



## *Pilbarana*, a new subterranean amphipod genus (Hadzioidea: Eriopisidae) of environmental assessment importance from the Pilbara, Western Australia

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### Abstract

The Pilbara and nearby regions in north-western Western Australia have an exceptionally high diversity of short-range endemic invertebrates inhabiting threatened groundwater-dependent habitats. Amphipod crustaceans, in particular, are dominant in these communities, but are poorly understood taxonomically, with many undescribed species. Recent molecular phylogenetic analyses of Pilbara eriopisid amphipods have, nonetheless, uncovered a previously unknown biodiversity. In this study, we formally establish a new genus, *Pilbarana* Stringer & King **gen. nov.**, and describe two new species, *P. grandis* Stringer & King **sp. nov.** from Cane River Conservation Park and *P. lowryi* Stringer & King **sp. nov.** from the Fortescue River Basin near the Hamersley Range, using a combination of molecular and morphological data. The new genus is similar morphologically to the two additional Western Australian eriopisid genera, *Nedsia* Barnard & Williams, 1995 and *Norcapensis* Bradbury & Williams, 1997, but represents a genetically divergent, reciprocally monophyletic lineage, which can be differentiated by its vermiform body shape, the presence of an antennal sinus, and by the length and form of the antennae and uropods. This research signifies an important contribution to knowledge of Pilbara subterranean communities and has critical implications for future environmental impact assessments and conservation management.

**Key words:** Amphipoda, arid zone, groundwater-dependent ecosystems, stygofauna, taxonomy

### Introduction

The Pilbara region in north-western Western Australia (WA) is widely considered a biodiversity hotspot for a unique and ancient subterranean invertebrate fauna with remarkably diverse assemblages (Eberhard *et al.* 2005; Humphreys 2008; Guzik *et al.* 2011; Halse *et al.* 2014). These subterranean taxa are typically restricted to distinct catchments and associated underground aquifers, arguably occurring in response to the aridification of Australia during the late Miocene and early Pliocene, which forced ancestral epigeal populations into groundwater refuges (Humphreys 2001, 2008; Byrne *et al.* 2008; Finston *et al.* 2009). This isolation, along with limited dispersal capabilities, likely led to the diversification of lineages and high levels of short-range endemism (Harvey 2002; Finston *et al.* 2007, 2009; Perina *et al.* 2019). Recent targeted molecular studies and environmental surveys have been critical in revealing the extent of this diversity (Finston *et al.* 2004, 2007; Perina *et al.* 2018, 2019; Abrams *et al.* 2019; Matthews *et al.* 2020); however, due to a deficiency in taxonomic expertise and the large degree of morphological convergence of characters exhibited by these subterranean taxa (Abrams *et al.* 2012; Perina *et al.* 2018) a substantial proportion of this biodiversity has not been formally described (Guzik *et al.* 2011).

Increased knowledge surrounding Pilbara subterranean biodiversity, the description of new taxa and documentation of their distribution is, nonetheless, critical. The Pilbara is impacted by major mining developments, which potentially represent a significant threat to the aquifer systems and their associated fauna through excavation and groundwater drawdown (Humphreys 2006; Stumpp & Hose 2013; Hose *et al.* 2015). Owing to the importance

of subterranean fauna, their narrow ranges and likely susceptibility to local impacts, this fauna must be considered in environmental impact assessments that involve mining and extraction of groundwater (EPA 2003; Halse *et al.* 2014). Despite the increase in environmental surveys resulting from this process, deficiencies in knowledge and the lack of formal taxonomic treatments has promoted inconsistent species identification, uncertainty in decision making, and has challenged effective conservation management of subterranean taxa (Costello *et al.* 2015). The need for more rapid taxonomic descriptions is, consequently, essential, particularly for subterranean fauna inhabiting threatened groundwater-dependent ecosystems. A planned, collaborative effort is currently underway to help build capacity using taxonomic and molecular resources to establish an enhanced and more rapid framework for Pilbara species identification (King *et al.* 2022; Sacco *et al.* 2022), which will ultimately assist with the environmental assessment process.

Pilbara subterranean aquatic communities principally consist of crustaceans, with many diverse amphipod families, in particular, acting as a significant component of these groundwater habitats (King *et al.* 2022). These amphipods exhibit adaptations associated with their underground existence, such as a reduction or loss of eyes, a lack of pigmentation and enhanced sensory appendages (Humphreys 2006). Four stygobiontic (subterranean, obligate groundwater inhabitants) amphipod families are known from the Pilbara and adjacent North West Cape and Barrow Island regions, including Bogidiellidae Hertzog, 1936, Eriopisidae Lowry & Myers, 2013, Hadziidae S. Karaman, 1943, and Paramelitidae Bousfield, 1977, with 40 described species. The Australian Eriopisidae, specifically, includes the widespread genus *Nedsia* Barnard & Williams, 1995 from the Pilbara, Barrow Island and North West Cape peninsula, the monotypic genus *Norcapensis* Bradbury & Williams, 1997 from North West Cape, and three additional genera from eastern Australia (Lowry & Springthorpe 2005; Yerman 2009; Myers *et al.* 2018). A recent study by King *et al.* (2022) provided a revision of *Nedsia*, revealing exceptional and previously unknown diversity, and described 13 new species distributed across the Pilbara and Barrow Island regions. Species were found to be functionally morphologically cryptic, in a similar way to many stygobiontic taxa, with molecular and distribution data required, in addition to morphology, to delineate clearly monophyletic lineages (King *et al.* 2022). Phylogenetic analyses using multiple genes further uncovered the presence of an apparent additional undescribed eriopisid amphipod genus from the Pilbara, which is examined further in the current study.

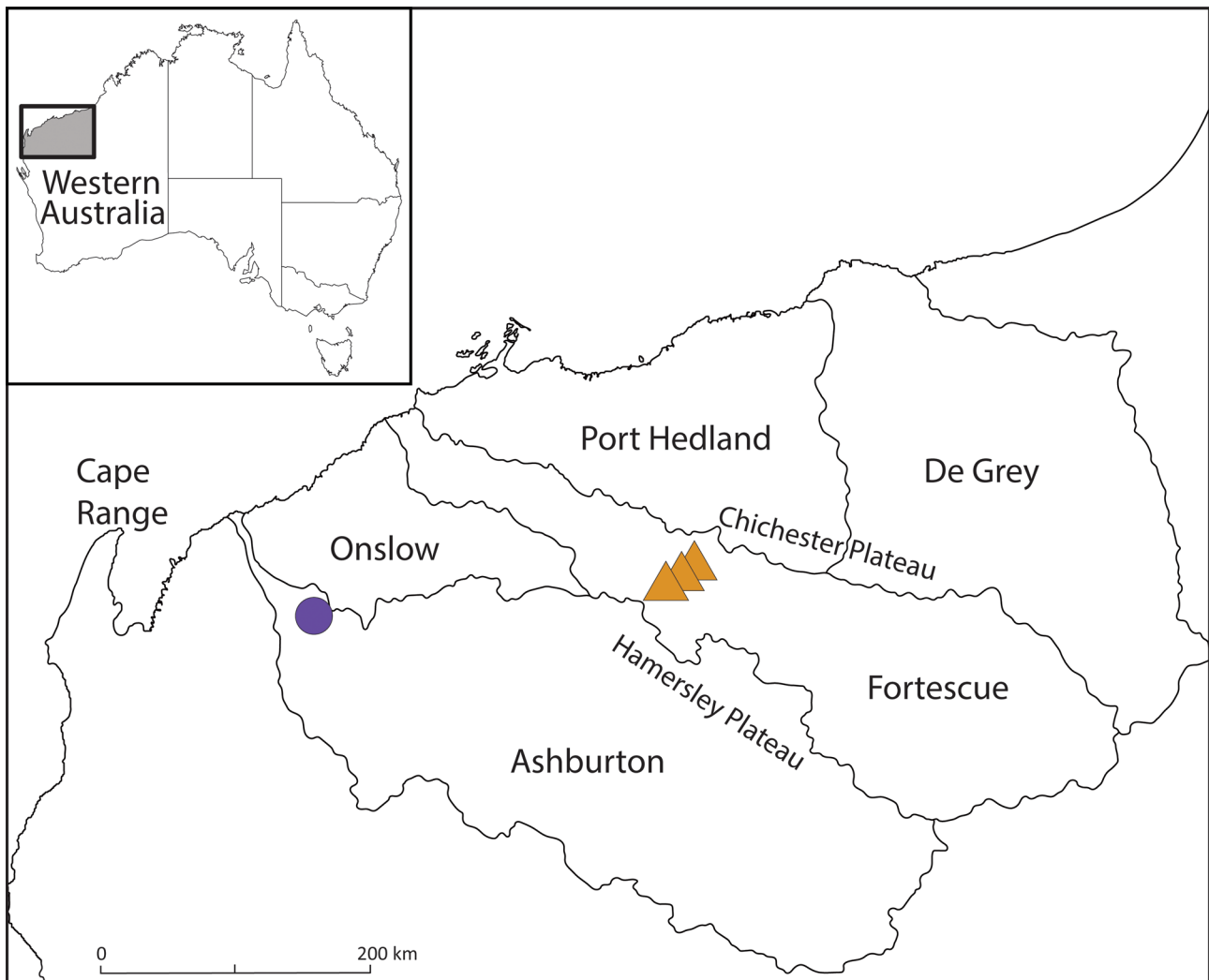
Here, we aim to increase understanding of the Pilbara Eriopisidae by describing this new genus, together with two new species from disparate locations, using an integrated approach. We implemented sequence data generated by King *et al.* (2022), as well as newly produced genetic divergence estimates, morphological analyses, and the General Lineage Species Concept (de Queiroz 1998, 2007) to support and inform species hypotheses. We provide much needed new information concerning Pilbara amphipod biodiversity by increasing the total number of known freshwater eriopisid genera, and further contribute vital taxonomic knowledge required for environmental impact assessments and monitoring of previously undocumented species.

