Bribiesca-Contreras *et al.* 2021: 1–15, figs 1, 4–6, table 1.

Valettietta cf. *anacantha*—Mohrbeck *et al.*, 2021: 1–12, figs 3, 5, 7, tables 2, 3.

ZooBank registration: http://zoobank.org/F787152D-237B-4C45-A6DA-C7A366D9D62A

Holotype: Immature, 16.1 mm, carcass and 22 slides, ECDS_AMP33, NHMUK 2024.63, Clarion-Clipperton Zone, Pacific Ocean (17.321 N, 122.833 W), expedition JC120, Station 039, depth 4230 m, genseq-1 *COI* (PP841420), genseq-1 16S (PP849019), genseq-1 *H3* (PP855322), genseq-1 28S (PP848491).

Paratypes: Immature, 9.23 mm, carcass, NHM_10315, NHMUK 2024.75, Clarion-Clipperton Zone, Pacific Ocean (10.33 N, 117.17 W), expedition C7B, Station AT-03, depth 4292 m, genseq-2 *COI* (PP841434), genseq-2 16S (PP849032), genseq-2 *H3* (PP855323), genseq-2 28S (PP848494). Mature male, 15.44 mm, carcass and 13 slides, ECDS-AMP32, NHMUK 2024.62, Clarion-Clipperton Zone, Pacific Ocean (17.321 N, 122.833 W), expedition JC120, Station 039, depth 4230 m, genseq-2 *COI* (PP841419), genseq-2 16S (PP849018), genseq-2 *H3* (PP855321), genseq-2 28S (PP848490). Immature, 11.33 mm, carcass, ECDS-AMP35, NHMUK 2024.65, Clarion-Clipperton Zone, Pacific Ocean (17.321 N, 122.833 W), expedition JC120, Station 039, depth 4230 m, genseq-2 *COI* (PP841422), genseq-2 16S (PP849021). Mature male, 14.35 mm, carcass, ECDS-AMP38, NHMUK 2024.68, Clarion-Clipperton Zone, Pacific Ocean (17.321 N, 122.833 W), expedition JC120, Station 039, depth 4230 m, genseq-2 *COI* (PP841424), genseq-2 16S (PP849024). Immature, 10.96 mm, carcass, ECDS-AMP16, NHMUK 2024.38, Clarion-Clipperton Zone, Pacific Ocean (16.891 N, 123.004 W), expedition JC120, Station 008, depth 4313 m, genseq-2 *COI* (PP841404), genseq-2 16S (PP849005).

Type locality: The Clarion-Clipperton Zone, central Pacific Ocean (17.321 N, 122.833 W), expedition JC120, Station 039, depth 4230 m.

Table 3. Estimates of K2P divergence values within and between species of *Valettietta*, estimated from *COI* sequence data. Sequence data from this study, Bribiesca-Contreras *et al.* (2021), Weston *et al.* (2021b), and Mohrbeck *et al.* (2021)

	Intraspecific			Interspecific		
Species	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Valettietta gracilis	0.0000	0.0068	0.0045	0.0804	0.1048	0.0914
Valettietta synchlys	0.0000	0.0049	0.0016	0.0804	0.1048	0.0949
Valettietta trottarum	0.0000	0.0018	0.0001	0.0901	0.1002	0.0952

Figure 4. Photographs of *Valettietta synchlys*: top, holotype, immature, NHMUK 2024.63, preserved specimen; bottom, paratype, immature, NHMUK 2024.75, fresh specimen showing ocular patch. Scale bar = 2mm.

Material examined: See Supporting information, Table S2.

Etymology: Synchlys, Greek, meaning mixed or 'washed together by the waves', alluding to the morphological characters of this species resembling a mixture of both *Valettietta anacantha* and *Valettietta gracilis*. Used as a noun in apposition.

Diagnosis: Coxa 1 not shorter or slightly shorter than coxa 2 and not strongly tapered distally. Gnathopods 1 and 2 weakly subchelate or simple, tapering distally, palms weakly defined or lacking. Gnathopod 1 propodus slender, tapering distally, carpus and propodus subequal. Gnathopod 2 propodus slender, length less than 5 × width. Basis of pereopod 7 with distinct posterodistal lobe. Maxilliped inner plate reaching halfway along palp article 1, with two short nodular robust setae on sub-apical medial margin. Inner ramus of uropod 1, 2, and 3 shorter than outer. Telson with four lateral spine groups, apices with a single subapical large robust seta and one small setae towards lateral margins.

Description (Figs 4–7) Based on holotype, immature, 16.1 mm length, NHMUK 2024.63

Head: Head large, rostrum absent. Pale yellow eyes present upon collection but faded in ethanol (Fig. 4). Lateral lobe triangular, apically rounded. *Antennae 1* (Fig. 5) elongate, 0.37 × as long as body length, flagellum 33-articulate, sparsely setose; accessory flagellum 6-articulate, reaching beyond end of basal conjoint article of flagellum; conjoint article subequal to length of peduncle article 1. *Antennae 2* (Fig. 5) 0.78 × the length of Antennae 1, 0.28 × as long as body, flagellum 31-articulate, proximal flagellar articles with slender setae.

Mouthparts: Upper lip asymmetrically rounded with small apical notch, distal surface minutely setose (not illustrated). *Mandible* (Fig. 6) left incisor 8-dentate and closely applied to 8-dentate lacinia mobilis, spine row with 12 large spines interspersed with long plumose setae, left molar damaged (not illustrated), palp robust, article 1 small, article 2 elongate, broadened medially/centrally, 14 A2 setae present, D2 setae present along distal half, B2 setae present proximally on medial margin. Article 3 oval, tapering distally; three A3 setae, two B3 setae, and five plumose E3 setae present, 19 plumose D3 setae on the posterodistal two-thirds of the margin. Right incisor 9-dentate, closely applied to 5-dentate lacinia, spine row with seven large spines interspersed with long plumose setae, molar strongly triturative; palp robust, article 1 small, article 2 elongate, broadened laterally/centrally, 21 A2 setae present, D2 setae present along distal half, B2 setae present proximally on lateral margin. Article 3 oval, tapering distally; two A3 setae, one central and two lateral B3 setae, and six plumose E3 setae present, 18 plumose D3 setae on the posterodistal two-thirds of the margin. *Maxilla 1* (Fig. 6) inner plate with 20 marginal plumose setae, outer plate with 6/5 setal-tooth crown arrangement; palp large, article 2 lateral margin with two long slender setae, distal margin with 12 robust setae and row of 11 long submarginal setae. *Maxilla 2* (Fig. 6) inner and outer plates subequal, distally setose, inner plate also with oblique row of 17 plumose facial setae. *Maxilliped* (Fig. 6) inner plate reaching halfway along palp article 1, with two short nodular robust setae at sub-apical medial margin; outer plate

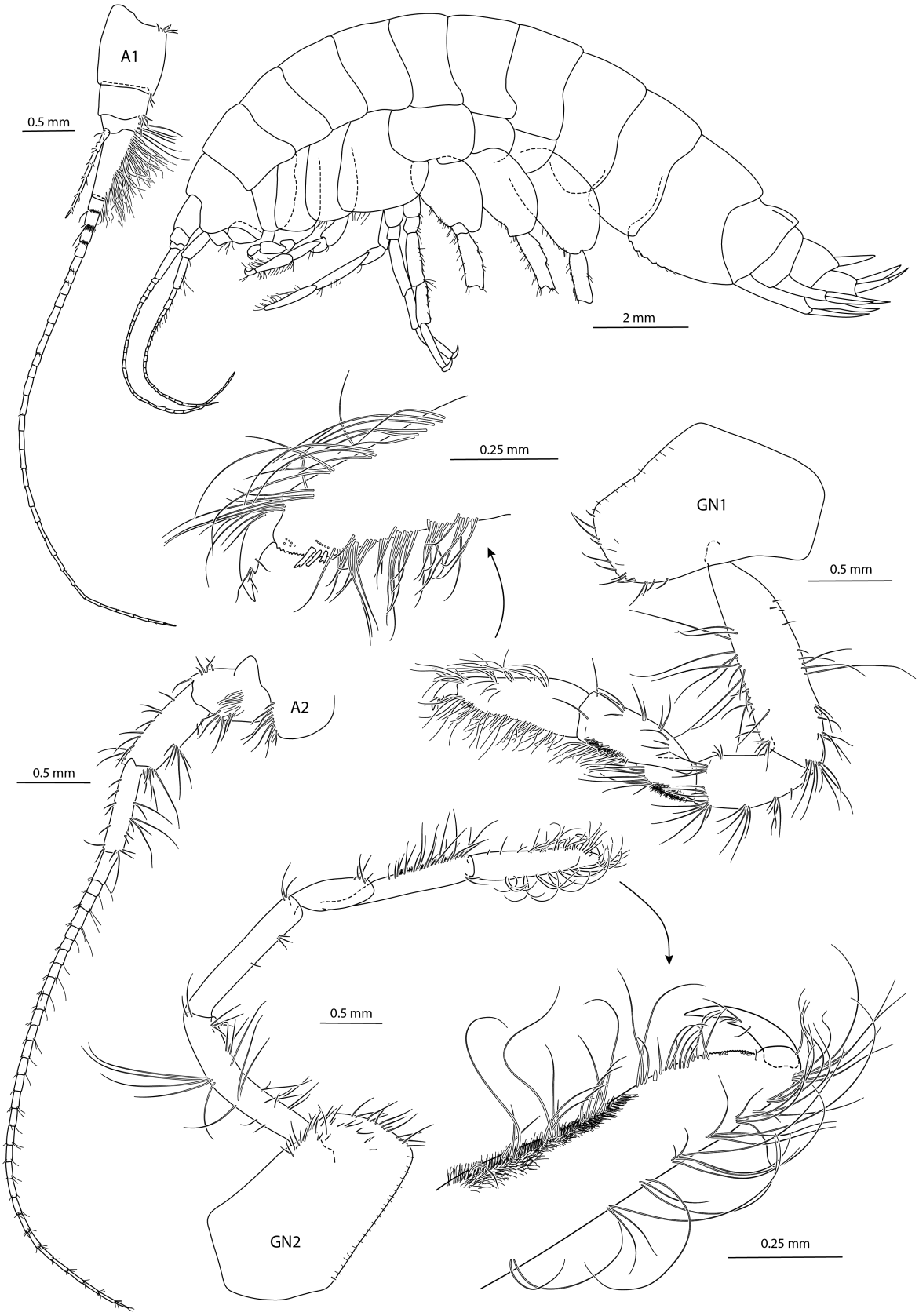

Figure 5. *Valettietta synchlys* holotype, immature, NHMUK 2024.63, 16.1 mm, Clarion-Clipperton Zone, 4230 m. A = Antenna; GN = Gnathopod. Unfilled circles indicate setal bases.

