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Understanding subterranean variability: the first genus of Bathynellidae (Bathynellacea, Crustacea) from Western Australia described through a morphological and multigene approach

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ABSTRACT

The number of subterranean taxa discovered in the north of Western Australia has substantially increased due to the requirements for environmental surveys related to mining development.

Challenges in estimating subterranean biodiversity are related to lack knowledge of subterranean taxa in a largely unobservable environmental setting, and convergent morphological characters. An integrated approach is warranted to understand such complexity.

Bathynellidae occur in most of Australian aquifers, but only one species has been described so far, and the group lacks a reliable taxonomic framework, which makes biodiversity and distribution assessment difficult. A new genus and one new species from the Pilbara, *Pilbaranella ethelensis* gen. et sp. nov., is described from an integrated approach using both morphological and molecular data. Three additional species of *Pilbaranella* are defined through mitochondrial and nuclear genes, using Automatic Barcode Gap Discovery (ABGD) and Poisson Tree Processes (PTP) species delimitation methods. A comparison of morphology and 18S ribosomal RNA sequences between *Pilbaranella* gen. nov. and known lineages provides the evidentiary basis for the decision to establish a new genus. This study provides a morphological and molecular framework to work with Bathynellidae, especially in Australia where a highly diverse fauna remains still undescribed.

Key words: ABGD - mitochondrial DNA - morphology - new species - nuclear DNA - Pilbara - *Pilbaranella* gen. nov. - PTP - species delimitation - stygofauna

INTRODUCTION

Despite the substantial increase in the number of subterranean taxa named in the past few decades and occurring in the arid zones of Western Australia (WA), it is estimated that 90% of species still await formal description (Guzik et al., 2010). Dytiscids, ostracods and copepods are the groups with the largest number of species described for stygofauna (organisms living in groundwater), while arachnids predominate in the troglofauna literature (Guzik et al., 2010) (animals living in the fissures and voids of rocks and sediments below the epigean environment (Barr & Holsinger, 1985; Culver & Sket, 2000; Giachino & Vailati, 2010). These results reflect the taxonomic work carried out by a few specialists on selected groups of fauna, but other taxa that could be equally diverse have not yet been studied (Guzik et al., 2010).

The surge in discovery of underground groups has largely been due to environmental surveys conducted on behalf of mining companies (Eberhard, Halse, & Humphreys, 2005; Guzik et al., 2010) under regulatory requirements in Western Australia for subterranean fauna to be considered, along with all other flora and fauna, in Environmental Impact Assessments (EIAs).

Estimating subterranean biodiversity can be challenging. Delimiting species and estimating their distributions are often difficult tasks given incomplete sampling and limited taxonomic knowledge, complicated by morphological convergence that occurs in the underground environment (Finston, Johnson, Humphreys, Eberhard, & Halse, 2007; Lefébure, Douady, Malard, & Gibert, 2007), lack of expertise for particular taxa, and the impossibility of observing and studying the whole subterranean habitat. Molecular tools have often been adopted to overcome morphological difficulties and support discrimination at both species and generic levels. For example, in WA, the mitochondrial COI gene has been used to reveal cryptic species of amphipods, (Finston et al., 2007), support new lineages and distribution of stygobitic isopods (Finston, Francis, & Johnson, 2009), and, together with the 12S mtDNA, define genetic diversity of subterranean oligochaetes (L. Brown, Finston, Humphreys, Eberhard, & Pinder, 2015). COI barcoding combined with two-dimensional geometric morphometrics has also

been used to analyse a subterranean species complex of harpacticoid copepods, finding congruence between the two data sets (T. Karanovic, Djurakic, & Eberhard, 2016). Morphometric analysis associated with a multilocus DNA-based species delimitation has also been employed to reveal the biodiversity of amphipods occurring in desert springs in South Australia (Nicholas P. Murphy, King, & Delean, 2015). An integrated approach, employing multiple loci, morphology, environmental and other data available where possible, is needed to have a comprehensive knowledge and a better understanding of complex population/species structures in such a unique environment (Camacho, Dorda, & Rey, 2011; N. P. Murphy, Adams, & Austin, 2009; N. P. Murphy, Guzik, & Worthington Wilmer, 2010; Sukumaran & Gopalakrishnan, 2015).