## Material and methods

The Pilbara bioregion is situated in the arid north-west of WA, encompassing an area of approximately 178,000 km<sup>2</sup>, and overlapping with the emergent part of the Pilbara craton (Eberhard *et al.* 2005). This region consists of five major hydrographic basins, including the Ashburton River Basin, De Grey River Basin, Fortescue River Basin, Port Hedland Coast Basin, and Onslow Coast Basin (Fig. 1). Specimens for this study were acquired from the material used in King *et al.* (2022, see collection methods), which were originally obtained through environmental and biological surveys of the Pilbara. *Pilbarana grandis* **sp. nov.** specimens were collected during a 2011 Bush Blitz of the Cane River Conservation Park, spanning the Ashburton River and Onslow Coast Basins (Fig. 1) (<https://bushblitz.org.au>, Commonwealth of Australia 2014). Bush Blitz is an Australian Government, BHP and Earthwatch partnership, with the aim of documenting Australia's plant and animal biodiversity from remote and hard to reach locations. *Pilbarana lowryi* **sp. nov.** specimens were collected by environmental consulting company, Subterranean Ecology Pty Ltd, on behalf of the Fortescue Metals Group Ltd for the Solomon Mining Project in the Fortescue Basin (Fig. 1) (Subterranean Ecology 2011) and were subsequently borrowed from the Western Australian Museum. Specimens from both surveys were preserved in 100% ethanol.

The new genus and species were delineated using an integrated approach involving phylogenetic and divergence analyses of sequence data, together with an analysis of fixed morphological differences and distribution

information, employing the General Lineage Species Concept (de Queiroz 1998, 2007). For molecular analyses, DNA extraction, sequencing of the *cytochrome c oxidase subunit I (COI)* and, for some individuals, 28S rRNA genes, and phylogenetic tree estimation were conducted by King *et al.* (2022). Additional estimation of *COI* divergences with the Kimura 2-parameter (K2P) model (Kimura 1980) in MEGA 11.0.11 (Tamura *et al.* 2021) was conducted here, with pertinent results included in the ‘Remarks’ section for each species. For morphological analyses, type specimens were dissected along the left side, where possible, and appendages were slide-mounted and illustrated with a camera lucida attachment to a Nikon Eclipse 80i (Nikon, Tokyo, Japan) compound microscope. Total body length was measured through the lateral midline from the head to the telson, and head length was measured from the rostrum to the posterior margin from illustrations in lateral view. Type material has been lodged at the Western Australian Museum, with sequence data also available on GenBank. The family and higher systematic classification follows Lowry & Myers (2013), with Eriopisidae separated by: the absence of sternal gills, one or multiple robust basofacial seta(e) on the peduncle of the first uropod, the presence of a biramous third uropod, and the lack of sexually dimorphic second gnathopods.



**FIGURE 1.** Map of the Pilbara region and associated major catchments indicating sampling locations for the new species of *Pilbarana*. Circle (purple): *P. grandis* **sp. nov.**, and Triangle (orange): *P. lowryi* **sp. nov.**

This work represents a contribution to Taxonomy Australia (2021), a national initiative organised under the auspices of the Australian Academy of Science that brings together the taxonomic community to develop approaches that will significantly increase the rate at which new species are discovered, resolved and named, with a view to completely documenting the Australian biota within a generation. Here, we present relatively reduced descriptions for brevity and clarity, with images kept to a minimum, in line with the goals of Taxonomy Australia.

## Taxonomy

### Infraorder Hadziida S. Karaman, 1932 (Lowry & Myers, 2013)

### Superfamily Hadzioidea S. Karaman, 1943 (Bousfield, 1983)

### Family Eriopisidae Lowry & Myers, 2013

#### *Pilbarana* Stringer & King gen. nov.

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Type species: *Pilbarana grandis* sp. nov.

Included species: *Pilbarana grandis* sp. nov. and *Pilbarana lowryi* sp. nov.

**Diagnosis.** Head with weakly concave antennal sinus. Antenna 1 not longer than half body length. Maxilla 1 inner plate with one distal robust seta. Coxae reduced, with coxae 1–4 lengths (depths) distinctly shorter than pereonite lengths; coxae 1–2 anteriorly projected/produced, coxae 3–4 with small to indistinct anterior lobe and associated seta(e), posterior lobe indistinct, coxae 5–7 with anterior lobe gradually less distinct (coxae 5–6 anterior lobe with associated setae), posterior lobe very small. Coxal gills present on coxae 3–6, sternal gills absent. Pereonite 1 with concave posterodistal corner; pereonites 2–7 laterally square-shaped, as broad as long, vermiform body shape. Gnathopod 1 carpus at least 3.5 times as long as broad, longer than propodus; propodus with palm distinctly transverse. Gnathopod 2 propodus approximately 4.5 times length of carpus, palm enlarged, strongly oblique. Pereopods 5–7 basis not distinctly expanded posteriorly, pereopod 7 without lobe on posterodistal corner. Uropod 1 peduncle 2 times length of rami, with one or more robust basofacial seta(e). Uropod 3 strongly extended, distinctly larger than uropod 1; outer ramus cylindrical, larger than inner ramus and apically concave.

**Description.** Head with rostrum weak to obsolete; lateral cephalic lobes moderately to strongly projecting, broad, antennal sinus present, weakly concave; eyes absent. Antenna 1 not longer than half body length; longer than antenna 2; flagellum about 1.2 times length of peduncle; accessory flagellum of two articles, second article tiny. Antenna 2 flagellum shorter than peduncle; calceoli absent. Mandible palp of three articles, terminal article linear or tapered with long apical setae; molar small and triturative. Maxilla 1 inner plate ovate with one distal robust seta; outer plate with denticulate robust setae; palp of two articles. Maxilla 2 inner plate with row of simple and plumose apical setae. Maxilliped inner and outer plates moderately setose, outer plate with smooth edge (not serrated as in *Nedsia*).