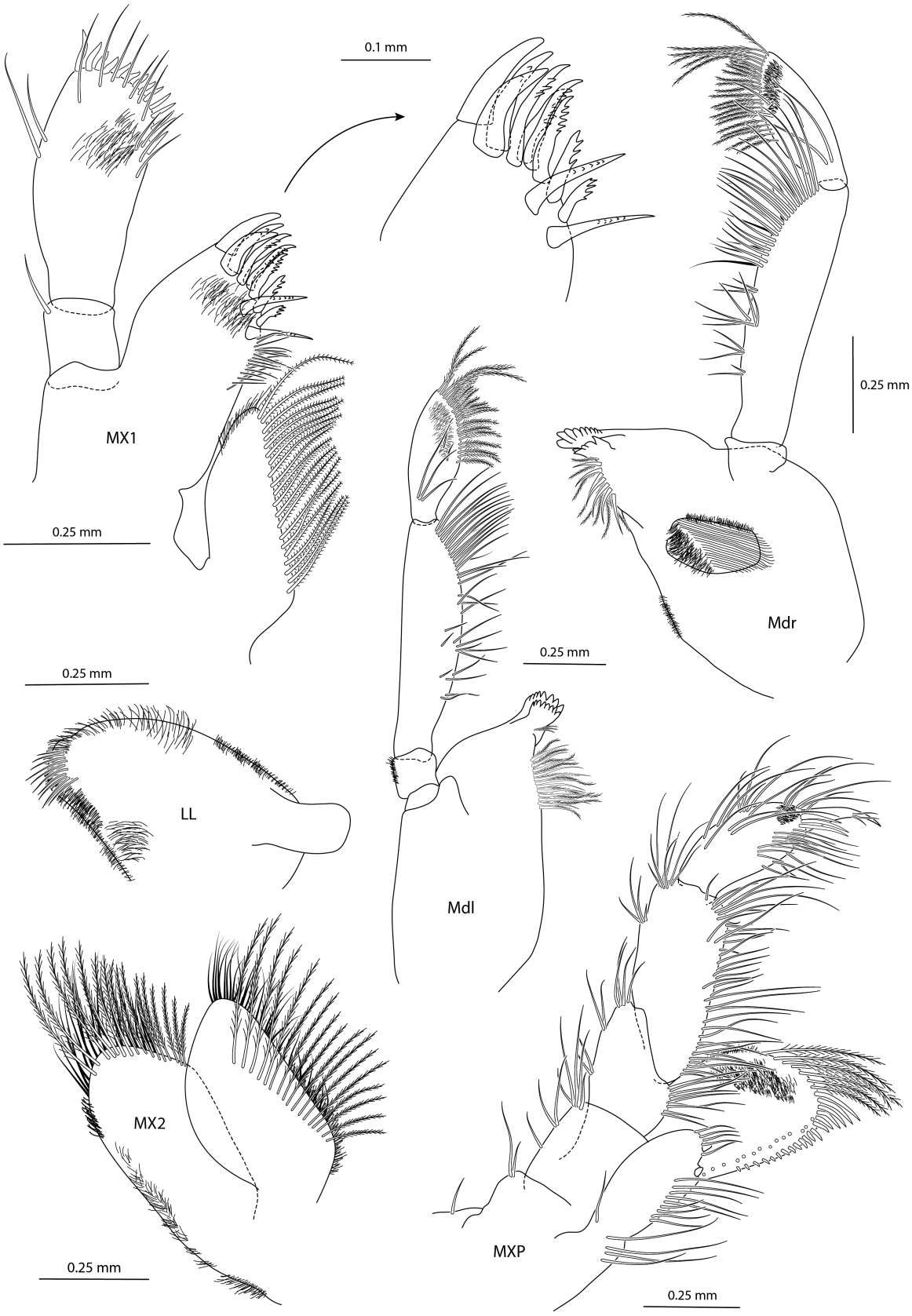

Figure 6. *Valettietta synchlys* holotype, immature, NHMUK 2024.63, 16.1 mm, Clarion-Clipperton Zone, 4230 m. MX = Maxilla; Md = Mandible; MXP = Maxilliped; LL = Lower lip; l = left; r = right. Unfilled circles indicate setal bases.

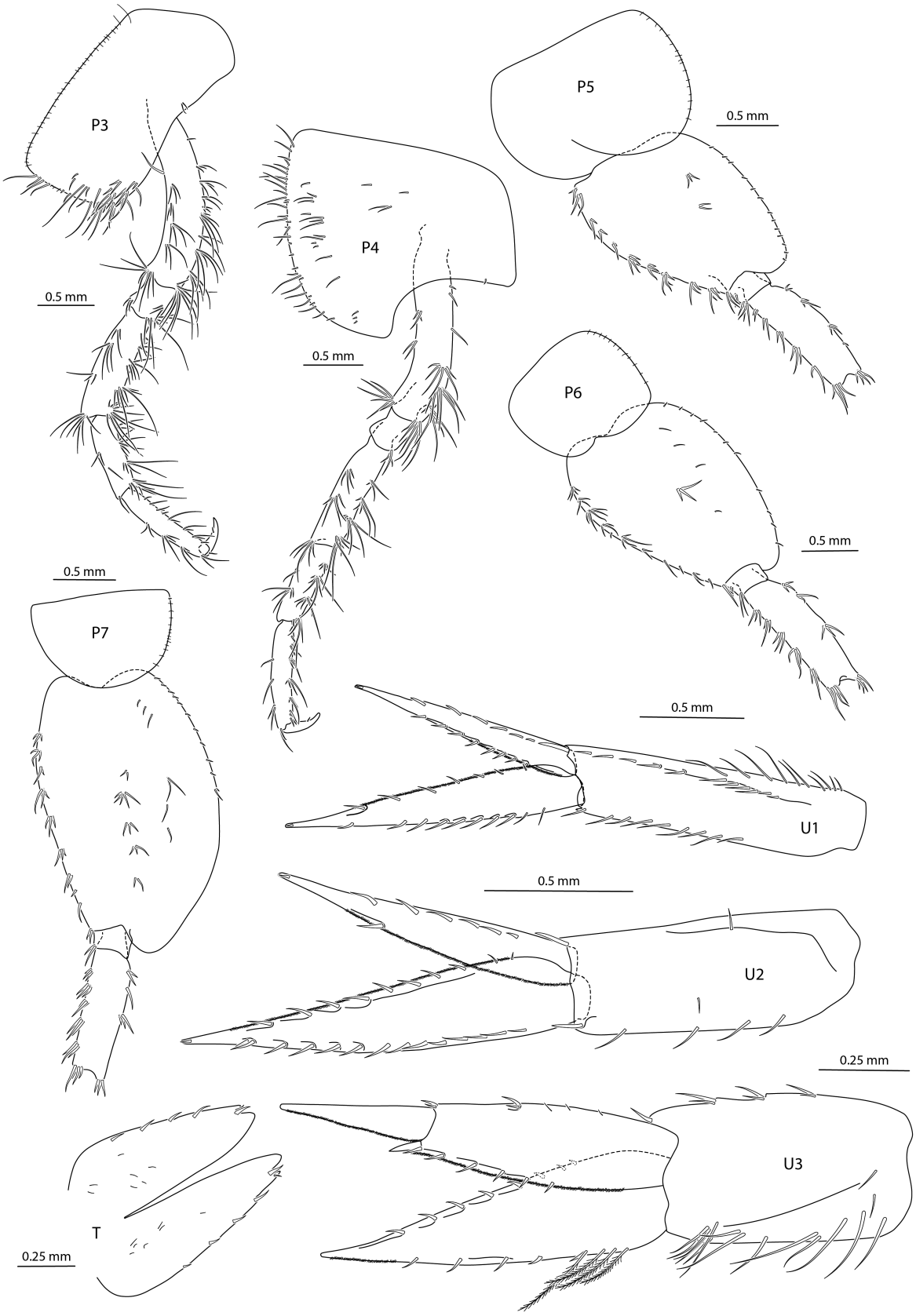

Figure 7. *Valettietta synchlys* holotype, immature, NHMUK 2024.63, 16.1 mm, Clarion-Clipperton Zone, 4230 m. P = Pleopod; U = Uropod; T = Telson.

reaching halfway along palp article 2, inner margin with row of 19 short nodular robust setae grading to seven elongate and plumose setae distally; palp 4-articulate, article 4 with three robust setae distally, dactylus well-developed.

Pereon: Gnathopod 1 (Fig. 5) subchelate; coxa 1 shorter than coxa 2, slightly tapering distally, proximal and distal margins sub-rectangular, setose; carpus and propodus subequal, propodus not strongly elongate or tapering distally, palm acute, palmar margin straight and dentate, delimited by three bifid robust setae, dactylus overlapping palm, inner margin toothed with a few fine setules. *Gnathopod 2* (Fig. 5) subchelate; anterodistal corner of coxa sub-rectangular, distal margin setose, basis curved, ischium extremely elongate (sub-equal to basis); carpus slightly shorter than propodus (1:0.85), both slender, propodus length 5 × width; palm acute, palmar margin straight and dentate, dactylus shorter than palm, not reaching single palmar defining robust seta, inner margin toothed with a few fine setules. *Pereopod 3* (Fig. 7) coxa rectangular, distal margin setose; basis curved; merus and propodus longer than carpus; all articles with long slender marginal setae. *Pereopod 4* (Fig. 7) coxa very broad with large posteroventral lobe, posterodistal margin broadly rounded and setose; merus and propodus subequal in length and longer than carpus. *Pereopods 5–7* (Fig. 7) coxa 5 broadly expanded and bilobate with posterodistal lobe largest, coxa 6 smaller than coxa 5 with posterior margin weakly lobate; coxa 7 smaller than coxa 6, not bilobate; basis broadly expanded distally, increasing from P5 to P7.