One of the neglected groups is the family Bathynellidae Grobben, 1905, which consists of small (about 1 mm) interstitial and subterranean crustaceans that inhabiting groundwater. Bathynellidae, along with other stygofauna taxa, have the important role in maintaining water quality through purification and nutrient cycling (Boulton, Fenwick, Hancock, & Harvey, 2008). Together with Parabathynellidae Noodt, 1965, they form the order Bathynellacea Chappuis, 1915, which has a worldwide distribution, excluding Antarctica. Bathynellidae family is represented thus far by 28 genera and 103 species (Camacho, 2015; Camacho et al., 2016), but the taxonomy of this group is poorly resolved, and, for some areas, such as Australia, almost completely unknown. The species-genera delineation is enigmatic, especially for the genus *Bathynella* Vejdovsky, 1882, which has created disagreements among different authors (Delachaux, 1920; Jakobi, 1954; Noodt, 1965; Schminke, 1973; Serban, 1970). The confusion is in part due to the poor description of the first species discovered in Prague, *Bathynella natans* Vejdovsky, 1882 (type genus and species of Bathynellidae), based on one specimen only mounted on a slide which has deteriorated over time (Serban, 1966a), and also in part due to convergent evolution resulting in a very conservative morphology with few characters available to distinguish species and genera (Camacho, Dorda, & Rey, 2013; Schminke, 1981).

Between 1950 and 1980, many new species from different countries were described as belonging to the genus *Bathynella* (Birstein & Ljovuschkin, 1964;

Jankowskaya, 1964; Morimoto, 1959; Noodt, 1971; Serban, 1971; Ueno, 1952; Ueno & Morimoto, 1956), but without a clear morphological framework to support the taxa, since specific and generic characters were not well defined and morphological convergence has misled taxonomists. The only species described from the Australian continent was included in this genus too: *Bathynella primaustraliaensis* Schminke, 1973. Serban (Serban, 1966a, 1966b, 1970, 1973, 2000) attempted to organize the group, emphasizing the importance of the male thoracopod VIII, which has simplified ambulatory structures (endo-exopod) and more complex protopodite that forms the penis region. Together with Coineau and Delamare Deboutteville, Serban revisited the species and distribution of the genus *Bathynella* in Europe, described new genera in detail and created three subfamilies (Austrobathynellinae Delamare Deboutteville & Serban, 1973, Bathynellinae Serban, Coineau & Delamare Deboutteville, 1971, Gallobathynellinae Serban, Coineau & Delamare Deboutteville, 1971) based mainly on the male and female thoracopos VIII, the structure of the mandibles, and the geographic occurrence (Delamare Deboutteville & Serban, 1973; Serban, 1973, 1977, 1989, 1992, 1993, 2000; Serban et al., 1971; Serban, Coineau, & Delamare Deboutteville, 1972).

The brief description of *Bathynella primaustraliensis* is based on one female only and the lack of the male thoracopod VIII prevents us from making a meaningful comparison with these above taxa, and other Australian material, including the work described herein.

Morphological convergence results in few useful taxonomic characters, and when they are found they appear extremely subtle (Camacho, 2015; Cho, 2005). Subtle morphological changes, combined with features such as a delicate exoskeleton and the size of individuals, make a morphological study and species identification for the Bathynellidae difficult and inherently prone to underestimating the diversity present in the group. Therefore we integrated molecular data to support species delineation. Sequences of mitochondrial DNA (mtDNA) cytochrome c oxidase I (COI) and 16S, and 28S and 18S ribosomal RNA (rRNA) were sought for 98 specimens. COI is commonly used for studies at species level (Hebert, Ratnasingham, & deWaard, 2003; Lefébure, Douady, Gouy, & Gibert, 2006),

while the nuclear 18S gene (from the small subunit of the 80S eukaryotic ribosome) is useful for older divergences (Boyko, Moss, Williams, & Shields, 2013). The mitochondrial 16S locus and the nuclear 28S fragment were attempted to be sequenced, and we expected those genes to have more information at species/genus level. Sequencing and alignment success of these markers represent a novelty for studies of the Bathynellidae family and we hypothesize that they will provide the additional data to support the species delimitation and the phylogeny of this group. In this study we used the morphological species concept integrated with statistical species delimitation using DNA sequence data.

The aims of this paper are to describe and characterize the first bathynellid genus and species from WA (*Pilbaranella ethelensis* gen. et sp. nov.) through integrative taxonomy, exploring the biodiversity of this group at the aquifer scale, and creating a molecular and morphological framework to understand Bathynellidae variability in Australia.

MATERIAL AND METHODS

Study area

The Pilbara bioregion is situated in the northwest of WA, between 20° and 23°30' latitude and 115° and 121°30' longitude (McKenzie, van Leeuwen, & Pinder, 2009) and covers a total area of 178,231 km² (Department of the Environment, 2013). The climate is subtropical dry with two well-defined seasons: hot summers and mild winters. The bioregion is characterised by average annual rainfall between 230 and 350 mm, but like most of Arid Australia, tropical cyclones and flooding events reflect intense rainfall events that are unpredictable interannually (Johnson & Wright, 2001).

The Pilbara bioregion IBRA7 (Interim Biogeographic Regionalisation for Australia, latest version) corresponds approximately with the Pilbara Craton, formed in the Archean about 3.80-3.53 billion years ago (Ga) (Hickman & Van Kranendonk, 2012), and it comprises five principal catchment basins. The Fortescue River crosses the whole region and flows towards the Indian Ocean, but its upper section drains internally (Barnett & Commander, 1986).