Coxae 1–7 short, broader than long with few or no posterior setae; coxae 1–4 lengths (or depths) distinctly shorter than pereonite lengths; coxae 1–2 anteriorly projected/produced, coxae 3–4 with small to indistinct anterior lobe and associated seta(e), posterior lobe indistinct, coxae 5–7 with anterior lobe gradually less distinct (coxae 5–6 anterior lobe with associated setae), posterior lobe very small; coxae 3–6 with simple ovate gills, coxa 6 gill smallest; coxae 2–5 with thin, poorly setose oostegites. Thoracic segments lacking sternal gills. Pereonite 1 with concave or ‘cut out’ posterodistal corner; pereonites 2–4 with distinctly lobed posterodistal corners; pereonites 5–7 slightly lobed at posterodistal corners with associated setae. Gnathopods 1–2 subchelate, not sexually dimorphic. Gnathopod 1 decidedly smaller than gnathopod 2; carpus at least 3.5 times as long as broad, with multiple rows of setae, longer than propodus; propodus with palm distinctly transverse with two robust setae at palm corner. Gnathopod 2 carpus much shorter than propodus, similar length to merus; propodus approximately 4.5 times length of carpus, with palm enlarged, strongly oblique, with robust setae, including one long robust seta, at palm corner and along palm margin. Pereopods 3–4 similar, basis not expanded posteriorly. Pereopods 5–6 basis with slight posterior expansion, remaining longer than broad, small lobe on posterodistal corner. Pereopod 7 longer than pereopods 5–6; basis longer than broad, not expanded posteriorly, no lobe present on posterodistal corner.

Epimera 1–3 with serrated or sculptured posterodistal corners and associated setae. Epimera 2–3 ventral margin with central seta. Uropod 1 peduncle approximately 2 times length of rami, with one or more robust basofacial seta(e). Uropod 2 smaller than uropod 1; outer ramus shorter than inner ramus. Uropod 3 strongly extended, not sexually dimorphic, distinctly larger than uropod 1, parviramous; outer ramus cylindrical with two articles, second article apically concave; inner ramus short and scale-like. Telson longer than broad, deeply cleft into two lobes, with lateral setae and long apical penicillate setae.

**Etymology.** The name *Pilbarana* references the Pilbara region of Western Australia where this genus is found. The gender should be considered as female.

**Remarks.** *Pilbarana* gen. nov. was placed within Eriopisidae by King *et al.* (2022, labelled ‘Eriopisidae gen. undet.’) as a reciprocally monophyletic lineage based on *COI* mtDNA and 28S rRNA molecular data and due to the presence of eriopisid morphological characters: biramous and enlarged third uropod, one or multiple robust basofacial seta(e) on the peduncle of the first uropod, the lack of sternal gills, and the absence of sexually dimorphic second gnathopods (Lowry & Myers 2013). King *et al.* (2022) further indicated that *Pilbarana*, together with *Nedsia* and *Norcapensis*, the two additional WA subterranean eriopisid genera, are genetically divergent from the morphologically similar Australian melitid genera, *Brachina* Barnard & Williams, 1995 and *Nurina* Bradbury & Eberhard, 2000, supporting the current morphological-based classification of Lowry & Myers (2013).

Morphologically, *Pilbarana* is expectedly more similar to *Nedsia* and *Norcapensis* than any of the east coast Australian marine and estuarine eriopisid genera: *Eriopisella* Chevreux, 1920, *Netamelita* J. L. Barnard, 1962, and *Victoriopisa* Karaman & Barnard, 1979. It appears to be closest to *Norcapensis* particularly due to the larger body size compared to *Nedsia*, a mandible palp of three articles, similarly enlarged second gnathopods, and strongly extended, cylindrical third uropods. *Pilbarana*, however, can be easily differentiated since *Norcapensis*: lacks an antennal sinus; comprises an elongate first antenna, reaching past half body length; maxilla 1 inner plate consists of a row of distal plumose setae; possesses a markedly robust rather than vermiform body shape with the first pereonite lacking a distinctly concave or ‘cut out’ posterodistal corner and pereonites 2–7 around 2 times as long as broad; comprises coxal gills on coxae 2–6; first gnathopod with carpus equal in length to propodus; pereopods 5–7 bases progressively more expanded posteriorly; uropod 1 peduncle is approximately equal in length to rami; and uropod 3 outer ramus second article is not apically concave. *Pilbarana* is, additionally, distinct from *Nedsia* as *Nedsia*, like *Norcapensis*, lacks an antennal sinus and possesses elongated first antennae, but further consists of comparably smaller second gnathopods, and third uropods that are leaf-shaped rather than cylindrical.

### ***Pilbarana grandis* Stringer & King sp. nov.**

(Figs. 2–4)

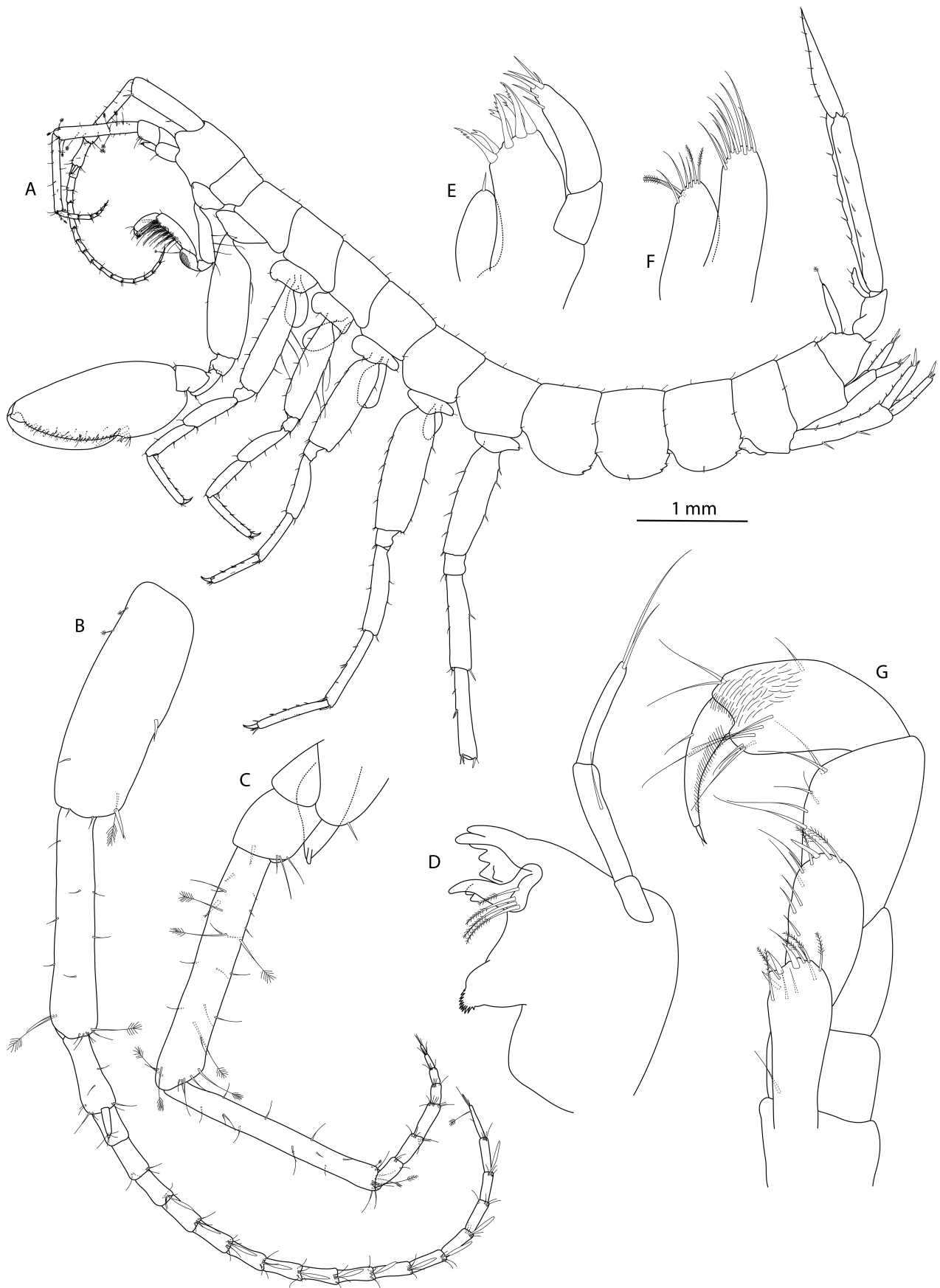
urn:lsid:zoobank.org:act:D7C6FEE5-FF95-49BB-83FB-47875CC57A15