Pleon and urosome: Epimeron (Fig. 5) 1 broadly rounded, 2 subquadrate, 3 produced to a subacute tooth. *Urosomite 1* with low rounded boss on dorsal margin. *Uropods* (Fig. 7) biramous, lanceolate. *Uropod 1* inner ramus shorter than outer (1:0.8), with both margins lined by short robust setae, adjacent margins minutely serrate, apex of both rami with inset small setule; peduncle with one apicolateral robust seta, 11 dorsolateral setae, nine dorsomedial setae, and 10 longer ventrolateral setae. *Uropod 2* inner ramus shorter than outer (1:0.75), both margins of outer ramus lined by short robust setae, inner margin of inner ramus lined by short robust setae, with one seta present on outer margin, adjacent margins minutely serrate, apex of both rami with inset small setule; peduncle with fewer setae than peduncle of uropod 1, one large robust seta present on peduncle at base of inner and outer ramus. *Uropod 3* inner ramus shorter than outer (1:0.9), distal article of outer ramus approximately two-thirds length of proximal article, apex of proximal article with robust setae, inner margin minutely serrate; proximal inner margin of inner rami with four plumose setae, distal inner margin of inner rami minutely serrate; peduncle with long fine setae on inner margin, and three groups of two short setae on outer margin. *Telson* (Fig. 7) triangular, 75% cleft, apices with a single subapical large robust seta and one small setae towards lateral margins; lobes with four lateral robust setae.

Habitat and ecology: Valettietta synchlys is a benthopelagic scavenger species, currently known only from the central Pacific Ocean in the Clarion-Clipperton Zone, at abyssal depths of 4230–4313 m.

Remarks: Valettietta synchlys most closely resembles *V. anacantha* in the shape of coxa 1, gnathopods 1 and 2, and uropod 3, and has been recorded as *V.* cf. *anacantha* in recent literature (Bribiesca-Contreras *et al.* 2021, Mohrbeck *et al.* 2021). *Valettietta synchlys* can be distinguished by characters of gnathopod 1, which more closely resembles that of *V. gracilis*, with propodus longer and more strongly tapering distally (shorter and broader, weakly tapering distally in *V. anacantha*), and a longer carpus (shorter in *V. anacantha*). It can be further distinguished by the angle of the anterodistal corner of coxa 2 which is sub-rectangular in *V. synchlys* (rounded in *V. anacantha*). The basis of pereopod 7 is narrower with a distinct posterodistal lobe (in *V. anacantha* this is significantly more broadly rounded with a shallow, rounded posterodistal lobe). Body size ranged from 9.23–16.10 mm between examined specimens.

***Valettietta trottarum* sp. nov.**

Figs 8–11

Valettietta gracilis—Ritchie *et al.*, 2015: 122–128, fig 2, table 1, 3.

Valettietta anacantha—Ritchie *et al.*, 2015: 122–128, fig 2.

Valettietta cf. *anacantha* Va1—Bribiesca-Contreras *et al.*, 2021: 1–15, figs 1, 4–6, table 1.

Valettietta cf. *anacantha*—Mohrbeck *et al.*, 2021: 1–12, figs 3, 5, 7, tables 2, 3.

ZooBank registration: http://zoobank.org/901CA16B-ECE3-4886-A72D-74960AA34027

Holotype: Immature, 10.5 mm, carcass and 22 slides, ECDS-AMP10, NHMUK 2024.37, Clarion-Clipperton Zone, Pacific Ocean (16.891° N, 123.004° W), expedition JC120, Station 008, depth 4313 m, genseq-1 *COI* (PP841401), genseq-1 *H3* (PP855319), genseq-1 28S (PP848488).

Paratypes: Immature, 8.79 mm, carcass and two slides, ECDS-AMP13, NHMUK 2024.53, Clarion-Clipperton Zone, Pacific Ocean (17.321° N, 122.833° W), expedition JC120, Station 039, depth 4230 m, genseq-2 *COI* (PP841403), genseq-2 16S (PP849004), genseq-2 *H3* (PP855320), genseq-2 28S (PP848489). Immature, 8.65 mm, carcass and 1 slide, ECDS-AMP17, NHMUK 2024.39, Clarion-Clipperton Zone, Pacific Ocean (16.891° N, 123.004° W), expedition JC120, Station 008, depth 4313 m, genseq-2 *COI* (PP841405). Immature, 9.09 mm, carcass, ECDS-AMP18, NHMUK 2024.40, Clarion-Clipperton Zone, Pacific Ocean (16.891° N, 123.004° W), expedition JC120, Station 008, depth 4313 m, genseq-2 *COI* (PP841406), genseq-2 16S (PP849006). Immature, 7.9 mm, carcass, ECDS-AMP21, NHMUK 2024.43, Clarion-Clipperton Zone, Pacific Ocean (16.891° N, 123.004° W), expedition JC120, Station 008, depth 4313 m, genseq-2 *COI* (PP841409), genseq-2 16S (PP849009). Immature, 7.92 mm, carcass and three slides, ECDS-AMP22, NHMUK 2024.44, Clarion-Clipperton Zone, Pacific Ocean (16.891° N, 123.004° W), expedition JC120, Station 008, depth 4313 m, genseq-2 *COI* (PP841410), genseq-2 16S (PP849010). Immature, 7.81 mm, carcass, ECDS-AMP44, NHMUK 2024.45, Clarion-Clipperton Zone, Pacific Ocean (16.891° N, 123.004° W), expedition JC120, Station 008, depth 4313 m, genseq-2 *COI* (PP841430).

Type locality: The Clarion-Clipperton Zone, central Pacific Ocean (16.891° N, 123.004° W), expedition JC120, Station 008, depth 4313 m.

Etymology: This species is named for the Trott family of Deal, Kent (UK). In their service as sailors of luggers, they saved many lives before the introduction of formal lifeboats (*c.*1750—*c.*1856). It is particularly named for Robert and Suzanne Trott, who spent their childhood in the city of Valetta, Malta. Used as a noun in apposition, gender feminine.

Diagnosis: Coxa 1 not shorter or slightly shorter than coxa 2 and not strongly tapered distally. Gnathopods 1 and 2 weakly subchelate or simple, tapering distally, palms weakly defined or lacking. Gnathopod 2 propodus extremely slender and elongate, length more than 6 × width, propodus with weak convex dentate palm. Uropod 3 outer ramus article 2 elongate, subequal to article 1. Maxilliped inner plate reaching two-thirds along palp article 1, with two short nodular robust setae, and three smaller nodular setae at sub-apical medial margin. Telson with three robust setae in large sub-apical notch.

Description (Figs 8–11) Based on holotype, immature female, 10.5 mm length, NHMUK 2024.37

Head: Head large, rostrum absent. Eyes not documented at collection and not apparent in preserved specimen (Fig. 8). Lateral lobe sub-rectangular. *Antennae 1* (Fig. 9) elongate, 0.39 × as long as body length, flagellum 22-articulate, sparsely setose; accessory flagellum 4-articulate, reaching to end of basal conjoint article of flagellum; conjoint article subequal to length of peduncle article 1. *Antennae 2* (Fig. 9) 0.84 × the length of Antennae 1, 0.33 × as long as body, flagellum 22-articulate, proximal flagellar articles with slender setae.

Mouthparts: Upper lip (Fig. 10) asymmetrically rounded with small apical notch, distal surface minutely setose. *Lower lip* (Fig. 10) as for genus. *Mandibles* (Fig. 10) asymmetric. Left incisor 7-dentate and closely applied to 7-dentate lacinia mobilis, spine row with 4 large spines interspersed with long plumose setae, molar strongly triturative; palp robust, article 1 small, article 2 elongate, broadened medially/distally, 11 A2 setae present, D2 setae present along distal three-quarters of medial margin. Article 3 oval, tapering distally; two A3 setae and four plumose E3 setae present, 15 plumose D3 setae on the posterodistal half of the margin. Right incisor 8-dentate, closely applied to 5-dentate lacinia mobilis, spine row with nine large spines interspersed with long plumose setae, molar strongly triturative; palp robust, article 1 small, article 2 elongate broadened medially/distally, 14 A2 setae present, D2 setae present along distal three-quarters of medial margin. Article 3 oval, tapering distally; three A3 setae and five plumose E3 setae present, 13 plumose D3 setae on the posterodistal half of the margin. *Maxilla 1* (Fig. 10) inner plate with 22 marginal plumose setae, outer plate with 6/5 crown arrangement; palp large, article 2 lateral margin with two long slender setae, distal margin with 13 robust setae and row of seven long submarginal setae. *Maxilla 2* (Fig. 10) inner and outer plates subequal, distally setose, inner plate also with oblique row of 22 plumose facial setae. *Maxilliped* (Fig. 10) inner plate reaching two-thirds along palp article 1, with two short nodular robust setae, and three smaller nodular setae at sub-apical medial margin; outer plate reaching halfway along palp article 2, inner margin with row of 15 short nodular robust setae grading to four elongate and plumose setae distally; palp 4-articulate, dactylus well developed.

Pereon: Gnathopod 1 (Fig. 9) subchelate; coxa 1 shorter than coxa 2, slightly tapering distally, proximal margin rounded, distal margin sub-rectangular and setose; carpus shorter than propodus, propodus not strongly elongate, tapers slightly distally; palm acute, palmar margin slightly rounded and dentate, delimited by three bifid robust setae, dactylus overlapping palm, inner margin smooth, outer margin with a few fine setules. *Gnathopod 2* (Fig. 9) subchelate; coxa rectangular, proximal margin rounded, distal margin setose, basis curved, ischium extremely elongate (equal to basis); carpus slightly shorter than propodus (1:0.81), both slender, propodus length seven times width; palm acute, palmar margin convex and minutely dentate, dactylus overlapping palm, inner margin toothed with a few fine setules, one robust palmar defining seta. *Pereopod 3* (Fig. 11)

Figure 8. *Valettietta trottarum* holotype, immature, NHMUK 2024.37, preserved specimen. Scale bar = 1mm.

