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A new species of *Eualus* Thallwitz, 1892 and new record of *Lebbeus antarcticus* (Hale, 1941) (Crustacea: Decapoda: Caridea: Hippolytidae) from the Scotia Sea

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ABSTRACT

Eleven specimens representing two hippolytid genera, *Eualus* Thallwitz, 1892 and *Lebbeus* White, 1847 were sampled recently from the Scotia Sea (1517–2598 m). Seven specimens are described and illustrated as *Eualus amandae* sp. nov., and its morphology is compared with those of previously described species. Four female specimens, morphologically consistent with *Lebbeus antarcticus* (Hale, 1941), are described and illustrated to supplement previous descriptions of this rarely collected bathyal species. Partial COI mtDNA and 18S rDNA sequences were generated for both species. Only limited DNA sequences are available for the Hippolytidae. COI phylogenetic trees are presented to illustrate that the new species is genetically distinct from all other species in GenBank. This record enhances existing knowledge of Antarctic invertebrate biodiversity and species richness of decapod crustaceans in the Southern Ocean.

1. Introduction

Knowledge of the distribution of decapod crustaceans from the Southern Ocean has increased considerably in recent years (e.g. Raso et al., 2008; Rogers et al., 2012; Thatje and Arntz, 2004; Thatje and Lörz, 2005). Caridean shrimps cover the largest known bathymetric range of the Southern Ocean decapod fauna, distributed from the intertidal zone to the deep sea (Gorny, 1999). They are one of few groups of decapod crustaceans found south of the Antarctic Convergence (Arntz et al., 1999; Kirkwood, 1984; Thatje and Arntz, 2004; Yaldwyn, 1965), where they are represented by at least five families, nine genera and ten species (Boschi and Gavio, 2005). The caridean family Hippolytidae Spence Bate, 1888 is the most diverse decapod family south of the Antarctic Convergence (Boschi and Gavio, 2005; Gorny, 1999), represented to date by three species: *Chorismus antarcticus* (Pfeffer, 1887); *Eualus kinzeri* Tiefenbacher, 1990; and *Lebbeus antarcticus* (Hale, 1941).

During a recent research cruise to the East Scotia Ridge and Kemp Caldera, Scotia Sea, a novel species of the hippolytid genus *Eualus* Thallwitz, 1892 was discovered and several specimens of *Lebbeus antarcticus* (Hale, 1941) were also sampled. The aims of this paper were to: (1) describe the new species and compare its morphology with those of previously described species; (2) describe and illustrate the new specimens of *L. antarcticus* to supplement previous descriptions (based on single or incomplete specimens) of this rarely collected bathyal species (Hale, 1941; Komai et al., 1996; Ward, 1985; Zarenkov, 1970); and (3) use the COI mtDNA region to determine if the species are genetically distinct from other hippolytid species in the GenBank database. This record enhances existing knowledge of Antarctic invertebrate biodiversity and species richness of decapod crustaceans in the Southern Ocean.

2. Materials and methods

2.1. Sample collection

The specimens were collected during the *RRS James Cook* cruise 042 (JC42) in January–February 2010 to the Scotia Sea (Fig. 1). All specimens were collected using sampling equipment attached to, or deployed by, the Remotely Operated Vehicle (ROV) *Isis*. Sampling data are summarized in Table 1.

2.2. Morphology

Prior to fixation, a pereopod was removed from each specimen for subsequent molecular analysis. Specimens were fixed in 10% neutralized formalin and then transferred to 75% industrial

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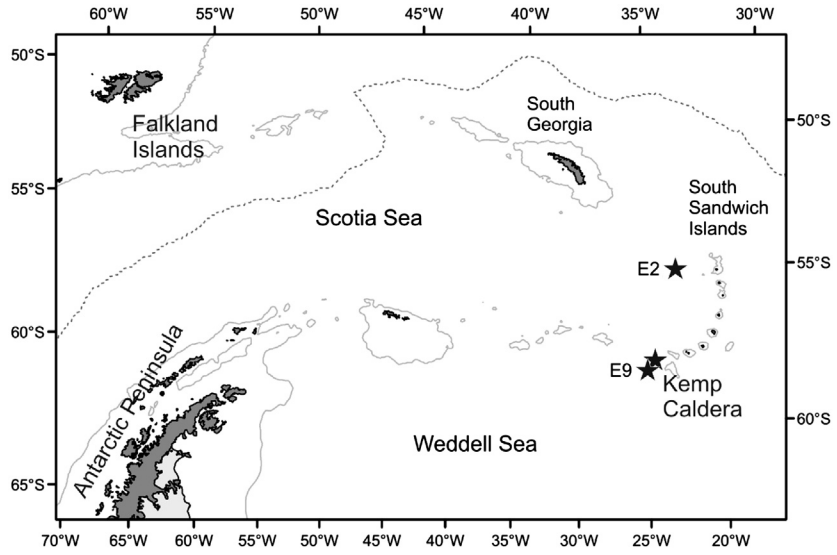

Fig. 1. Map showing sampling locations E2, E9, and Kemp Caldera in the Scotia Sea. Dashed line represents the Antarctic Convergence.