The Ethel Gorge aquifer system is situated in the southeast of the Pilbara region, upstream in the internally drainig part of the Fortescue River Basin, near the town of Newman (Figure 1). Individual aquifers are between 1 to 8 km wide, for a total area of 200 km^2 and a 300 GL aquifer reserve (Middlemis, 2006). The four main surface tributaries, Homestead creek, Whaleback creek, Warrawanda creek, and Shovelanna creek, and the Fortescue River flow north through the Ophthalmia Range, north of Ophthalmia Dam, creating Ethel Gorge, which is approximately 400 m wide (Johnson & Wright, 2001). In this area aquifers are present in the calcrete, alluvium and basement rocks, and groundwater salinity ranges between 540 and 2700 mg/L (Johnson & Wright, 2001).

Mining operations in the Gorge commenced in 1992, and in 1998 BHP Billiton Iron Ore Pty Ltd proposed to mine Orebody 23 (see Figure 3) below the water table. Stygofauna studies conducted in the area revealed a rich community (Eberhard & Humphreys, 1999) which influenced the provision to assess the conservation significance. As per Ministerial Condition (Minister for the Environment; Employment and Training, 1998), the proponent put in place a management plan to regulate impacts on subterranean fauna, aquifers and surrounding vegetation. The BHP Iron Ore Environmental Management Plan included: sampling, identification, and mapping the stygofauna species present in the groundwater, and their distribution and conservation significance (Environmental Protection Authority, 1998). In 2001, the Ethel Gorge stygofauna community was listed as an Endangered Threatened Ecological Community (TEC) by the Western Australian Minister for the Environment, and over the last decade annual monitoring of the stygofauna has revealed about 80 stygofauna species present in, or within the proximity of, this aquifer system (Halse et al., 2014; Tang & Eberhard, 2016) comprising oligochaetes, amphipods, copepods, ostracods, isopods and syncarids (L. Brown et al., 2015; Finston, Bradbury, Johnson, & Knott, 2004; Hong & Cho, 2009; I. Karanovic, 2007; T. Karanovic, 2006; Wilson, 2003). The bathynellids recovered from this monitoring programme represent the material used for this study. Specifically, the material used in this study comes from stygofaunal surveys conducted between 2009 and

2015 by different environmental consultancy companies (Bennelongia, Subterranean Ecology, MWH Global (now part of Stantec)).

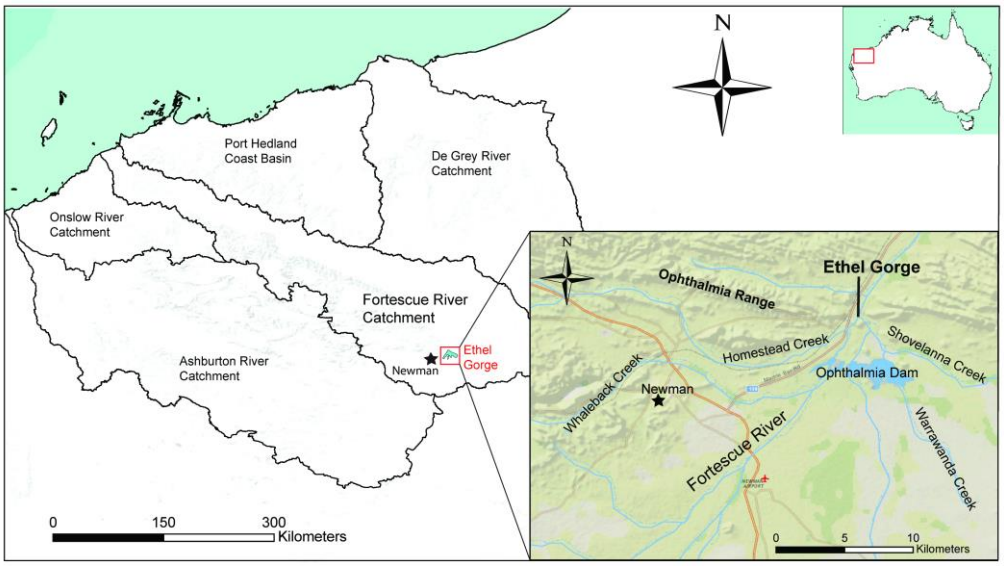

Figure 1 The Pilbara bioregion with the five major catchments. Zoomed in: the Ethel Gorge study area near the town of Newman and the main creeks that flows towards the gorge.

Groundwater Sampling Methods

Pre-established bore holes were sampled, following EPA guidelines (Environmental Protection Authority, 2003, 2007, 2013), using plankton nets of different diameters, with mesh pore size between 50-150 µm. The net was lowered to the bottom of the bore, pulled up and down several times, for a short distance, to dislodge the sediment and the invertebrates at the base of the hole, and hauled at least six times through the water column (Environmental Protection Authority, 2007). The samples were fixed and preserved in 100% ethanol and, in some cases, refrigerated (Subterranean Ecology and MWH Global environmental consultants, personal communication, 2014).