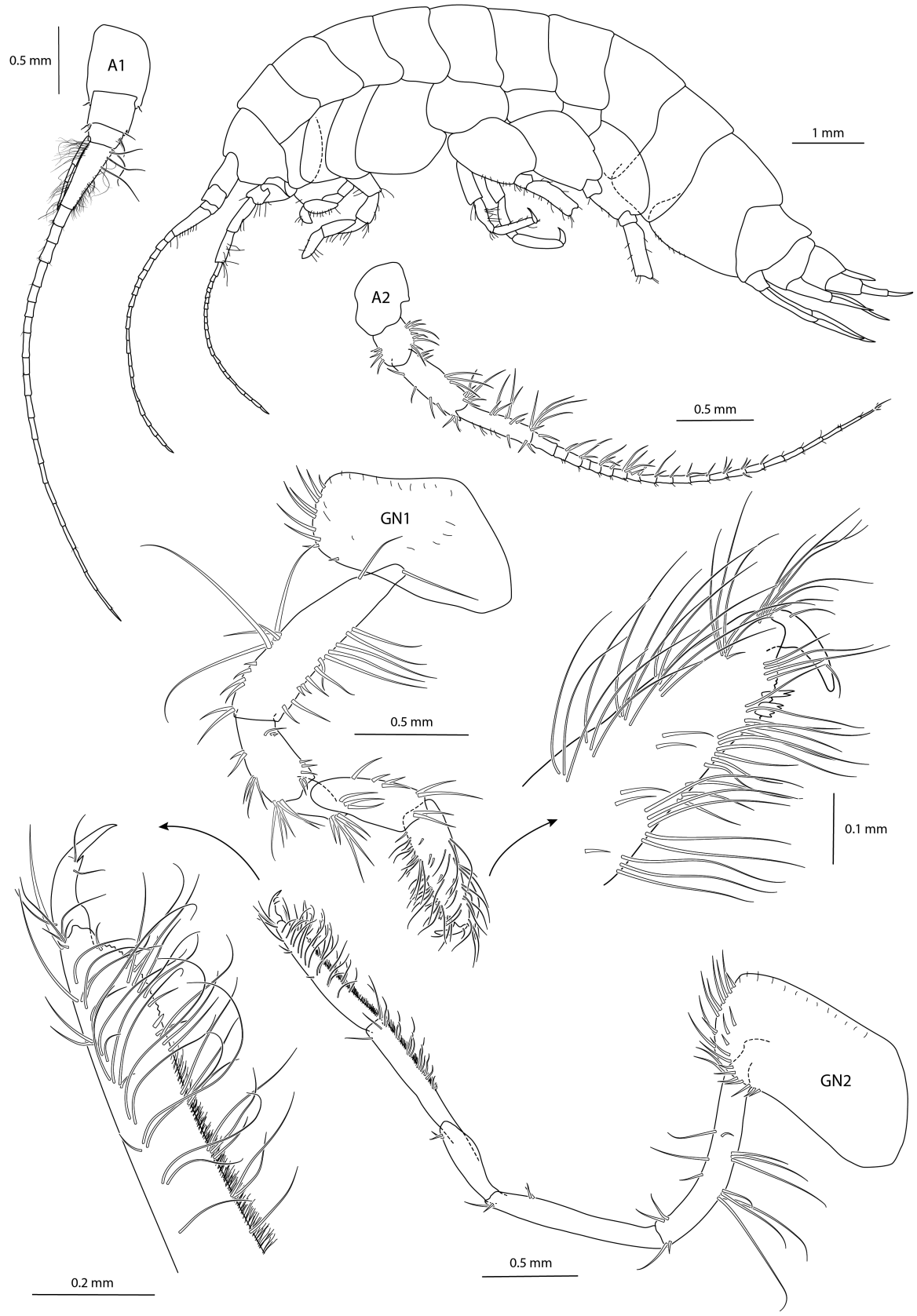

Figure 9. *Valettietta trottarum* holotype, immature, 10.5 mm, NHMUK 2024.37, Clarion-Clipperton Zone, 4313 m. A = Antenna; GN = Gnathopod.

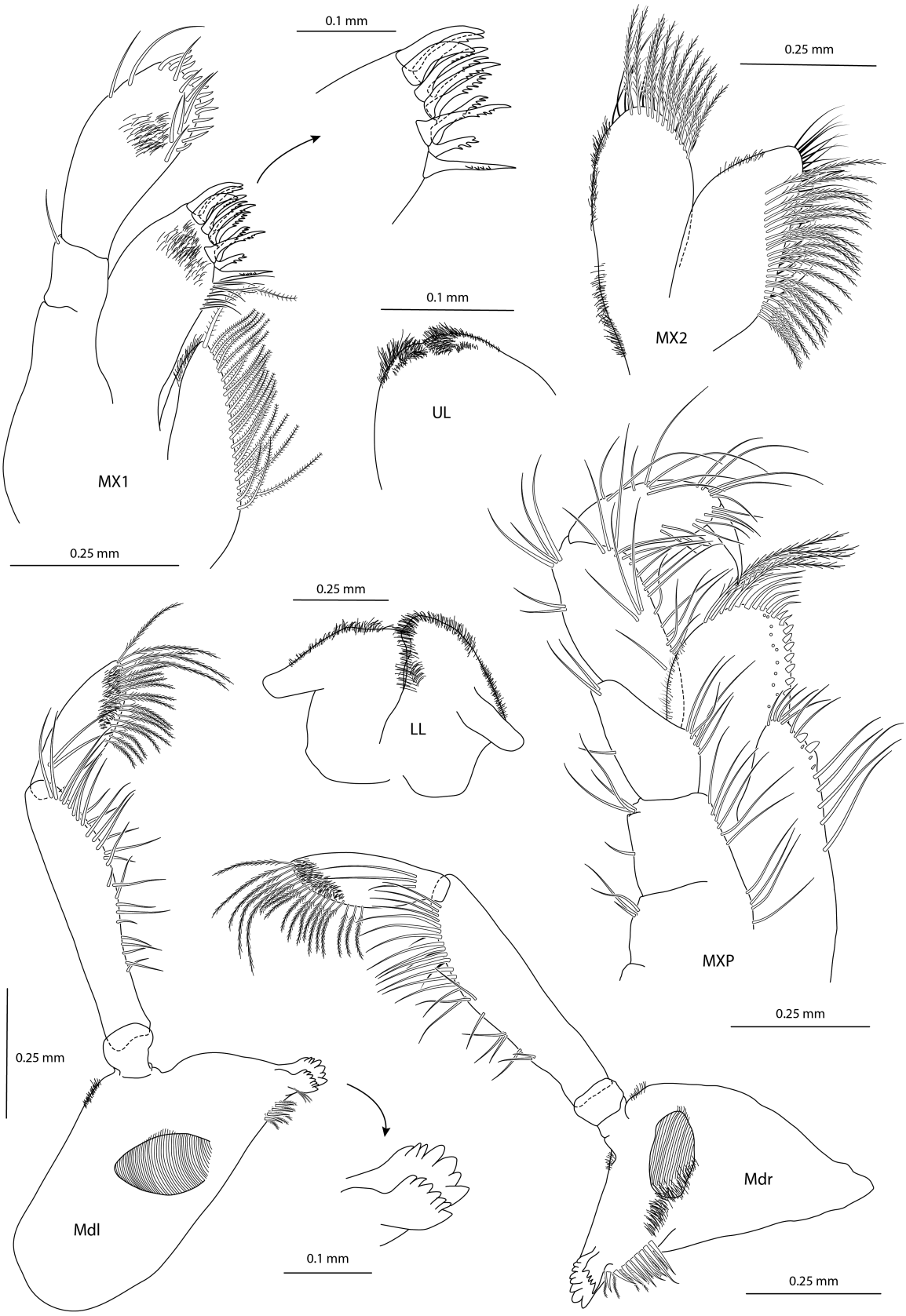

Figure 10. *Valettietta trottarum* holotype, immature, 10.5 mm, NHMUK 2024.37, Clarion-Clipperton Zone, 4313 m. MX = Maxilla; Md = Mandible; MXP = Maxilliped; LL = Lower lip; UL = Upper lip; l = left; r = right. Unfilled circles indicate setal bases.

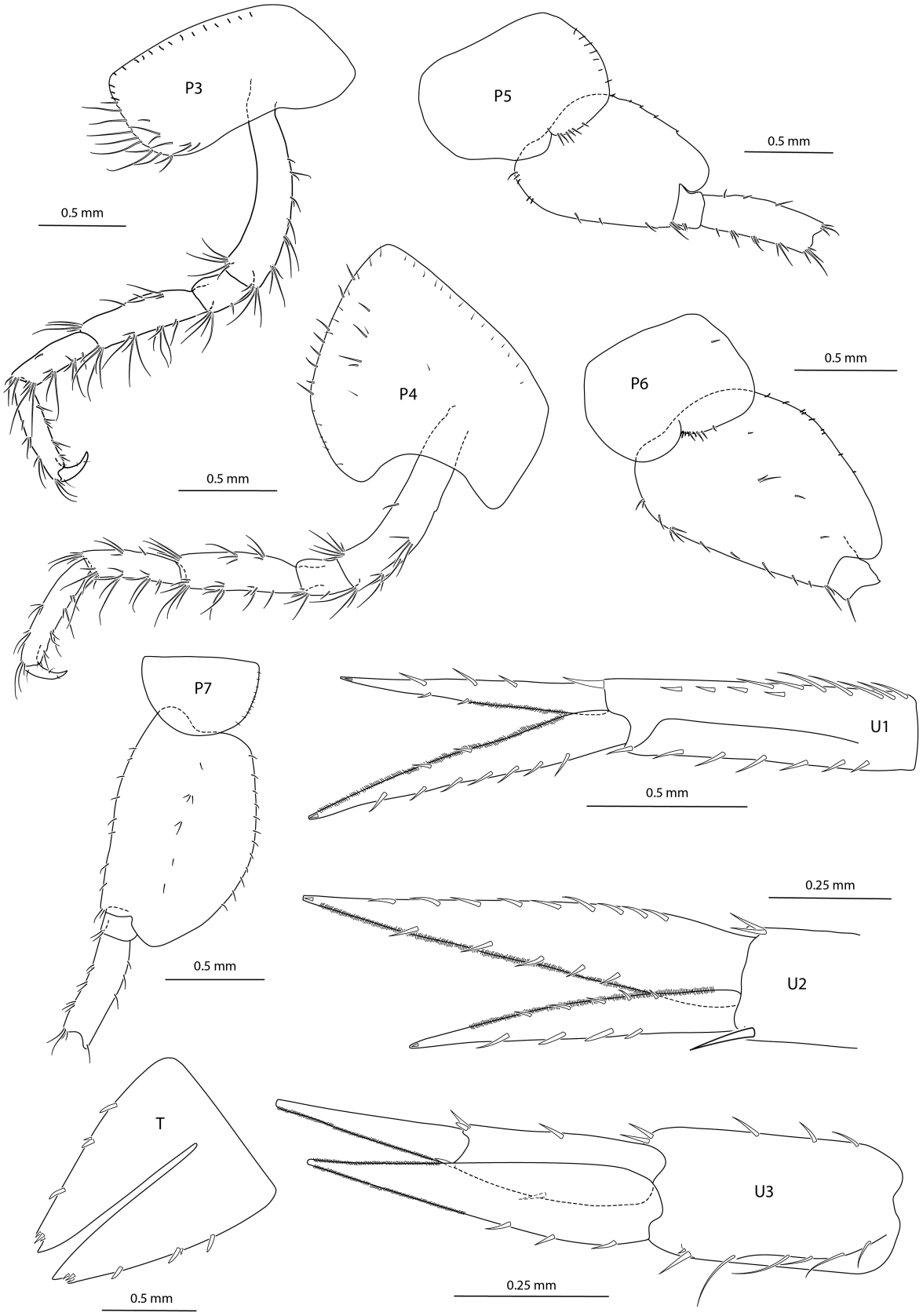

Figure 11. *Valettietta trottarum* holotype, immature, 10.5 mm, NHMUK 2024.37, Clarion-Clipperton Zone, 4313 m. P = Pleopod; U = Uropod; T = Telson. P7 and U3 are illustrated from the paratype, immature, 8.79 mm, NHMUK 2024.53.

coxa rectangular, distal margin setose, ventral proximal margin corner angular rather than rounded; basis curved; merus longer than propodus, and both longer than carpus; all articles with long slender marginal setae. *Pereopod 4* (Fig. 11) coxa very broad with large posteroventral lobe, posterodistal margin broadly rounded and setose; merus and propodus subequal in length and longer than carpus. *Pereopods 5–7* (Fig. 11) coxa 6 smaller than coxa 5, both weakly bilobate; coxa 7 smaller than coxa 6, not bilobate; basis broadly expanded distally, increasing from P5 to P7.