**Material examined.** Holotype: male, WAM C78830 (RL1779), Cork Tree Well, Cane River Conservation Park, WA, 22°21'22.0"S 115°30'35.4"E, coll. R. Leijs and R. A. King, 28 June 2011. Paratypes: 3 males, 1 female, 1 juvenile, WAM C78831 (RL1779; GenBank *COI*: OK170022, OK170023), collection data as for holotype; 1 male, WAM C78832 (RL1750), Cork Tree Well, Cane River Conservation Park, WA, 22°21'22.0"S 115°30'35.4"E, coll. R. Leijs and R. A. King, 24 June 2011.

**Diagnosis.** Head with antennal sinus square-shaped. Antenna 1 peduncular article 1 approximately equal in length to article 2. Antenna 2 peduncular articles 4–5 distinctly longer than length of head; flagellum shorter than peduncular article 5. Gnathopod 1 propodus approximately 2.5 times as long as broad; carpus greater than 4 times as long as broad. Pereopods 3–4 coxae with small anterior lobe. Uropod 1 peduncle with two or more robust basofacial setae. Uropod 2 peduncle similar in length to inner ramus.

**Description.** Holotype male. Length 7.0 mm. Head (Fig. 2A) with antennal sinus square-shaped, anteroventral corner rounded. Antenna 1 (Fig. 2B) peduncular article 1 approximately equal in length to article 2; peduncular article 3 around one third length of article 2; primary flagellum of 16 articles, with one ventral aesthetasc on proximal margin of most articles. Antenna 2 (Fig. 2C) slender, around two thirds length of antenna 1; peduncular article 4 approximately equal in length to peduncular article 5, both articles each longer than length of head; flagellum of 7 articles, shorter than peduncular article 5. Mandible (Fig. 2D) palp article 1 twice as long as broad, around half length of articles 2 and 3, articles 2 and 3 approximately equal in length. Maxilla 1 (Fig. 2E) outer plate with seven denticulate robust setae. All other mouthparts (Figs. 2F, G) as in generic description.

Gnathopod 1 (Fig. 3A) coxa anteriorly projected with one associated seta, posterodistal corner reduced and somewhat concave; propodus approximately 2.5 times as long as broad; carpus greater than 4 times as long as broad. Gnathopod 2 (Figs. 2A, 3B) coxa anteriorly projected with associated seta, propodus approximately 2 times as long as broad. Pereopods 3–4 (Figs. 3C, D) coxae with small anterior lobe and associated setae. Pereopods 5–7 (Figs. 2A, 3E–G) coxae anterior lobe gradually less distinct (coxae 5–6 lobe with associated seta), posterior lobe very small.



**FIGURE 2.** *Pilbarana grandis* sp. nov. holotype male WAM C78830, 7mm. A, whole animal with scale; B, antenna 1; C, antenna 2; D, mandible; E, maxilla 1; F, maxilla 2; G, maxilliped.



**FIGURE 3.** *Pilbarana grandis* **sp. nov.** holotype male WAM C78830, 7 mm. A, gnathopod 1; B, gnathopod 2; C, pereopod 3; D, pereopod 4; E, pereopod 5; F, pereopod 6; G, pereopod 7.

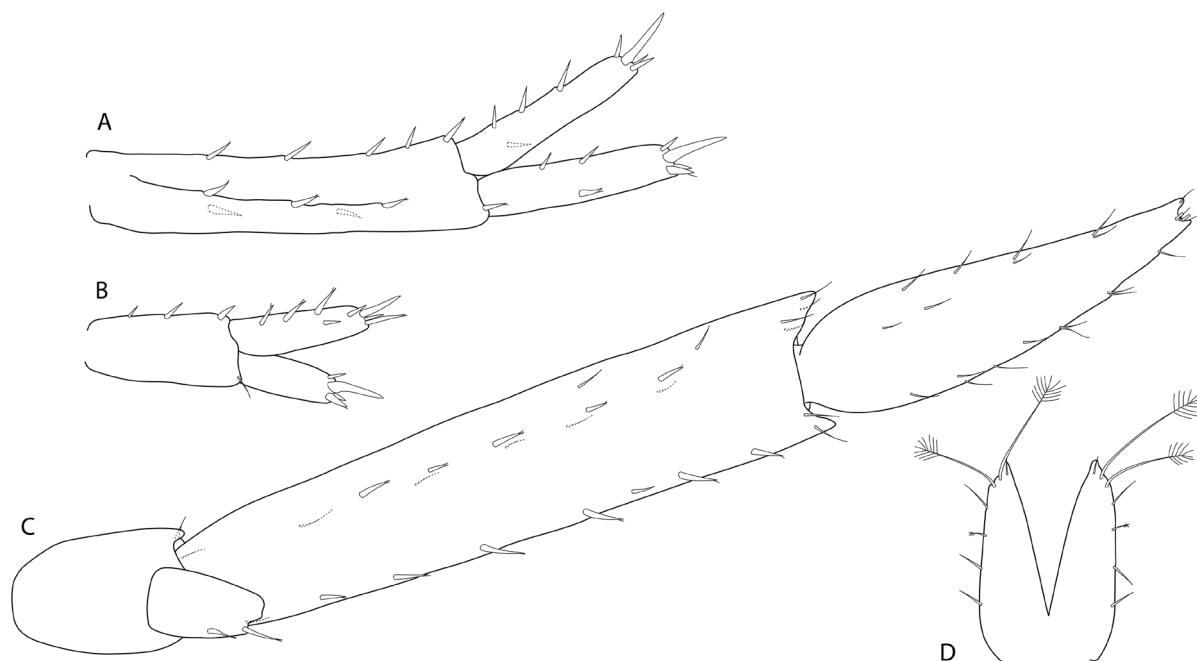
Uropod 1 (Fig. 4A) peduncle with two robust basofacial setae, row of robust setae along dorsal margin. Uropod 2 (Fig. 4B) peduncle similar in length to inner ramus, row of setae along dorsal margin. Uropod 3 (Fig. 4C) outer ramus cylindrical with 2 articles, first article approximately 1.6 times length of second article.

**Distribution.** Cane River Conservation Park, Ashburton River Basin, Pilbara, Western Australia.

**Etymology.** This species name is taken from the Latin word ‘grandis’, and refers to the large body size of this and other species of *Pilbarana*.

**Remarks.** Overall, the two new species of *Pilbarana* can be distinguished morphologically based on the shape of the antennal sinus, the length and elongation of antennal articles, differences in the shape of coxae, the length of the first gnathopod propodus, the number of robust basofacial setae on the peduncle of the first uropod, and the length of the peduncle of the second uropod versus the length of the rami. Examination of specimens of *P. grandis* **sp. nov.** has revealed that individuals may possess between two (most common) and four robust basofacial setae on the peduncle of the first uropod, with the number of basofacial setae further fluctuating, in some cases, between the pair of uropods for a single specimen. Specimens (except for juveniles), nonetheless, never possess only a single basofacial seta, contrasting with specimens of *P. lowryi* **sp. nov.** that always exhibit only one robust basofacial seta on the peduncle of the first uropod. The number of robust basofacial setae on the peduncle of uropod 1 is one of the few morphological characters that separate distinct lineages of *Nedsia* from the Pilbara (King *et al.* 2022) and, consequently, appears to represent a useful and consistent distinguishing eriopisid trait.