DNA Extraction, Amplification, and Sequencing

Specimens used for genomic DNA extractions were stored in 100% ethanol. The animals were placed in a drop of propylene glycol (this liquid allows the dissection of the animal without compromising the DNA (Moreau, Wray, Czekanski-Moir, & Rubin, 2013)) in a concave slide and a few body segments of the abdomen bearing no useful morphological characters were dissected under a stereo microscope using tungsten needles. Some specimens were labelled

individually with a registration number, measured through an eye piece micrometer and photographed with an Olympus E30 digital SLR camera attached to an Olympus BX50/BX43 compound microscope. Each tissue dissected for DNA extraction was placed directly into a 1.5ml Eppendorf containing 180µl of Qiagen ATL buffer solution (tissue lysis buffer). Subsequently 20 µl of proteinase K was added to each vial, then incubated overnight at 56°C. After incubation, nucleic acids were extracted from the digestion buffer using Qiagen DNeasy kits, following the manufacturer's specifications (Alda, Rey, & Doadrio, 2007). Markers and primers used in this study are summarised in table 1. The universal primers 16SarL and 16SbrH (Palumbi et al., 1991) were utilised first to amplify the 16S gene with very low success (only two sequences from the study area were obtained), so new primers were designed with much higher success (16SBathy-21F and 16SBathy- 453R). Similarly, the first fragment of the 18S was amplified initially using the universal primers 1F and 5R (Giribet, Carranza, Baguna', Riutort, & Ribera, 1996) obtaining only 3 sequences, while the new designed primers (18SiBathy-30F and 18SiBathy- 634R) achieved better results (see table 1).

Table 1 List of markers and primers with relative sequences utilised.

Marker	Primer	Sequence	Reference/Designed by	No. of specimens amplified
COI	C1-J-1718F	5-GGAGGATTTGGAAATTGATTAGTTCC-3	(Simon et al., 1994)	75
	C1-J-2329R	5-ACTGTAAATATATGATGAGCTCA-3		
	LCO1490F	5-GGTCAACAAATCATAAAGATATTGG-3	(Folmer, Black, Hoeh, Lutz, & Vryenhoek, 1994)	1
	HCO2198R	5-TAAACTTCAGGGTGACCAAAAAATCA-3		
16S	16SBathy-21F	5-ARTAHAATCTGCCCGGTGAT-3	G. Perina	77
	16SBathy-453R	5-TCCAACATCGAGGTCGHAAAC-3		
	16SarL F	5-CGCCTGTTTAACAAAAACAT-3	(Palumbi et al., 1991)	2
	16SbrHR	5-CCGGTCTGAACTCAGATCACGT-3		
18Si	1F	5-TACCTGGTTGATCCTGCCAGTAG-3	(Giribet et al., 1996)	3
	5R	5-CTTGGCAAATGCTTTCGC-3		
	18SiBathy-30F	5-GGCGAAACCGCGAATGGCTC-3	G. Perina	72
	18SiBathy-634R	5-GCTGCGGTTAAAAAGCTCGTAG-3		
18Sii	3F	5-GTTCGATTCCGGAGAGGGA-3	(Giribet et al., 1996)	
	18Sbi	5-GAGTCTCGTTCGTTATCGGA-3	(Whiting, Carpenter, & Wheeler, 1997)	
18Siii	18Sa2.0	5-ATGGTTGCAAAGCTGAAAC-3	(Whiting et al., 1997)	
	9R	5-GATCCTTCCGCAGGTTCACCTAC-3	(Giribet et al., 1996)	
28S	28S-D1F	5-GGGACTACCCCCTGAATTTAAGCAT-3	(Park & Foighil, 2000)	
	28Sb	5-TCGGAAGGAACCAGCTACTA-3	(Nunn, Theisen, Christensen, & Arctander, 1996)	