Pleon and urosome: Epimeron (Fig. 9) 1 weakly rounded, 2 subquadrate, 3 produced to a subacute tooth. *Uropods* (Fig. 11) biramous, lanceolate. *Uropod 1* (Fig. 11) inner ramus shorter than outer (1:0.8), outer margin with three short robust setae, inner margin with two on distal half, proximal half of inner margin minutely serrate; outer margin of outer rami lined by short robust setae, inner margin with two setae on central margin, inner margin minutely serrate; apex of both rami with inset small setule; peduncle with row of robust setae on both margins, one large robust seta present on peduncle at base of inner ramus. *Uropod 2* (Fig. 11) inner ramus shorter than outer (1:0.75), both margins of inner and outer ramus lined by short robust setae, adjacent margins minutely serrate; apex of both rami with inset small setule; peduncle damaged, one large robust seta present on peduncle at base of inner ramus and two at base of outer ramus. *Uropod 3* (Fig. 11) inner ramus shorter than outer (1:0.9), distal and proximal article of outer ramus subequal in length, apex of proximal article with robust setae, inner margin of distal article minutely serrate, centre of outer and inner margin of outer rami with one robust seta; inner and outer distal margin of inner rami minutely serrate, outer margin with three robust setae on proximal half of margin; peduncle with long fine setae on inner margin, two robust setae present at base of outer rami. Outer ramus article 2 elongate, subequal to article 1. *Telson* (Fig. 11) triangular, over 75% cleft, apices with three short robust setae in subapical notch; lobes with three lateral robust setae.

Habitat and ecology: Valettietta trottarum is an abyssal benthopelagic scavenger species. It is currently known to occur in high numbers in both the east and west Clarion-Clipperton Zone (abyssal central Pacific), at depths of 4082–4313 m. Based on available genetic sequences, the species is also present in both the Mariana and New Hebrides Trenches, in the western and southern Pacific, respectively, at depths down to 5467 m (Ritchie *et al.* 2015).

Remarks: Valettietta trottarum morphologically most closely resembles *V. gracilis* in the distally narrowing propodus of gnathopod 1, the sub-rectangular shape of coxa 2, and the presence of three spine groups along the telson. However, *V. trottarum* can be distinguished from *V. gracilis* by the convex dentate palm of gnathopod 2 (concave in *V. gracilis*), and the large sub-apical notch on the telson with three robust setae (vs. a small notch with one robust seta in *V. gracilis*). Key morphological differences distinguishing *V. trottarum* from *V. synchlys* and *V. anacantha* can be found in the key to the genus (below). Ritchie *et al.* (2015) identified specimens of *V. gracilis* and *V. anacantha* morphologically in the west and south Pacific Ocean; however, based on our phylogenetic analyses of the associated genetic sequences, some of these specimens actually belong to the species *V. trottarum* (Supporting information, Figs S1S2). Modified records can be found in Supporting information, Table S1. Body size in examined specimens ranged from 7.81 to 10.50 mm, which was notably smaller than the body size range of measured specimens of *V. synchlys*. This, however, should not be deemed as a defining characteristic of the species, as we only collected immature individuals and so the full body size range is not currently known.

Key to *Valettietta* species:

1. Coxa 1 much shorter than coxa 2 and strongly tapered distally..... *Valettietta cavernicola* (Anchialine cave habitat)
   Coxa 1 not shorter or slightly shorter than coxa 2 and not strongly tapered distally 2
2. Gnathopods 1 and 2 subchelate, robust, with sub-rectangular broadened carpus and propodus, palms weakly oblique 3
   Gnathopods 1 and 2 weakly subchelate or simple, tapering distally, palms weakly defined or lacking 4
3. Tegument ornamented with small conical setules, single tooth on the posterodistal margin of coxae 1, 2, and 3..... *Valettietta punctata*
   Tegument without ornamentation, two teeth on the posterodistal margin of coxae 2 and 3..... *Valettietta lobata*
4. Gnathopod 2 propodus extremely slender and elongate, length more than 6 × width..... 5
   Gnathopod 2 propodus slender, length less than 5 × width 6
5. Gnathopod 2 propodus lacking palm, very strongly narrowed distally; uropod 3 outer ramus article 2 shorter than article 1 (0.74 × length of article 1)..... *Valettietta gracilis*
   Gnathopod 2 propodus with weak convex palm; uropod 3 outer ramus article 2 elongate, subequal to article 1 ..... *Valettietta trottarum*
6. Gnathopod 1 propodus robust, tapering distally, broadly rounded proximally, carpus, short and broad, clearly shorter than propodus ..... *Valettietta anacantha*
   Gnathopod 1 propodus slender, tapering distally, carpus and propodus subequal ..... *Valettietta synchlys*

DISCUSSION

Species delimitation

The salient finding of this study is that paired molecular and morphological data provide congruent support for establishing two new species within *Valettietta*, *V. trottarum*, and *V. synchlys*. While morphologically quite cryptic, these species can be distinguished by the shape of the propodus of gnathopod 1, and the relative lengths of the articles of the outer ramus of uropod 3. These two new species also formed two well-supported monophyletic clades in multiple phylogenetic analyses. A clear 'barcoding gap' was found in the *COI* data (Table 3), with mean interclade divergences of around 9.5% being concordant with divergences previously reported for lysianassoid amphipods (Havermans *et al.* 2011, 2013, Havermans 2016, Mohrbeck *et al.* 2021). Hebert *et al.* (2004) proposed a standard sequence threshold of ten times the mean intraspecific divergence (K2P distance) to delimit animal species, a value which has since been used in the study of marine amphipods (Havermans *et al.* 2011, 2013, Havermans 2016, Mohrbeck *et al.* 2021). Based on our data, for *Valettietta*, the value to delimit species is recommended between 1–4%. However, while this may present a useful guideline, the use of an absolute threshold for delimiting species based on molecular data has the potential to ignore differing patterns of intraspecific genetic variation. It may be the case that larger intraspecific divergences are present within *V. trottarum* and *V. synchlys*; however, the species are currently not well sampled, and so the true genetic diversity is unknown.

Integrative taxonomy aims to use varied data (including, but not limited to: morphological, molecular, karyological, distributional, and behavioural) to create complementary lines of evidence to propose and consolidate species hypotheses (Will *et al.* 2005). The relative weight, however, of each complementary data type largely depends on the taxa of interest (Miralles *et al.* 2024). The importance of including both mitochondrial and nuclear genetic markers in species delimitation has been highlighted for a number of invertebrate taxa, including many freshwater amphipod species (Hupało *et al.* 2023, Knüsel *et al.* 2023). However, among marine amphipod species the current, albeit limited, evidence suggests that nuclear genetic markers (including 28S) may over-lump otherwise well-delimited operational taxonomic units due to conflicting evolutionary histories among genes (Verheye *et al.* 2016). While the three *Valettietta* species with genetic data presented here exhibit only marginal divergence in *H3* and 28S sequences, their significant mitochondrial genetic distances, distinct monophyletic clades in concordance with the phylogenetic species concept (*sensu* Donoghue 1985), and morphological variation support the establishment of two new species. In addition, while behavioural experiments are currently unfeasible with deep-sea scavenging amphipods, the co-occurrence of two populations in sympatry or parapatry with un-ambiguous differentiation of multiple traits (e.g. molecular markers and morphology), combined with the absence of individuals with intermediate traits, strongly suggests the absence of genetic admixture, thereby fulfilling the biological species criteria (Hillis *et al.* 2021, Miralles *et al.* 2024).

Phylogenetics

Based on their phylogenetic reconstructions, Ritchie *et al.* (2015) suggested that *Valettietta* is not a monophyletic genus and that the morphology of *V. gracilis* needed to be reviewed as it did not fall in the same clade as *V. anacantha*. However, both of our mitochondrial and concatenated mitochondrial and nuclear phylogenies support *Valettietta* as a monophyletic genus, and Valettiopsidae as a monophyletic family with the inclusion of both 16S and *COI* sequences of a *Valettiopsis* species. This supports the morphological phylogenetic results of Weston *et al.* (2020b). As highlighted by Jażdżewska *et al.* (2021a, b), the inclusion of misidentified sequences is the likely cause of the polyphyletic *Valettietta* clade presented in Ritchie *et al.* (2015). The 16S sequence provided for the individual identified as *V. gracilis* (GenBank accession number: KP456130) does not show similarity to any *Valettietta* species, while the *COI* sequence (GenBank accession number: KP713951) bears 100% similarity to new sequences provided for *V. trottarum*. We therefore suggest this record to be updated with the new identification of *V. trottarum*. Another individual identified as *V. anacantha* has a 16S and *COI* sequence which bear less than 60% similarity to other *Valettietta* sequences (*COI* accession number: KP713950, 16S accession number: KP456094), and so we also recommend these records be updated on GenBank to a broader identification of Amphipoda. A full list of updated identifications based on our new data can be found in Supporting information, Table S1.