Table 1
Sampling data.

Species	NHMUK Reg. no.	Site	Area	Latitude (S)	Longitude (W)	Depth (m)	Date	Dive no.	Method
E. amandae sp. nov.	2012.1522	JC42-4-15	E9	60°02.580	29°58.837	2401	04/02/2010	145	Suction sampler
E. amandae sp. nov.	2012.1523	JC42-5-15	Kemp Caldera	59°41.973	28°21.01	1517	11/02/2010	152	Suction sampler
E. amandae sp. nov.	2012.1524	JC42-5-15	Kemp Caldera	59°41.973	28°21.01	1517	11/02/2010	152	Suction sampler
E. amandae sp. nov.	2012.1525	JC42-5-15	Kemp Caldera	59°41.973	28°21.01	1517	11/02/2010	152	Suction sampler
E. amandae sp. nov.	2012.1526	JC42-5-15	Kemp Caldera	59°41.973	28°21.01	1517	11/02/2010	152	Suction sampler
E. amandae sp. nov.	2012.1527	JC42-5-15	Kemp Caldera	59°41.973	28°21.01	1517	11/02/2010	152	Suction sampler
E. amandae sp. nov.	2012.1528	JC42-5-15	Kemp Caldera	59°41.973	28°21.01	1517	11/02/2010	152	Suction sampler
L. antarcticus	2012.1529	JC42-3-15	E2	56°05.307	30°19.094	2598	23/01/2010	133	Baited trap
L. antarcticus	2012.1530	JC42-3-18	E2	56°05.324	30°19.107	2139	25/01/2010	135	Baited trap
L. antarcticus	2012.1531	JC42-3-18	E2	56°05.324	30°19.107	2139	25/01/2010	135	Baited trap
L. antarcticus	2012.1532	JC42-4-7	E9	60°02.568	29°58.89	2402	30/01/2010	140	Biobox

methylated spirits. Individuals were measured to the nearest 0.1 mm using Vernier callipers. Postorbital carapace length (CL) was measured from the posterior margin of the orbit to the posterior margin of the carapace and is used herein as an indication of specimen size. Individuals were sexed under a dissecting microscope. Illustrations were prepared with the aid of a cameral lucida mounted onto a Leica MZ8 stereomicroscope, scanned and inked digitally using a WACOM™ digitizer and Adobe® Illustrator® software. Specimens were deposited in the invertebrate collection at the Natural History Museum, London (NHMUK). Morphological terminology generally follows Komai and Hayashi (2002) and Komai et al. (2012).

2.3. Molecular

A pereopod from each specimen was immediately placed in 100% ethanol for molecular analysis. Genomic DNA was isolated from the pereopods. DNA was extracted with the DNeasy Tissue Extraction Kit (Qiagen, Crawley, West Sussex, United Kingdom) as directed by the manufacturer. Reactions were performed in 10 μl volumes, containing 0.5 μl of each primer (forward and reverse) at a concentration of 10 nmol, 5 μl of Qiagen 10× PCR buffer, 1.5 μl of $MgCl_2$ (25 mM), 1 μl dNTPs (2 nmol, Bioline), 0.25 μl of Taq (5 U/μl) and 1 μl of DNA template (~ 30 ng). Partial 18S rDNA (~580 bps) was amplified using SSUA NSF4 (Hendriks et al., 1989; NSF4 5′-CTGGTTGATYCTGCCAGT-3′) and SSUA NSR581 (Wilmotte et al., 1993 SSUA NSR581 5′ATTACCGCGGCTGCTGGC-3′) under the following conditions: initial denaturation at 96 °C for 0.5 min, followed by 40 cycles of 94 °C for 0.5 min, 55 °C for 0.5 min, 72 °C for 1 min, and a final extension of 5 min at 72 °C. Partial COI mtDNA (~700 bps) was amplified using LCO 1490 and HCO 2198 (Folmer et al., 1994; LCO 1490 5′GGTCAACAA-ATCATAAAGATATTGG-3′ HCO 2198 5′-TAAACTTCAGGGTGAC-CAAAAAATCA-3′) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 5 cycles of 94 °C for 1 min, 45 °C for 1.5 min, 72 °C for 1.5 min, then 30 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final extension of 5 min at 72 °C.

DNA sequencing was performed at LGC Berlin, Germany. All sequences were edited and proofread in CodonCode Aligner Version 3.5.6 (CodonCode Corporation 2006). Sequence quality was evaluated using "Phred" quality scores, excluding sequences with values <300 (Ewing et al., 1998a, 1998b). Electropherograms were also manually examined for sequencing errors and, where possible, variable positions were confirmed by reference to the corresponding reverse sequences. The partial 18S sequences were blast search (blastn) to find the closest matching sequences. The partial COI mtDNA were checked for open-reading frames and blast searched (tblastx) to assess gene homology. The edited partial COI mtDNA (650–685 bps) were aligned with all hippolytid species available in GenBank (Table 2). If numerous COI sequences were available for a species, a maximum of six