Two microlitres of the DNA template were used in a 25-µL PCR reaction containing: 1x MyTaq Reaction Buffer (1 mM dNTPs, 3 mM $MgCl_2$, stabilisers and enhancers), 0.2 µM of each primer (in some cases 0.3 µM), and 1 unit of MyTaq DNA Polymerase. Thermal cycling was performed in a Biorad T100 Thermal Cycler using different conditions. For COI: enzyme activation at 95°C for 3 minutes, follow by 7 cycles of denaturation at 95°C for 30 seconds, annealing at 40°C (30 sec) and extension at 72°C (45 sec); follow by 34 cycles of denaturation at 95°C (30 sec), annealing at 49°C (30 sec) and extension at 72°C (45 sec). The final extension step was carried out at 72°C for 10 minutes. For 16S and 28S: enzyme activation at 95°C for 3 minutes, follow by 34 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C (30 sec) and extension at 72°C (45 sec); the final extension step was carried out at 72°C for 10 min. Samples showing weak bands were amplified again, using the same conditions, but increasing the number of cycles to 38/40. For the 18S-fragment 1: enzyme activation at 95°C for 3 minutes, follow by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 49/51°C (30 sec) and extension at 72°C (45 sec); the final extension step was carried out at 72°C for 10 min. For the 18S-fragments 2-3: enzyme activation at 95°C for 3 minutes, follow by 34 cycles of denaturation at 95°C for 30 seconds, annealing at 49°C (30 sec) and extension at 72°C (45 sec); the final extension step was carried out at 72°C for 10 min. Samples showing weak bands were amplified again, using the same conditions, but increasing the number of cycles to 38/40.

Five microliters of PCR products were run through Invitrogen E-gel and visualized under ultraviolet light. Successfully amplified PCR products were sent to the Australian Genome Research Facility (AGRF) in Perth for Sanger sequencing (forward and reverse). The workflow was managed through the LIMS (Laboratory Information Management Software) Biocode plug-in (http://www.mooreabiocode.org). The raw chromatograms were imported into Geneious 8.1.4 software (Kearse et al., 2012). Forward and reverse reads were assembled, checked by eye and edited. The consensus sequences were extracted, blasted against GenBank and aligned using the MAFFT algorithm (Multiple

Alignment using Fast Fourier Transform) (Katoh, Misawa, Kuma, & Miyata, 2002) with default parameters.

DNA analysis and species delimitation methods

COI fragments were translated into amino acid chains to ensure no stop codons were present, while the online server GBlocks 0.91b (Castresana, 2000) was used to eliminate poorly aligned positions of the 16S, 18S and 28S alignments using the less stringent options.

Phylogenetic reconstruction of COI, 16S, 18S and 28S were conducted using Bayesian and Maximum Likelihood (ML) methods. Single-gene trees and combined datasets for COI, 16S and 28S were constructed using the sequences from one specimen collected in the De Grey River catchment as outgroup. We chose this particular outgroup as it represents a distinct taxon, but close to the lineages considered, and it has been successfully sequenced for all the markers tested. The 18S phylogeny was constructed using: representatives of the Ethel Gorge material, one specimen from the De Grey River catchment, sequences of genera and unidentified lineages of Bathynellidae downloaded from GenBank, and *Iberobathynella imuniensis* Camacho, 1987 and *I. celiana* Camacho, 2003 (Parabathynellidae) as outgroups.

jModelltest 2.1.9 (Posada, 2008) was implemented to select the best models of nucleotide substitution using the Akaike information criterion. RaxML_HPC_BlackBox (Randomised Axelerated Maximum Likelihood) in CIPRES online server (Miller, Pfeiffer, & Schwartz, 2010) and MrBayes 3.2.5 (Ronquist et al., 2012) were used respectively for the maximum likelihood and the Bayesian analysis. RaxML analyses were conducted using RaxML_HPC_BlackBox default values, including the recommended automatic bootstrapping stop, which determines the number of replicates sufficient to get stable support value using the MRE-based bootstrapping criterion (Pattengale, Alipour, Bininda-Emonds, Moret, & Stamatakis, 2009). For the concatenated tree, the data were partitioned by gene, using the GTR model for all partitions. In the Bayesian analyses the data were partitioned by gene and the following models

were used: GTR+G for COI, 18S and 28S, and GTR+I for 16S. Each partition had unlinked models. The program Tracer 1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) was used to assess the convergence of the Bayesian analysis, making sure that the Effective Sample Size was above 200. Between 200 000 and 600 000 Markov Chain Monte Carlo generations were run for the single-gene trees, and 1 300 000 generations were run for the concatenated tree. A burn-in fraction of 0.25 was chosen and the consensus tree was built from the remaining trees.

Automatic Barcode Gap Discovery (ABGD) (Puillandre, Lambert, Brouillet, & Achaz, 2012) and Poisson Tree Processes (PTP) (Zhang, Kapli, Pavlidis, & Stamatakis, 2013)species-delimitation methods were implemented, using the online websites available (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.htmL and http://sco.h-its.org/exelixis/web/software/PTP/), to assess putative species boundaries using, respectively, alignments and phylogenetic trees constructed for COI, 16S and 28S. Kimura 80 (K80) and Jukes–Cantor (JC69) distance, and default values were used in the ABGD analysis, while rooted trees and the exclusion of distant outgroups to improve the delimitation results were applied to the PTP method, leaving the other parameters unchanged.