The phylogenetic relationships between families of the Alicelloidea and Lysianassoidea found here contrast with recent large-scale published amphipod phylogenies (Lowry and Myers 2017, Copilaş-Ciocianu *et al.* 2020). Both the morphological phylogeny of Lowry and Myers (2017) and the molecular phylogeny of Copilaş-Ciocianu *et al.* (2020) found the families Alicellidae and Valettiopsidae to form a clade basal to the Lysianassoidea. In comparison, all four of our phylogenies found these two families to be nested among lysianassoid families. This result does, however, align with other phylogenetic analyses of deep-sea scavenger amphipod taxa, which have reported varied placements of the Alicelloidea among the Lysianassoidea, supporting previous suggestions of the Alicellidae being paraphyletic (Corrigan *et al.* 2014, Ritchie *et al.* 2015, Bribiesca-Contreras *et al.* 2021, Weston *et al.* 2021b). While a thorough systematic discussion is beyond the scope of this paper, our results suggest the need for additional molecular data from across the Alicellidae and Lysianassoidea to increase the robustness of future phylogenetic work.

Biogeographic patterns

Our data and phylogenetic analyses support the notion of a broad biogeographic range in *V. gracilis*, covering both the North Atlantic and the Indian Oceans (Lincoln and Thurston 1983, Weston *et al.* 2020b, 2021b). *Valettietta gracilis* was described from the Bay of Biscay abyssal plain and north-west of the Cape Verde Islands, in the north and central Atlantic Ocean, and has been recorded across the North Atlantic (Thurston 1990). When described, Lincoln and Thurston (1983) considered it to be a vicarious species with *V. anacantha*, owing to their 'disjunct distributions, one from the Atlantic Ocean and the other from the Pacific Ocean'. Records have since been published tentatively identifying *V.* cf. *gracilis* in the New Hebrides Trench (south-west Pacific Ocean; Lacey *et al.* 2016). However, based on the potentially cryptic morphological characters found between *V. gracilis* and *V. trottarum*, with the latter being molecularly identified from the Mariana Trench (west Pacific Ocean), it

is likely that this individual is *V. trottarum*, though without molecular data this cannot be said for certain. Similar can be said for the specimens identified as *V.* cf. *gracilis* in the CCZ by Patel *et al.* (2020)—these are likely to be individuals of *V. trottarum* and/or *V. synchlys*; however, without examining the material or molecular data this cannot be said for certain.

Weston *et al.* (2020b, 2021b) reported individuals identified as *Valettietta* sp. from the Wallaby Zenith Fracture Zone, East Indian Ocean, and highlighted them as a potential new species. However, further morphological examination of these specimens and comparison with specimens from the type locality suggests that they are in fact *V. gracilis*, confirmed by the phylogenetic placement of this specimen within the *V. gracilis* clade. This is the first true confirmation of *V. gracilis* outside of the Atlantic Ocean, adding it to the growing number of deep-sea amphipod species with pan-oceanic distributions (e.g. *Hirondellea dubia* Dahl, 1959, Weston & Jamieson, 2022; *Paralicella tenuipes* Chevreux, 1908, Jażdżewska *et al.*, 2021a; *Eurythenes gryllus*, Havermans *et al.*, 2013; *Abyssorchomene distinctus* (Birstein & Vinogradov, 1960), Dupont *et al.*, 2024; *Rhachotropis abyssalis* Lörz, 2010, Lörz *et al.*, 2023).

The presence of a species across multiple ocean basins is a strong indicator that gene flow has occurred or is ongoing, on evolutionary or potentially ecological time scales. Population genetics studies offer the means to investigate patterns of genetic connectivity and diversity; however, the logistical difficulties of deep-sea sampling combined with often low sample numbers, make connectivity studies of abyssal invertebrates challenging (Taylor and Roterman 2017). Available population genetics studies of abyssal Amphipoda have shown some species to have limited genetic differentiation across multiple ocean basins, with haplotype networks of *Abyssorchomene distinctus* suggesting a single population expansion event across distances up to 24 000 km (Dupont *et al.* 2024). Long range dispersal in the abyss is suggested to be facilitated by abyssal currents, such as the Pacific Arctic Intermediate Water across the Pacific Ocean (Havermans 2016). The distribution of *Valettietta gracilis* between the Atlantic and Indian Oceans may potentially be facilitated by occasional westward transport of warm water of the Agulhas Current around southern Africa (Bowen *et al.* 2016). However, as it stands there is no direct evidence for any deep-ocean migration corridors. It is also possible that *V. gracilis* has a far more continuous range across these ocean basins than is currently known, due to infrequent and spatially patchy sampling efforts. Future molecular studies using fast-evolving nuclear markers will be able to test the levels of connectivity between these populations of *V. gracilis*, and determine whether they are demographically independent, or experience high levels of gene flow.

CONCLUSION

Significant policy decisions on deep-sea mining are on the near horizon, and law makers need access to accurate biodiversity data to make informed choices. Fundamental to this is the identification and formal description of new species, with systematic archiving of faunal data with accessible, vouchered, and databased material in open, curated collections (Glover *et al.* 2018). Here, we contribute two new species of necrophagous amphipod to the growing list of species inhabiting the CCZ, and highlight the continued need for applying a fully integrative taxonomic approach. Even with this addition of two new species, the total number of formally described amphipod species recorded in the CCZ is remarkably low (Rabone *et al.* 2023). The use of integrative taxonomic methods will ultimately lead to increased understanding of the biogeographic ranges of these ecologically-important deep-sea organisms, and the eco-evolutionary drivers of speciation in the world's largest ecosystem.

SUPPORTING INFORMATION

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

ACKNOWLEDGEMENTS

We would like to thank the masters, crew, and technical staff aboard the RRS *James Cook*, and the MV *Island Pride* for their outstanding support, with particular thanks to the chief scientist aboard expedition JC120 Dr Daniel Jones (National Oceanography Centre). Thanks also to Dr Lauren Hughes at the Natural History Museum (UK) for the use of her microscope and camera lucida, and for the accessioning of specimens, and to Dr Kirill Mikhailov from the Zoological Museum of Moscow University for facilitating digital access to the type material of *Valettietta anacantha*.

CREDIT STATEMENT

Eva C.D. Stewart (Conceptualisation, Methodology, Formal analysis, Investigation, Data curation, Visualisation, Writing—Original Draft), Guadalupe Bribiesca-Contreras (Methodology, Investigation, Writing—Review and Editing), Johanna N.J. Weston (Resources, Investigation, Writing—Review and Editing), Adrian G. Glover (Project administration, Supervision, Writing—Review and Editing), and Tammy Horton (Conceptualisation, Resources, Methodology, Investigation, Data curation, Writing—Original draft)

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research was supported by funding to A.G.G., G.B-.C., and E.C.D.S. from the UK Natural Environment Research Council (NERC) Seabed Mining And Resilience To EXperimental impact (SMARTEX) project (Grant Reference NE/T003537/1), and funding to A.G.G., G.B-.C., E.C.D.S., and T.H. from The Metals Company Inc. (TMC) through its subsidiary Nauru Ocean Resources Inc. (NORI). NORI holds exploration rights to the NORI-D contract area in the CCZ regulated by the International Seabed Authority and sponsored by the government of Nauru. E.C.D.S. was also supported through funding from NERC (grant number NE/S007210/1). The funders had no influence on the data or interpretations presented in this paper.

DATA AVAILABILITY

This article is registered in ZooBank under http://zoobank.org/E64C1409-DD34-4196-ACDB-B72DCE516D4A. The data underlying this article are available in the article and in its Supporting information. Genetic data are available on GenBank under the accession

numbers: PP841397– PP841434 (*COI*); PP848990– P849032 (16S); PP848466– PP848494 (28S); PP855309– PP855337 (*H3*).

REFERENCES

Bellan-Santini D. Amphipodes profonds de Méditerrannée (Campagnes Biomede I, Polymede I et II). *Bolletino del Museo Civico di Storia Naturale di Verona* 1985;**10**:263–313.

Birstein YA, Vinogradov ME. Pelagischeskie gammaridy tropischeskoi chasti Ticogo Okeana. *Akademi nauk SSSR* 1960;**34**:165–241.

Birstein YA, Vinogradov ME. The deep-sea pelagic amphipods of the Philippine Trench. *Trudy Instituta Okeanologii Akademi nauk SSSR* 1963;**71**:81–93.

Bouckaert R, Heled J, Kühnert D *et al.* BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 2014;**10**:e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Bowen BW, Gaither MR, DiBattista JD *et al.* Comparative phylogeography of the ocean planet. *Proceedings of the National Academy of Sciences of the United States of America* 2016;**113**:7962–9. https://doi.org/10.1073/pnas.1602404113

Brandt A, Gooday AJ, Brandão SN *et al.* First insights into the biodiversity and biogeography of the Southern Ocean deep sea. *Nature* 2007;**447**:307–11. https://doi.org/10.1038/nature05827

Brasier MJ, Wiklund H, Neal L *et al.* DNA barcoding uncovers cryptic diversity in 50% of deep-sea Antarctic polychaetes. *Royal Society Open Science* 2016;**3**:160432. https://doi.org/10.1098/rsos.160432

Bribiesca-Contreras G, Dahlgren TG, Horton T *et al.* Biogeography and connectivity across habitat types and geographical scales in Pacific abyssal scavenging amphipods. *Frontiers in Marine Science* 2021;**8**:705237.

Chakrabarty P, Warren M, Page LM *et al.* GenSeq: an updated nomenclature and ranking for genetic sequences from type and non-type sources. *ZooKeys* 2013;**346**:29–41. https://doi.org/10.3897/zookeys.346.5753

Chevreux E. Diagnoses d'amphipodes nouveaux provenant des campagnes de la 'Princesse-Alice' dans l' Atlantique nord. *Bulletin de l'Institut Océanographique de Monaco* 1908;**117**:1–13.

Chevreux E. Deuxième expédition dans l'Antarctique, dirigée par le Dr. Charcot, 1908-1910. Diagnoses d'amphipodes nouveaux. *Bulletin du Museum National d'Histoire Naturelle* 1912;**18**:208–18.

Coleman C. 'Digital inking': how to make perfect line drawings on computers. *Organisms Diversity and Evolution* 2003;**3**:303–4. https://doi.org/10.1078/1439-6092-00081

Copilaş-Ciocianu D, Borko S, Fišer C. The late blooming amphipods: global change promoted post-Jurassic ecological radiation despite Palaeozoic origin. *Molecular Phylogenetics and Evolution* 2020;**143**:106664. https://doi.org/10.1016/j.ympev.2019.106664

Corrigan LJ, Horton T, Fotherby H *et al.* Adaptive evolution of deep-sea amphipods from the superfamily Lysiassanoidea in the North Atlantic. *Evolutionary Biology* 2014;**41**:154–65. https://doi.org/10.1007/s11692-013-9255-2

d'Udekem d'Acoz C, Havermans C. Contribution to the systematics of the genus *Eurythenes* S.I. Smith in Scudder, 1882 (Crustacea: Amphipoda: Lysianassoidea: Eurytheneidae). *Zootaxa* 2015;**3971**:1–80.