Molecular analyses further revealed that the two new species of *Pilbarana* are highly divergent genetically, with approximately 13–14.5% *COI* divergence estimated (with 0.2% between sequences of *P. grandis* individuals). This level of divergence is well above the 5–10% threshold principally followed by King *et al.* (2022) in their description of new *Nedsia* species, and is indicative of long-term isolation in discrete (and distant) subterranean habitats. *Pilbarana grandis* has, thus far, only been sampled from one well in the Cane River Conservation Park in the Pilbara region and is located approximately 250 km from bores in the Fortescue Basin where *P. lowryi* was collected (Fig. 1).



**FIGURE 4.** *Pilbarana grandis* **sp. nov.** holotype male WAM C78830, 7 mm. A, uropod 1; B, uropod 2; C, uropod 3; D, telson.

#### *Pilbarana lowryi* Stringer & King **sp. nov.**

(Figs. 5–7)

urn:lsid:zoobank.org:act:35C8B0FC-98EE-411E-8AD8-C93784B618B0

**Material examined.** Holotype: female, WAM C78833 (10:7951; GenBank *COI*: OK170015, 28S: OK257013), Solomon Mine Project, Valley of the Kings Iron Deposit, 60 km north of Tom Price, WA, 22°09'45.36"S 117°53'39.36"E, coll. P. Bell and E. S. Volschenk, 20 January 2010. Paratypes: 1 female, WAM C78834 (10:0087;



GenBank *COI*:OK170006, 28S: OK257012), Cappers Well, 70 km north of Tom Price, WA, 22°03'44.62"S 117°59'57.77"E, coll. S. Eberhard and S. Catomore, 7 October 2010; 1 male WAM C76988 (10:0082; GenBank *COI*: OP160221), Pigeon Well Bore, 70 km north of Tom Price, WA, 22°01'51.04"S 118°03'32.81"E, coll. S. Eberhard and S. Catomore, 7 October 2010.

**Diagnosis.** Head with antennal sinus concave. Antenna 1 peduncular article 1 slightly longer than article 2. Antenna 2 peduncular articles 4–5 distinctly shorter than length of head; flagellum equal in length to peduncular article 5. Gnathopod 1 propodus approximately 2 times as long as broad; carpus approximately 3.5 times as long as broad. Pereopods 3–4 coxae with indistinct anterior lobe. Uropod 1 peduncle with one robust basofacial seta. Uropod 2 peduncle longer than inner ramus.

**Description.** Holotype female. Length 8.7 mm. Head (Fig. 5A) with antennal sinus concave or rounded, anteroventral corner rounded. Antenna 1 (Fig. 5B) peduncular article 1 slightly longer than article 2; peduncular article 3 around one third length of article 2; flagellum of 18 articles, with one ventral aesthetasc on proximal margin of most articles. Antenna 2 (Fig. 5C) slender, around two thirds length of antenna 1; peduncular article 4 approximately equal in length to peduncular article 5, both articles shorter than length of head; flagellum of 7 articles, equal in length to peduncular article 5. Mandible (Fig. 5D) palp article 1 twice as long as broad, around half length of articles 2 and 3, articles 2 and 3 approximately equal in length. Maxilla 1 (Fig. 5E) outer plate with six denticulate robust setae. All other mouthparts (Figs. 5F, G) as in generic description.

Gnathopod 1 (Fig. 6A) coxa anteriorly projected with one associated seta, posterodistal corner reduced; propodus approximately 2 times as long as broad; carpus approximately 3.5 times as long as broad. Gnathopod 2 (Figs. 6B) coxa anteriorly projected with associated setae; propodus approximately 2 times as long as broad. Pereopods 3–4 (Figs. 6C, D) coxae with indistinct anterior lobe. Pereopod 5 (Fig. 6E) coxa anterior lobe gradually less distinct, with associated seta, posterior lobe very small.

Uropod 1 (Fig. 7A) peduncle with one robust basofacial seta, row of robust setae along dorsal margin. Uropod 2 (Fig. 7B) peduncle longer than inner ramus, row of setae along dorsal margin. Uropod 3 (Fig. 7C) outer ramus cylindrical with 2 articles, first article approximately 1.5 times length of second article.

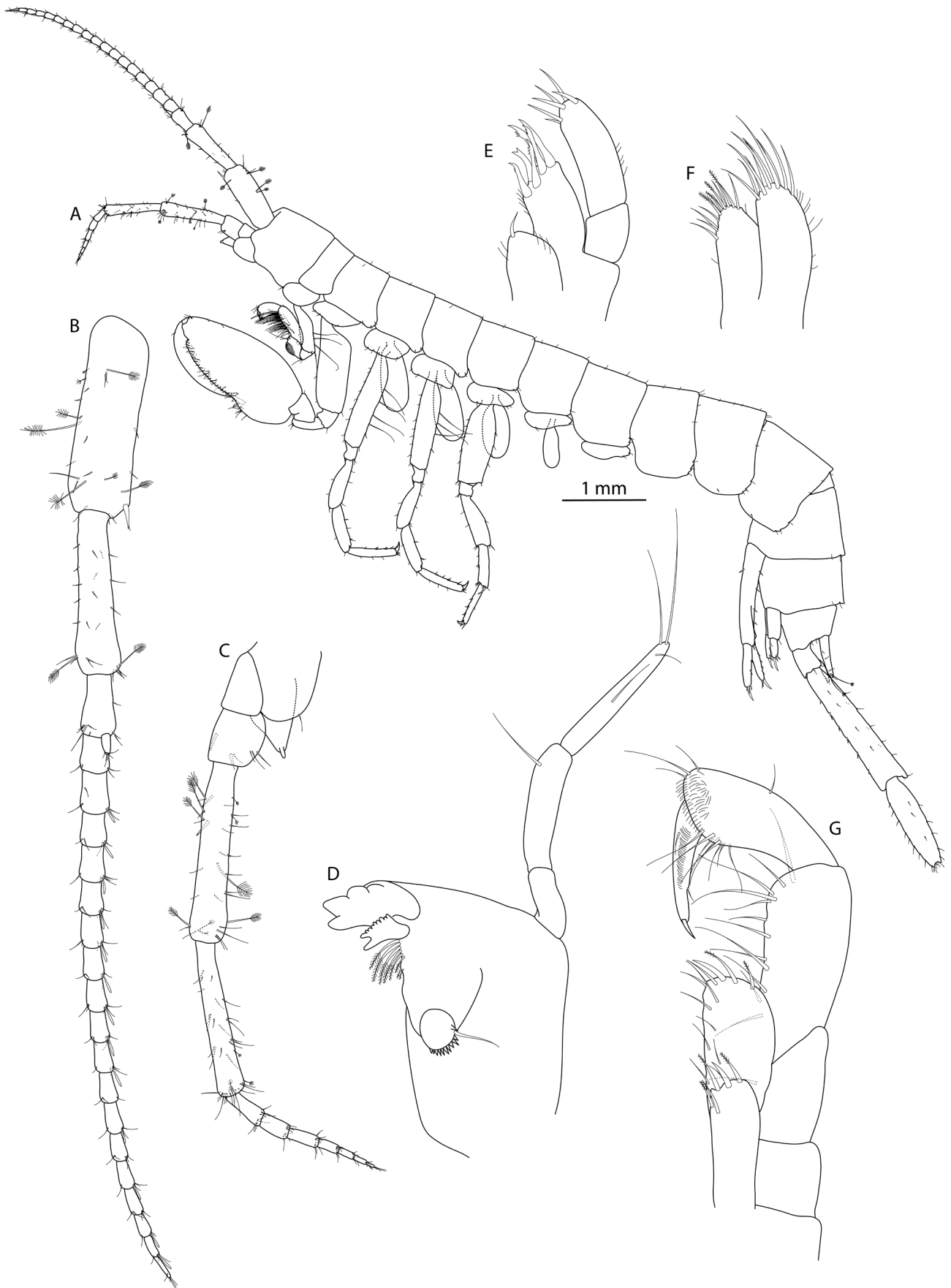
**Distribution.** 60–70 km north of Tom Price, Fortescue River Basin, Pilbara, Western Australia.