Morphological study

Partially or completely dissected specimens were mounted on permanent slides following the methods outlined in Perina and Camacho (2016). Morphology was examined using an oil immersion object (100X) on a standard Zeiss microscope with phase contrast, and an Olympus BX50 or BX43 interference microscope. Drawings were done using a drawing tube, digitalized using a WACOM tablet and retouched using drawing software. The material is vouchered at the Western Australian Museum.

We used the terminology proposed by Serban (1972). The morphological and molecular descriptions are based on the type series (Appendix 1).

Abbreviations used in text and figures after Camacho (1986): Th, thoracopod; A.I, antennule; A.II, antenna; Md, mandible, Mx.I, maxillule and Mx.II maxilla.

RESULTS

Molecular results

Ninety-five specimens from Ethel Gorge and one from the De Grey River catchment were sequenced and included in the phylogeny. The total number of specimens tested and successfully amplified are summarised in table 2 for each marker. Seventy-five sequences (of about 610 bp) and one (of 658 bp) were obtained for COI. All COI fragments were translated and revealed no stop codons. Seventy-nine sequences of about 390 bp for the 16S mitochondrial fragment, and 56 sequences of about 1050 bp of the 28S rRNA were obtained. Six representative sequences, of about 1700 bp, were used in the 18S phylogeny. Sequences are deposited in GenBank (see Appendix 3 for accession numbers). Alignments of the rRNA genes produced gaps, therefore GBlocks online server was used to eliminate poorly aligned positions, returning new blocks of 385 bp, 1042 bp, and 1353 bp for 16S, 28S and 18S respectively. All alignments, except for 18S, include the sequences of the specimen from the De Grey River as an outgroup.

Table 2 Number of tested and successfully amplified specimens per marker, and percentage success

Marker	No. of specimens tested	No. of successful specimens	%
COI	108	76	70
16S	83	79	95
28S	79	56	71
18Si	77	75	97
18Sii	74	67	91
18Siii	53	47	89

Molecular phylogeny

Single COI, 16S and 28S gene trees (see supplementary material) produced congruent clades, showing the five lineages represented in Figure 2, with slightly different topologies (trees not shown). The consensus concatenated tree for the three markers is shown in Figure 2. Maximum-likelihood and Bayesian analysis provided the same topology defined by five major lineages representing the

possible five congeneric species occurring in the Ethel Gorge aquifer system (*Pilbaranella ethelensis* gen. et sp. nov., *Pilbaranella* spp. A, B., C, and possibly *Pilbaranella* sp. D), all except one of which occur in multiple bores (Figure 3). Posterior probability (PP) and bootstrap (BS) values strongly support each lineage (PP = 1; 84 < BS < 100), while deeper nodes defining the relationships among the species have very low support. Forty-six specimens of *P. ethelensis* have been used for DNA extraction and are part of the type series. The extractions were obtained from: seven whole specimens, 11 half specimens, and 28 selected body parts (few body segments where no morphological characters are present and/or a piece of the animal's upper part of pereionites and pleonites). For *Pilbaranella* spp. A, B and C, 30, 12 and five individuals have been used respectively in the molecular study. Two sequences, from the only two specimens available, represent a possible fifth species, which is left as uncertain due to limited data. For comparison with other studies conducted on stygofauna, COI within and between species mean distances (computed through Molecular Evolutionary Genetics Analysis (MEGA) 7.0 for bigger datasets (Kumar, Stecher, & Tamura, 2016) using default values and 1000 bootstraps replications) are shown in Table 3. P-distances between all sequences are provided as a supplementary Excel file. COI distances among species of *Pilbaranella* range between 11.9 and 15.9%, while divergences among the De Grey River taxon and *Pilbaranella* species are over 20% (20.5–22.9%). Divergences within lineages range between 0.2 and 3.6%.

The more conservative 18S rRNA region was used to compare *Pilbaranella* with other sequences retrieved from GenBank. The 18S RaxML and MrBayes trees have highly congruent topologies, forming two well supported monophyletic clades (Figure 4). One is represented by the Australian Bathynellidae and the other clade includes the European genera and the lineage from Texas. The three European genera (*Vejdovskybathynella* Serban & Leclerc, 1984; *Paradoxiclamousella* Camacho, Dorda & Rey, 2013; *Gallobathynella* Serban, Coineau & Delamare Deboutteville, 1971) were originally defined based on morphology. The 18S phylogeny forms different monophyletic lineages corresponding to those genera corroborating the morphological analysis. Within

the Australian clade, the new genus *Pilbaranella* appears monophyletic and quite distinctive with respect to the undescribed taxon from the De Grey River (which represents a new genus currently under description by the authors), supporting the morphology and therefore the placement of *Pilbaranella* at the generic level. 18S sequences from the Ethel Gorge material, lineages retrieved from GenBank, including Australian lineages from Queensland and South Australia, were aligned separately, and ML and Bayesian trees were constructed.