Dahl E. Amphipoda from depths exceeding 6000 m. *Galathea Report* 1959;**1**:211–41.

Dana JD. Synopsis of the genera of Gammaracea. *The American Journal of Science and Arts (ser. 2)* 1849;**8**:135–40.

Danovaro R, Snelgrove PVR, Tyler P. Challenging the paradigms of deep-sea ecology. *Trends in Ecology & Evolution* 2014;**29**:465–75. https://doi.org/10.1016/j.tree.2014.06.002

Donoghue MJ. A critique of the biological species concept and recommendations for a phylogenetic alternative. *The Bryologist* 1985;**88**:172–81. https://doi.org/10.2307/3243026

Downey RV, Fuchs M, Janussen D. Unusually diverse, abundant and endemic deep-sea sponge fauna revealed in the Sea of Okhotsk (NW Pacific Ocean). *Deep Sea Research Part II: Topical Studies in Oceanography* 2018;**154**:47–58. https://doi.org/10.1016/j.dsr2.2018.02.005

Duffy GA, Horton T, Billett DSM. Deep-sea scavenging amphipod assemblages from the submarine canyons of the Western Iberian Peninsula. *Biogeosciences* 2012;**9**:4861–9. https://doi.org/10.5194/bg-9-4861-2012

Dupont DW, Patel T, Kochzius M *et al.* Evidence for a single population expansion event across 24 000 km: the case of the deep-sea scavenging amphipod *Abyssorchomene distinctus*. *Hydrobiologia* 2024;**851**:1–19.

Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 2004;**32**:1792–7. https://doi.org/10.1093/nar/gkh340

Eustace RM, Ritchie H, Kilgallen NM *et al.* Morphological and ontogenetic stratification of abyssal and hadal *Eurythenes gryllus sensu lato* (Amphipoda: Lysianassoidea) from the Peru–Chile Trench. *Deep Sea Research Part I: Oceanographic Research Papers* 2016;**109**:91–8. https://doi.org/10.1016/j.dsr.2015.11.005

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 1994;**35**:294–9.

Georgieva MN, Wiklund H, Bell JB *et al.* A chemosynthetic weed: the tubeworm *Sclerolinum contortum* is a bipolar, cosmopolitan species. *BMC Evolutionary Biology* 2015;**15**:280. https://doi.org/10.1186/s12862-015-0559-y

Glover AG, Smith CR, Paterson GLJ *et al.* Polychaete species diversity in the central Pacific abyss: local and regional patterns, and relationships with productivity. *Marine Ecology Progress Series* 2002;**240**:157–70. https://doi.org/10.3354/meps240157

Glover AG, Wiklund H, Chen C *et al.* Managing a sustainable deep-sea 'Blue Economy' requires knowledge of what actually lives there. *eLife* 2018;**7**:e41319. https://doi.org/10.7554/eLife.41319

Grassle JF, Maciolek NJ. Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *The American Naturalist* 1992;**139**:313–41. https://doi.org/10.1086/285329

Havermans C. Have we so far only seen the tip of the iceberg? Exploring species diversity and distribution of the giant amphipod *Eurythenes*. *Biodiversity* 2016;**17**:12–25. https://doi.org/10.1080/14888386.2016.1172257

Havermans C, Nagy ZT, Sonet G *et al.* DNA barcoding reveals new insights into the diversity of Antarctic species of *Orchomene sensu lato* (Crustacea: Amphipoda: Lysianassoidea). *Deep Sea Research Part II: Topical Studies in Oceanography* 2011;**58**:230–41. https://doi.org/10.1016/j.dsr2.2010.09.028

Havermans C, Sonet G, d'Acoz C *et al.* Genetic and morphological divergences in the cosmopolitan deep-sea amphipod *Eurythenes gryllus* reveal a diverse abyss and a bipolar species. *PLoS One* 2013;**8**:e74218.

Hebert PDN, Stoeckle MY, Zemlak TS *et al.* Identification of birds through DNA barcodes. *PLoS Biology* 2004;**2**:e312. https://doi.org/10.1371/journal.pbio.0020312

Hein JR, Mizell K, Koschinsky A *et al.* Deep-ocean mineral deposits as a source of critical metals for high- and green-technology applications: comparison with land-based resources. *Ore Geology Reviews* 2013;**51**:1–14. https://doi.org/10.1016/j.oregeorev.2012.12.001

Hillis DM, Chambers EA, Devitt TJ. Contemporary methods and evidence for species delimitation. *Ichthyology & Herpetology* 2021;**109**:895–903.

Hoang DT, Chernomor O, von Haeseler A *et al.* UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 2018;**35**:518–22. https://doi.org/10.1093/molbev/msx281

Holmes SJ. The Amphipoda collected by the U.S. Bureau of Fisheries Steamer 'Albatross' off the West Coast of North America, in 1903 and 1904, with descriptions of a new family and several new genera and species. *Proceedings of the United States National Museum* 1908;**35**:489–543. https://doi.org/10.5479/si.00963801.35-1654.489

Horton T, Cooper H, Vlierboom R *et al.* Molecular phylogenetics of deep-sea amphipods (*Eurythenes*) reveal a new undescribed species at the Porcupine Abyssal Plain, North East Atlantic Ocean. *Progress*

in Oceanography 2020;**183**:102292. https://doi.org/10.1016/j.pocean.2020.102292

Hupało K, Copilaş-Ciocianu D, Leese F *et al.* Morphology, nuclear SNPs and mate selection reveal that *COI* barcoding overestimates species diversity in a Mediterranean freshwater amphipod by an order of magnitude. *Cladistics* 2023;**39**:129–43. https://doi.org/10.1111/cla.12520

Hurley DE. Amphipoda of the family Lysianassidae from the West Coast of North and Central America. *Allan Hancock Foundation Publications* 1963;**25**:1–160.

Jamieson AJ, Kilgallen NM, Rowden AA *et al.* Bait-attending fauna of the Kermadec Trench, SW Pacific Ocean: evidence for an ecotone across the abyssal–hadal transition zone. *Deep Sea Research Part I: Oceanographic Research Papers* 2011;**58**:49–62. https://doi.org/10.1016/j.dsr.2010.11.003

Jażdżewska AM, Mamos T. High species richness of Northwest Pacific deep-sea amphipods revealed through DNA barcoding. *Progress in Oceanography* 2019;**178**:102184. https://doi.org/10.1016/j.pocean.2019.102184

Jażdżewska AM, Horton T, Hendrycks E *et al.* Pandora's box in the deep sea – intraspecific diversity patterns and distribution of two congeneric scavenging amphipods. *Frontiers in Marine Science* 2021a;**8**:750180.

Jażdżewska AM, Tandberg AHS, Horton T *et al.* Global gap-analysis of amphipod barcode library. *PeerJ* 2021b;**9**:e12352. https://doi.org/10.7717/peerj.12352

Jones DOB. RRS James Cook Cruise JC120 15 Apr–19 May 2015. Manzanillo to Manzanillo, Mexico. Managing Impacts of DeepseA resource exploitation (MIDAS): Clarion-Clipperton Zone North Eastern Area of Particular Environmental Interest. *British Oceanographic Data Centre* 2015.

Kalyaanamoorthy S, Minh BQ, Wong TKF *et al.* ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 2017;**14**:587–9. https://doi.org/10.1038/nmeth.4285

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 2013;**30**:772–80. https://doi.org/10.1093/molbev/mst010

Kearse M, Moir R, Wilson A *et al.* Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 2012;**28**:1647–9. https://doi.org/10.1093/bioinformatics/bts199

Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 1980;**16**:111–20. https://doi.org/10.1007/BF01731581

Kniesz K, Jażdżewska AM, Martínez Arbizu P *et al.* DNA barcoding of scavenging amphipod communities at active and inactive hydrothermal vents in the Indian Ocean. *Frontiers in Marine Science* 2022;**8**:752360.

Knüsel M, Borko S, Alther R *et al.* Phylogenetic structure and molecular species delimitation hint a complex evolutionary history in an Alpine endemic *Niphargus* clade (Crustacea, Amphipoda). *Zoologischer Anzeiger* 2023;**306**:27–36. https://doi.org/10.1016/j.jcz.2023.07.001

Kumar S, Stecher G, Li M *et al.* MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 2018;**35**:1547–9. https://doi.org/10.1093/molbev/msy096

Lacey NC, Rowden AA, Clark MR *et al.* Community structure and diversity of scavenging amphipods from bathyal to hadal depths in three south Pacific trenches. *Deep Sea Research Part I: Oceanographic Research Papers* 2016;**111**:121–37. https://doi.org/10.1016/j.dsr.2016.02.014

Lampitt RS. RRS Discovery Cruise DY077, 14 Apr-01 May 2017. Cruise to the Porcupine Abyssal Plain sustained observatory. *British Oceanographic Data Centre* 2017.

Lanfear R, Frandsen PB, Wright AM *et al.* PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 2017;**34**:772–3. https://doi.org/10.1093/molbev/msw260

Leigh JW, Bryant D. PopART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 2015;**6**:1110–6. https://doi.org/10.1111/2041-210x.12410

Lester SE, Ruttenberg BI, Gaines SD *et al.* The relationship between dispersal ability and geographic range size. *Ecology Letters* 2007;**10**:745–58. https://doi.org/10.1111/j.1461-0248.2007.01070.x

Lichtenstein H. In: Mandt MG (ed.), *Observationes in historiam naturalem et anatomiam comparatam in itinere Groenlandico factae. Dissertatio in auguralis quam consensu et auctoritate gratiosi medicorum ordinis in universitate literaria berolinensi ut summi in medicina et chirurgia honores rite sibi concedantur die XXII.* M. Iulii A. MDCCCXXII H.L.Q.S., publice defendet auctor Martinus Gulielmus Mandt Beyenburgensis. Opponentibus: J.Th. v. Brandt Med. Cd., J. Ollenroth Med. Cd., E. Gabler Med. Cd.; Berlin: Formis Brueschckianis, 1822, 31–7.

Lincoln RJ, Thurston MH. *Valettietta*, a new genus of deep-sea amphipod (Gammaridea: Lysianassidae) with descriptions of two new species from the North Atlantic Ocean. *Bulletin of the British Museum (Natural History)* 1983;**44**:85–101.

Lonsdale P. Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. *Deep-Sea Research* 1977;**24**:857–63. https://doi.org/10.1016/0146-6291(77)90478-7

Lörz AN. Deep-sea *Rhachotropis* (Crustacea: Amphipoda: Eusiridae) from New Zealand and the Ross Sea with key to the Pacific, Indian Ocean and Antarctic species. *Zootaxa* 2010;**2482**:22–48.