**Etymology.** Named in honour of Dr Jim Lowry for his substantial and valuable contribution to Australian amphipod taxonomy.

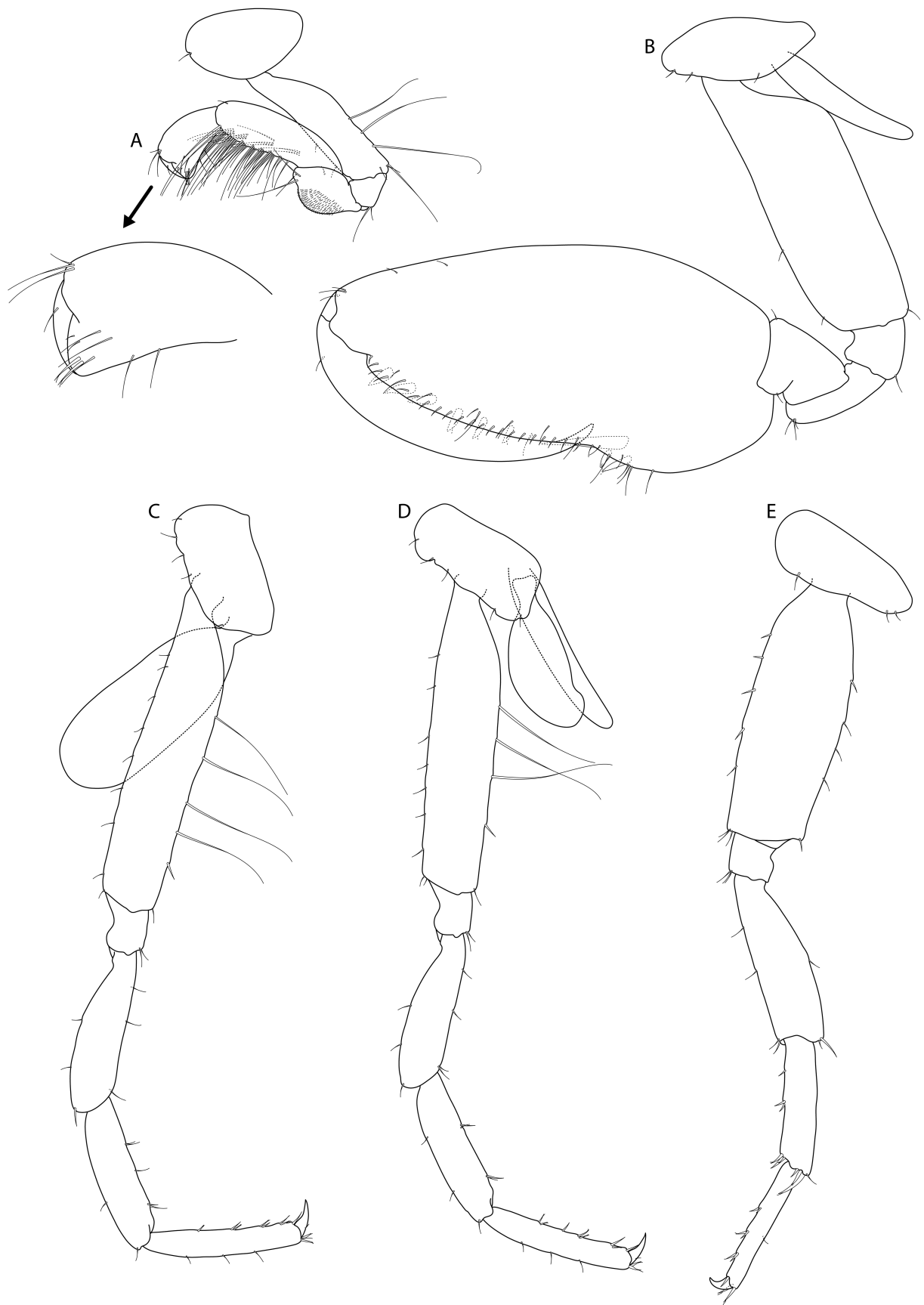
**Remarks.** Specimens of *P. lowryi* **sp. nov.** were sampled from three bores within the Fortescue River Basin near the Hamersley Range in the central area of the Pilbara, approximately 60–70 km north of the town of Tom Price (Fig. 1). The holotype specimen was collected from a deposit within the Solomon Mining Project, while paratypes were sampled from bores outside the impacted region. These specimens were found to be morphologically identical, and molecular analyses revealed relatively low molecular *COI* divergence estimates of between 2.8–3.1%. These divergences suggest some slight phylogeographic structuring of populations, potentially due to distance between collection sites, but are, nonetheless, within the range of amphipod intraspecific variation (Tempestini *et al.* 2018; King *et al.* 2022). In addition, no juveniles were examined and so should be assessed in further collections, and no morphological differences were observed between males and females (as for *P. grandis* **sp. nov.**).

## Discussion

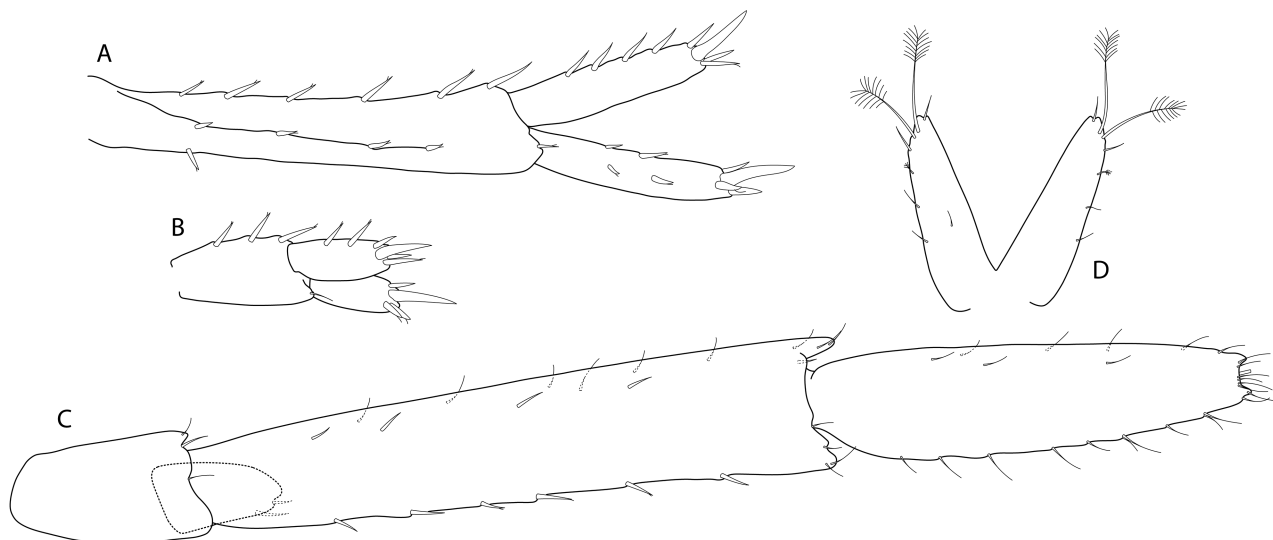
Here, we describe a new genus and two new species, *P. grandis* and *P. lowryi*, of eriopisid amphipods endemic to threatened subterranean habitats in the Pilbara region, WA using multiple lines of evidence. These findings elevate the total number of described eriopisid genera from the region as well as adjacent areas in north-western WA to three (six Australia wide), and the number of formally described eriopisid species from these locations to 19, with 23 known from across Australia. Nevertheless, we predict that a greater diversity of *Pilbarana* species persists within additional aquifers and catchments of the Pilbara region. Further sampling and sequencing work applying multiple genes is required to better understand the distribution of species, particularly since morphological convergence is prevalent in subterranean-adapted fauna (Finston *et al.* 2007; Perina *et al.* 2018; King *et al.* 2022). The species delimited here can only be distinguished using a relatively limited number of characters, with morphological convergence apparent across traits, such as mouthparts and pereopods, which are traditionally used to differentiate species in amphipod taxonomy (King *et al.* 2022). The DNA sequencing data is, therefore, integral to delimiting these species and the wider availability of the molecular data used here provides an important reference for species identification.