The small fragments (~700 bp) of the Australian lineages publicly available are not included in the phylogeny since the topology of the tree, if included, is not congruent with the outcome of a more complete analysis performed with additional Australian groups, which are currently under study by the authors and will be published later. However, the taxa from Queensland and South Australia retrieved from GenBank formed a distant and distinctive clade in the 18S analysis compared with the *Pilbaranella*/De Grey River clade, so they most likely represent distinct species/genera.

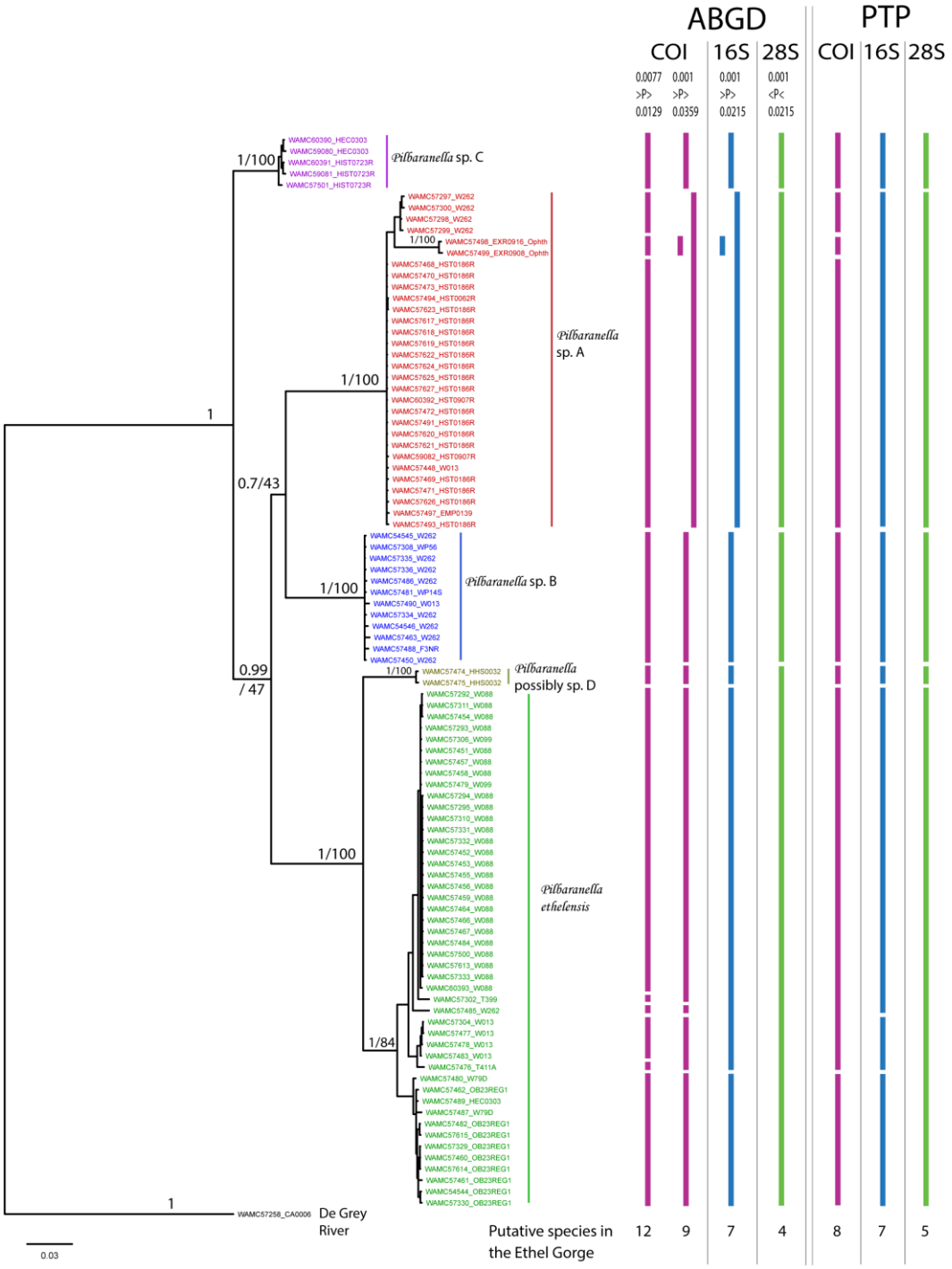

Figure 2 Bayesian consensus tree constructed using concatenated data for COI, 16S and 28S and model partitioning, implemented in MrBayes. Numbers on the branches represent Bayesian posterior probabilities followed by maximum likelihood bootstrap percentages. ABGD and PTP results are reported next to the tree. ABGD method: major partitions are shown; PTP: partitions with the highest support for each group are represented.