Lörz AN, Tandberg AHS, Willassen E *et al.* *Rhachotropis* (Eusiroidea, Amphipoda) from the North East Atlantic. *ZooKeys* 2018;**731**:75–101. https://doi.org/10.3897/zookeys.731.19814

Lörz AN, Schwentner M, Bober S *et al.* Multi-ocean distribution of a brooding predator in the abyssal benthos. *Scientific Reports* 2023;**13**:15867. https://doi.org/10.1038/s41598-023-42942-0

Lowry JK, De Broyer C. Alicellidae and Valettiopsidae, two new callynophorate families (Crustacea: Amphipoda). *Zootaxa* 2008;**1843**:57–66.

Lowry JK, Myers AA. A phylogeny and classification of the Amphipoda with the establishment of the new order Ingolfiellida (Crustacea: Peracarida). *Zootaxa* 2017;**4265**:1–89. https://doi.org/10.11646/zootaxa.4265.1.1

Lowry JK, Stoddart HE. Crustacea Amphipoda: lysianassoids from Philippine and Indonesian waters. *Résultats des campagnes MUSORSTOM* 1993;**10**:55–109.

Lowry JK, Stoddart HE. New lysianassoid genera and species from south-eastern Australia (Crustacea: Amphipoda). *Records of the Australian Museum* 1995;**47**:7–25. https://doi.org/10.3853/j.0067-1975.47.1995.5

Lowry JK, Stoddart HE. Amphipoda Crustacea IV. Families Aristiidae, Cyphocarididae, Endevouridae, Lysianassidae, Scopelocheiridae, Uristidae. *Memoirs of the Hourglass Cruises* 1997;**10**:1–148.

Lowry JK, Stoddart HE. The deep-sea scavenging genus *Hirondellea* (Crustacea: Amphipoda: Lysianassoidea: Hirondelleidae fam. nov.) in Australian waters. *Zootaxa* 2010;**2329**:37–55.

Lowry JK, Stoddart HE. The new deep-sea families Cebocaridae fam. nov., Cyclocaridae fam. nov. and Thoriellidae fam. nov. (Crustacea: Amphipoda: Lysianassoidea). *Zootaxa* 2011;**2747**:53–68.

Madsen FJ. On the zoogeography and origin of the abyssal fauna. *Galathea Report* 1961;**4**:177–218.

McClain CR, Hardy SM. The dynamics of biogeographic ranges in the deep sea. *Proceedings Biological Sciences* 2010;**277**:3533–46. https://doi.org/10.1098/rspb.2010.1057

Medlin L, Elwood HJ, Stickel S *et al.* The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* 1988;**71**:491–9. https://doi.org/10.1016/0378-1119(88)90066-2

Minh BQ, Schmidt HA, Chernomor O *et al.* IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 2020;**37**:1530–4. https://doi.org/10.1093/molbev/msaa015

Miralles A, Puillandre N, Vences M. DNA barcoding in species delimitation: from genetic distances to integrative taxonomy. In: DeSalle R (ed.), *DNA barcoding: methods and protocols.* New York, NY: Springer US, 2024, 77–104.

Mohrbeck I, Horton T, Jażdżewska AM *et al*. DNA barcoding and cryptic diversity of deep-sea scavenging amphipods in the Clarion-Clipperton Zone (Eastern Equatorial Pacific). *Marine Biodiversity* 2021;**51**:26.

Narahara-Nakano Y, Nakano T, Tomikawa K. Deep-sea amphipod genus *Eurythenes* from Japan, with a description of a new *Eurythenes* species from off Hokkaido (Crustacea: Amphipoda: Lysianassoidea). *Marine Biodiversity* 2018;**48**:603–20. https://doi.org/10.1007/s12526-017-0758-4

Nygren A, Sundberg P. Phylogeny and evolution of reproductive modes in Autolytinae (Syllidae, Annelida). *Molecular Phylogenetics and Evolution* 2003;**29**:235–49. https://doi.org/10.1016/s1055-7903(03)00095-2

Palumbi SR. Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* 2003;**13**:146–58. https://doi.org/10.1890/1051-0761(2003)013[0146:pgdcat]2.0.co;2

Patel T, Robert H, D'Udekem D'Acoz C *et al*. Biogeography and community structure of abyssal scavenging Amphipoda (Crustacea) in the Pacific Ocean. *Biogeosciences* 2020;**17**:2731–44. https://doi.org/10.5194/bg-17-2731-2020

Paull CK, Hecker B, Commeau R *et al*. Biological communities at the Florida escarpment resemble hydrothermal vent taxa. *Science* 1984;**226**:965–7. https://doi.org/10.1126/science.226.4677.965

Puillandre N, Brouillet S, Achaz G. ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources* 2021;**21**:609–20. https://doi.org/10.1111/1755-0998.13281

Rabone M, Wiethase JH, Simon-Lledó E *et al*. How many metazoan species live in the world's largest mineral exploration region? *Current Biology* 2023;**33**:2383–96.e5. https://doi.org/10.1016/j.cub.2023.04.052

Riehl T, Wölfl AC, Augustin N *et al*. Discovery of widely available abyssal rock patches reveals overlooked habitat type and prompts rethinking deep-sea biodiversity. *Proceedings of the National Academy of Sciences of the United States of America* 2020;**117**:15450–9. https://doi.org/10.1073/pnas.1920706117

Ritchie H, Jamieson AJ, Piertney SB. Phylogenetic relationships among hadal amphipods of the Superfamily Lysianassoidea: implications for taxonomy and biogeography. *Deep Sea Research Part I: Oceanographic Research Papers* 2015;**105**:119–31. https://doi.org/10.1016/j.dsr.2015.08.014

Ritchie H, Jamieson AJ, Piertney SB. Genome size variation in deep-sea amphipods. *Royal Society Open Science* 2017;**4**:170862. https://doi.org/10.1098/rsos.170862

Sanders HL. Marine benthic diversity: a comparative study. *The American Naturalist* 1968;**102**:243–82. https://doi.org/10.1086/282541

Smith SI. *Eurythenes* Lillgeborg. In: Scudder SH (ed.), *Nomenclator Zoologicus. An alphabetical list of all generic names that have been employed by naturalists for recent and fossil animals from the earliest times to the close of the year 1879. 1. Supplemental list of Genera in Zoology. Washington: Bulletin of the United States National Museum,* 1882;**21**:376.

Steiner G, Dreyer H. Molecular phylogeny of Scaphopoda (Mollusca) inferred from 18S rDNA sequences: support for a Scaphopoda–Cephalopoda clade. *Zoologica Scripta* 2003;**32**:343–56. https://doi.org/10.1046/j.1463-6409.2003.00121.x

Stewart ECD, Bribiesca-Contreras G, Taboada S *et al*. Biodiversity, biogeography, and connectivity of polychaetes in the world's largest marine minerals exploration frontier. *Diversity and Distributions* 2023;**29**:727–47.

Stock JH, Iliffe TM. Amphipod crustaceans from anchihaline cave waters of the Galapagos Islands. *Zoological Journal of the Linnean Society* 1990;**98**:141–60. https://doi.org/10.1111/j.1096-3642.1990.tb01213.x

Taylor ML, Roterman CN. Invertebrate population genetics across Earth's largest habitat: the deep-sea floor. *Molecular Ecology* 2017;**26**:4872–96. https://doi.org/10.1111/mec.14237

Thurston MH. A new species of *Valettia* (Crustacea: Amphipoda) and the relationship of the Valettidae to the Lysianassoidea. *Journal of Natural History* 1989;**23**:1093–107. https://doi.org/10.1080/00222938900770991

Thurston MH. Abyssal necrophagous amphipods (Crustacea: Amphipoda) in the northeast and tropical Atlantic Ocean. *Progress in Oceanography* 1990;**24**:257–74. https://doi.org/10.1016/0079-6611(90)90036-2

Verheye ML, Backeljau T, d'Udekem d'Acoz C. Looking beneath the tip of the iceberg: diversification of the genus *Epimeria* on the Antarctic shelf (Crustacea, Amphipoda). *Polar Biology* 2016;**39**:925–45. https://doi.org/10.1007/s00300-016-1910-5

Vrijenhoek RC. Cryptic species, phenotypic plasticity, and complex life histories: assessing deep-sea faunal diversity with molecular markers. *Deep Sea Research Part II: Topical Studies in Oceanography* 2009;**56**:1713–23. https://doi.org/10.1016/j.dsr2.2009.05.016

Weston JNJ, Jamieson AJ. The multi-ocean distribution of the hadal amphipod, *Hirondellea dubia* Dahl, 1959 (Crustacea, Amphipoda). *Frontiers in Marine Science* 2022;**9**:155.

Weston JNJ, Carrillo-Barragan P, Linley TD *et al*. New species of *Eurythenes* from hadal depths of the Mariana Trench, Pacific Ocean (Crustacea: Amphipoda). *Zootaxa* 2020a;**4748**:163–81.

Weston JNJ, Peart RA, Jamieson AJ. Amphipods from the Wallaby-Zenith Fracture Zone, Indian Ocean: new genus and two new species identified by integrative taxonomy. *Systematics and Biodiversity* 2020b;**18**:57–78. https://doi.org/10.1080/14772000.2020.1729891

Weston JNJ, Espinosa-Leal L, Wainwright JA *et al*. *Eurythenes atacamensis* sp. nov. (Crustacea: Amphipoda) exhibits ontogenetic vertical stratification across abyssal and hadal depths in the Atacama Trench, eastern South Pacific Ocean. *Marine Biodiversity* 2021a;**51**:51. https://doi.org/10.1007/s12526-021-01182-z

Weston JNJ, Peart RA, Stewart HA *et al*. Scavenging amphipods from the Wallaby-Zenith Fracture Zone: extending the hadal paradigm beyond subduction trenches. *Marine Biology* 2021b;**168**:1.

Weston JNJ, Stewart ECD, Maroni PJ *et al*. *Eurythenes sigmiferus* and *Eurythenes andhakarae* (Crustacea: Amphipoda) are sympatric at the abyssal Agulhas Fracture Zone, South Atlantic Ocean, and notes on their distributions. *Deep Sea Research Part I: Oceanographic Research Papers* 2023;**196**:104050.

Will KW, Mishler BD, Wheeler QD. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* 2005;**54**:844–51. https://doi.org/10.1080/10635150500354878

Zhang J, Kapli P, Pavlidis P *et al*. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 2013;**29**:2869–76. https://doi.org/10.1093/bioinformatics/btt499