**FIGURE 5.** *Pilbarana lowryi* sp. nov. holotype female WAM C78833, 8.7 mm. A, whole animal with scale; B, antenna 1; C, antenna 2; D, mandible; E, maxilla 1; F, maxilla 2; G, maxilliped.



**FIGURE 6.** *Pilbarana lowryi* sp. nov. holotype female WAM C78833, 8.7 mm. A, gnathopod 1; B, gnathopod 2; C, pereopod 3; D, pereopod 4; E, pereopod 5.



**FIGURE 7.** *Pilbarana lowryi* sp. nov. holotype female WAM C78833, 8.7 mm. A, uropod 1; B, uropod 2; C, uropod 3; D, telson.

Formal description of *Pilbarana* and two new species will enable more effective documentation of biodiversity in environmental impact assessments and for conservation management (Costello *et al.* 2015). *Pilbarana* individuals have typically been listed in environmental survey reports as species of Melitidae (rather than Eriopisidae), with significant inconsistencies evident in the species names applied by different consulting companies. Taxonomic studies, such as this one, serve to improve the accuracy of identifications, permitting more informed and appropriate decision-making regarding likely impacts on subterranean fauna and providing increased knowledge surrounding the composition of groundwater communities. These two new species likely have exceedingly small geographical ranges, with *P. lowryi* currently known from three bores near the Hamersley Range and *P. grandis* only collected from a single bore in the Cane River Conservation Park. Their likely limited dispersal ability along with their narrow ranges makes them susceptible to environmental changes and localised impacts. While additional work is required to fully comprehend the distribution of this genus, this study signifies a valuable contribution to the development of a broader taxonomic framework for better identification of Pilbara subterranean fauna and for enhanced protection for the persistence of these species.

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## References

Abrams, K.M., Guzik, M.T., Cooper, S.J.B., Humphreys, W.F., King, R.A., Cho, J.-L. & Austin, A.D. (2012) What lies beneath: molecular phylogenetics and ancestral state reconstruction of the ancient subterranean Australian Parabathynellidae

- (Syncarida, Crustacea). *Molecular Phylogenetics and Evolution*, 64 (1), 130–144.  
<https://doi.org/10.1016/j.ympev.2012.03.010>
- Abrams, K.M., Huey, J.A., Hillyer, M.J., Humphreys, W.F., Didham, R.K. & Harvey, M.S. (2019) Too hot to handle: Cenozoic aridification drives multiple independent incursions of Schizomida (Hubbardiidae) into hypogean environments. *Molecular Phylogenetics and Evolution*, 139, 106532.  
<https://doi.org/10.1016/j.ympev.2019.106532>
- Barnard, J.L. (1962) Benthic marine Amphipoda of southern California: families Tironidae to Gammaridae. *Pacific Naturalist*, 3, 73–115.
- Barnard, J.L. & Williams, W.D. (1995) The taxonomy of Amphipoda (Crustacea) from Australian fresh waters: part 2. *Records of the Australian Museum*, 47, 161–201.  
<https://doi.org/10.3853/j.0067-1975.47.1995.236>
- Bousfield, E.L. (1977) A new look at the systematics of gammaroidean amphipods of the world. *Crustaceana*, Supplement 4, 282–316.
- Bousfield, E.L. (1983) An updated phyletic classification and palaeohistory of the Amphipoda. In: Schram, F.R. (Ed.), *Crustacean Phylogeny*. Museum of Natural History, San Diego, pp. 257–277.
- Bradbury, J.H. & Williams, W.D. (1997) The amphipod (Crustacea) stygofauna of Australia: description of new taxa (Melitidae, Neoniphargidae, Paramelitidae), and a synopsis of known species. *Records of the Australian Museum*, 49, 249–341.  
<https://doi.org/10.3853/j.0067-1975.49.1997.1270>
- Bradbury, J.H. & Eberhard, S. (2000) A new stygobiont melitid amphipod from the Nullarbor Plain. *Records of the Western Australian Museum*, 20, 39–50.
- Byrne, M., Yeates, D.K., Joseph, L., Kearney, M., Bowler, J., Williams, M.A.J., Cooper, S., Donnellan, S.C., Keogh, J.S., Leys, R., Melville, J., Murphy, D.J., Porch, N. & Wyrwoll, K.-H. (2008) Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology*, 17, 4398–4417.  
<https://doi.org/10.1111/j.1365-294X.2008.03899.x>
- Chevreaux, E. (1920) Sur quelques amphipodes nouveaux ou peu connus provenant des côtes de Bretagne. *Bulletin de la Société Zoologique de France*, 45 (1–2), 75–87.
- Commonwealth of Australia (2014) Cane River Conservation Park WA 2011. A Bush Blitz survey report, pp. 68. Available from: <https://bushblitz.org.au/wp-content/uploads/2016/03/bb-CaneRiver-WA-2011.pdf> (accessed 12 June 2022)
- Costello, M.J., Vanhoorne, B. & Appeltans, W. (2015) Conservation of biodiversity through taxonomy, data publication, and collaborative infrastructures. *Conservation Biology*, 29 (4), 1094–1099.  
<https://doi.org/10.1111/cobi.12496>
- de Queiroz, K. (1998) The general lineage concept of species, species criteria, and the process of speciation. In: Howard, D.J. & Berlocher, S.H. (Eds.), *Endless forms: species and speciation*. Oxford University Press, New York, pp. 57–75.
- de Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56, 879–886.  
<https://doi.org/10.1080/10635150701701083>
- Eberhard, S.M., Halse, S.A. & Humphreys, W.F. (2005) Stygofauna in the Pilbara region, north-west Western Australia: a review. *Journal of the Royal Society of Western Australia*, 88, 167–176.
- EPA. (2003) *Guidance for the assessment of environmental factors: consideration of subterranean fauna in groundwater and caves during environmental impact assessment in Western Australia*. Environmental Protection Authority, Perth, 12 pp.
- Finston, T.L. & Johnson, M.S. (2004) Geographic patterns of genetic diversity in subterranean amphipods of the Pilbara, Western Australia. *Marine and Freshwater Research*, 55, 619–628.  
<https://doi.org/10.1071/MF04033>
- Finston, T.L., Johnson, M.S., Humphreys, W.F., Eberhard, S.M. & Halse, S.A. (2007) Cryptic speciation in two widespread subterranean amphipod genera reflects historical drainage patterns in an ancient landscape. *Molecular Ecology*, 16, 355–365.  
<https://doi.org/10.1111/j.1365-294X.2006.03123.x>
- Finston, T.L., Francis, C.J. & Johnson, M.S. (2009) Biogeography of the stygobitic isopod *Pygolabis* (Malacostraca: Tainisopoidae) in the Pilbara, Western Australia: evidence for multiple colonisation of the groundwater. *Molecular Phylogenetics and Evolution*, 52, 448–460.  
<https://doi.org/10.1016/j.ympev.2009.03.006>
- Halse, S.A., Scanlon, M.D., Cocking, J.S., Barron, H.J., Richardson, J.B. & Eberhard, S.M. (2014) Pilbara stygofauna: deep groundwater of an arid landscape contains globally significant radiation of biodiversity. *Records of the Western Australian Museum*, 78, 443–483.  
[https://doi.org/10.18195/issn.0313-122x.78\(2\).2014.443-483](https://doi.org/10.18195/issn.0313-122x.78(2).2014.443-483)
- Harvey, M.S. (2002) Short-range endemism amongst the Australian fauna: some examples from non-marine environments. *Invertebrate Systematics*, 16 (4), 555–570.  
<https://doi.org/10.1071/IS02009>
- Hertzog, L. (1936) Crustacés de biotopes hypogées de la vallée du Rhin d'Alsace. *Bulletin de la Société Zoologique de France*, 61, 356–372.
- Hose, G.C., Asmyhr, M.G., Cooper, S.J.B. & Humphreys, W.F. (2015) Down under down under: Austral groundwater life. In:

- Stow, A., Maclean, N. & Howell, G. (Eds.), *Austral Ark: The State of Wildlife in Australia and New Zealand*. Cambridge University Press, Cambridge, pp. 512–536.  
<https://doi.org/10.1017/CBO9781139519960.026>
- Humphreys, W.F. (2001) Groundwater calcrete aquifers in the Australian arid zone, the context to an unfolding plethora or stygal biodiversity. *Records of the Western Australian Museum*, 64, 63–83.  
<https://doi.org/10.18195/issn.0313-122x.64.2001.063-083>
- Humphreys, W.F. (2006) Aquifers: the ultimate groundwater-dependent ecosystems. *Australian Journal of Botany*, 54 (2), 115–132.  
<https://doi.org/10.1071/BT04151>
- Humphreys, W.F. (2008) Rising from Down Under: developments in subterranean biodiversity in Australia from a groundwater fauna perspective. *Invertebrate Systematics*, 22 (2), 85–101.  
<https://doi.org/10.1071/IS07016>
- Guzik, M.T., Austin, A.D., Cooper, S.J.B., Harvey, M.S., Humphreys, W.F., Bradford, T., Eberhard, S.M., King, R.A., Leys, R., Muirhead, K.A. & Tomlinson, M. (2011) Is the Australian subterranean fauna uniquely diverse? *Invertebrate Systematics*, 24 (5), 407–418.  
<https://doi.org/10.1071/IS10038>
- Karaman, G.S. & Barnard, J.L. (1979) Classificatory revisions in gammaridean Amphipoda (Crustacea), part 1. *Proceedings of the Biological Society of Washington*, 92, 106–165.
- Karaman, S.L. (1932) 5. Beitrag zur Kenntnis der Süßwasser-Amphipoden (Amphipoden unterirdischer Gewässer). *Prorodoslovne Razprave*, 2, 179–232.
- Karaman, S.L. (1943) Die unterirdischen Amphipoden Südserbiens. *Srpska Kraljevska Akademija*, Posebna izdanja, knj. 135, Prirodnjački i matematički spisi, knj. 34, *Ohridski Zbornik*, 4, 159–312.
- Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.  
<https://doi.org/10.1007/BF01731581>
- King, R.A., Fagan-Jeffries, E.P., Bradford, T.M., Stringer, D.N., Finston, T.L., Halse, S.A., Eberhard, S.M., Humphreys, G., Humphreys, B.F., Austin, A.D. & Cooper, S.J.B. (2022) Cryptic diversity down under: defining species in the subterranean amphipod genus *Nedsia* Barnard & Williams, 1995 (Hadzioidea: Eriopisidae) from the Pilbara, Western Australia. *Invertebrate Systematics*, 36 (2), 113–159.  
<https://doi.org/10.1071/IS21041>
- Lowry, J.K. & Springthorpe, R.T. (2005) New and little-known melitid amphipods from Australian waters (Crustacea: Amphipoda: Melitidae). *Records of the Australian Museum*, 57, 237–302.  
<https://doi.org/10.3853/j.0067-1975.57.2005.1463>
- Lowry, J.K. & Myers, A.A. (2013) A phylogeny and classification of the Senticaudata subord. nov. (Crustacea: Amphipoda). *Zootaxa*, 3610 (1), 1–80.  
<https://doi.org/10.11646/zootaxa.3610.1.1>
- Matthews, E.F., Abrams, K.M., Cooper, S.J.B., Huey, J.A., Hillyer, M.J., Humphreys, W.F., Austin, A.D. & Guzik, M.T. (2020) Scratching the surface of subterranean biodiversity: molecular analysis reveals a diverse and previously unknown fauna of Parabathynellidae (Crustacea: Bathynellacea) from the Pilbara, Western Australia. *Molecular Phylogenetics and Evolution*, 142, 106643.  
<https://doi.org/10.1016/j.ympev.2019.106643>
- Myers, A.A., Lowry, J.K. & Barnes, R.S.K. (2018) First record of the genus *Eriopisella* Chevreaux, 1920 (Crustacea, Amphipoda, Senticaudata, Eriopisidae) from Australia, with the description of a new species, *Eriopisella moretoni* sp. nov. *Zootaxa*, 4514 (2), 256–262.  
<https://doi.org/10.11646/zootaxa.4514.2.8>
- Perina, G., Camacho, A.I., Huey, J., Horwitz, P. & Koenders, A. (2018) Understanding subterranean variability: the first genus of Bathynellidae (Bathynellacea, Crustacea) from Western Australia described through a morphological and multigene approach. *Invertebrate Systematics*, 32 (2), 423–447.  
<https://doi.org/10.1071/IS17004>
- Perina, G., Camacho, A.I., Huey, J., Horwitz, P. & Koenders, A. (2019) The role of allopatric speciation and ancient origins of Bathynellidae (Crustacea) in the Pilbara (Western Australia): two new genera from the De Grey River catchment. *Contributions to Zoology*, 88, 452–497.  
<https://doi.org/10.1163/18759866-20191412>
- Sacco, M., Guzik, M.T., van der Heyde, M., Nevill, P., Cooper, S.J.B., Austin, A.D., Coates, P.J., Allentoft, M.E. & White, N.E. (2022) eDNA in subterranean ecosystems: applications, technical aspects, and future prospects. *Science of the Total Environment*, 820.  
<https://doi.org/10.1016/j.scitotenv.2022.153223>
- Stumpp, C. & Hose, G.C. (2013) The impact of water table drawdown and drying on subterranean aquatic fauna in in-vitro experiments. *PLoS ONE*, 8 (11), e78502.  
<https://doi.org/10.1371/journal.pone.0078502>
- Subterranean Ecology (2011) *Solomon Project: Regional Subterranean Fauna Survey (Report No. 2010/20)*. Perth, prepared for

Fortescue Metals Group.

- Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38 (7), 3022–3027.  
<https://doi.org/10.1093/molbev/msab120>
- Taxonomy Australia (2021) *Discovering Our Biodiversity*. Australian Academy of Science, Canberra. Available form: <https://www.taxonomyaustralia.org.au/> (accessed 12 June 2022)
- Tempestini, A., Rysgaard, S. & Dufresne, F. (2018) Species identification and connectivity of marine amphipods in Canada's three oceans. *PLoS ONE*, 13 (5), e0197174.  
<https://doi.org/10.1371/journal.pone.0197174>
- Yerman, M.N. (2009) Melitidae, the *Eriopisella* group. In: Lowry, J.K. & Myers, A.A. (Eds.), Benthic Amphipoda (Crustacea: Peracarida) of the Great Barrier Reef, Australia. *Zootaxa*, 2260, 713–717  
<https://doi.org/10.11646/zootaxa.2260.1.36>