Species delimitation

The COI–16S–28S Bayesian concatenated tree together with the species-delimitation results are represented in Figure2. The tree identifies at least five lineages corresponding to the five putative species occurring in the area: *Pilbaranella ethelensis*, gen. et sp. nov., *Pilbaranella* spp. A, B and C, and possibly *Pilbaranella* sp. D. All present subtle morphological differences to distinguish them, except possibly *Pilbaranella* sp. D, which is represented by two juveniles only with no useful morphological characters available. Considering that we have sequences from only two specimens, which come from the same bore hole, and therefore we do not have additional geographic/distributional data or environmental/ecological information, we prefer to treat this lineage as a 'possible sp. D', awaiting more evidence to confirm its status. The ABGD method applied to the COI alignment found two major barcoding gaps in the Ethel Gorge community and partitioned the data into 12 and 10 putative species with prior intraspecific divergence (P) ranging between 0.0077 and 0.0129, and 0.001 and 0.0359 respectively. Using the 16S alignment, the method divided the data into seven groups with $0.001 < P < 0.0215$, while only four assemblages were delineated though the 28S alignment with $0.001 < P < 0.0215$. K80 and JC69 distances produced the same results for each marker.

The PTP method found different ranges of estimated numbers of species for different markers, but it returned specifically eight, seven and five putative species for COI, 16S and 28S respectively as the partitions with the highest support for each group. All methods and markers identified lineages B and C. *Pilbaranella* sp. A is defined by the ABGD analyses of 28S and PTP of 16S and 28S, but the other markers/methods split this species into two or three groups corresponding to the geographical area where the haplotypes were sampled (the Gorge, Homestead Creek, Western Ophthalmia Range). *P. ethelensis* is detected by PTP and ABGD of the 28S, but the latter includes also the possible sp. D. 16S ABGD and COI PTP split *P. ethelensis* in two lineages, 16S PTP in three, while the ABGD of COI creates four and six groups: almost a different species per bore hole. *Pilbaranella* possible sp. D is identified by all methods, except for the ABGD of the 28S.

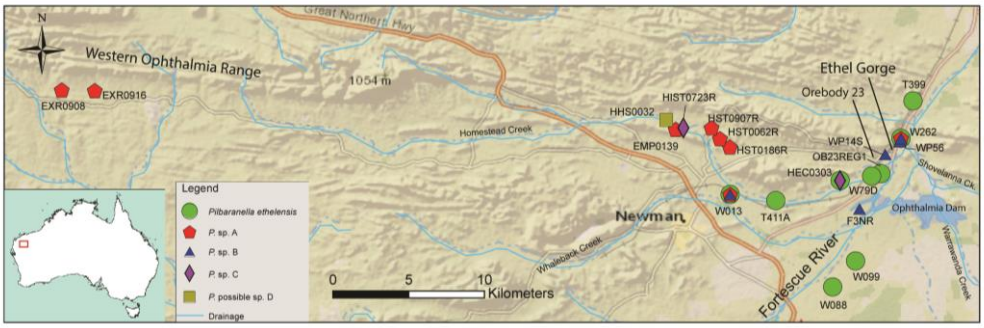

Figure 3 Bathynellid species distribution in the Ethel Gorge and surrounding area, and in the western Ophthalmia Range

We adopted a conservative approach to the number of species recognised here for several reasons. Given the high substitution rate and rate of fixation of mitochondrial genes (W. M. Brown, George, & Wilson, 1979; Oliveira, Raychoudhury, Lavrov, & Werren, 2008); and the limited dispersal abilities of the Bathynellacea (Humphreys, 2008; Schminke, 1974), we expect that COI and 16S variability represents, in this case, population structure more than species delimitation. Therefore slower nuclear markers are likely to reflect more accurate species boundaries. In addition, once other lines of evidence are taken into account, namely morphological differences, the connectivity of the aquifers, with the species-delimitation results of both methods applied on the 28S, we accept four species (possibly five) in Ethel Gorge, which are well identified by the lineages of the concatenated tree (Figure 2).

Table 3 Estimates of evolutionary divergence over sequence pairs between *Pilbaranella* species

	Pilbaranella ethelensis	*Pilbaranella* poss. sp. D	*Pilbaranella* sp. A	*Pilbaranella* sp. B	*Pilbaranella* sp. C	Bathynellidae 'De Grey'
Pilbaranella ethelensis	**0.036 (s.e. = 0.005)**	0.013	0.015	0.015	0.015	0.018
Pilbaranella poss. sp. D	0.119	**0.002 (s.e. = 0.002)**	0.016	0.015	0.016	0.018
Pilbaranella sp. A	0.151	0.137	**0.014 (s.e. = 0.002)**	0.015	0.016	0.019
Pilbaranella sp. B	0.146	0.135	0.142	**0.005 (s.e. = 0.002)**	0.016	0.019
Pilbaranella sp. C	0.145	0.159	0.146	0.154	**0.005 (s.e. = 0.003)**	0.019
Bathynellidae 'De Grey'	0.208	0.205	0.229	0.216	0.215	-

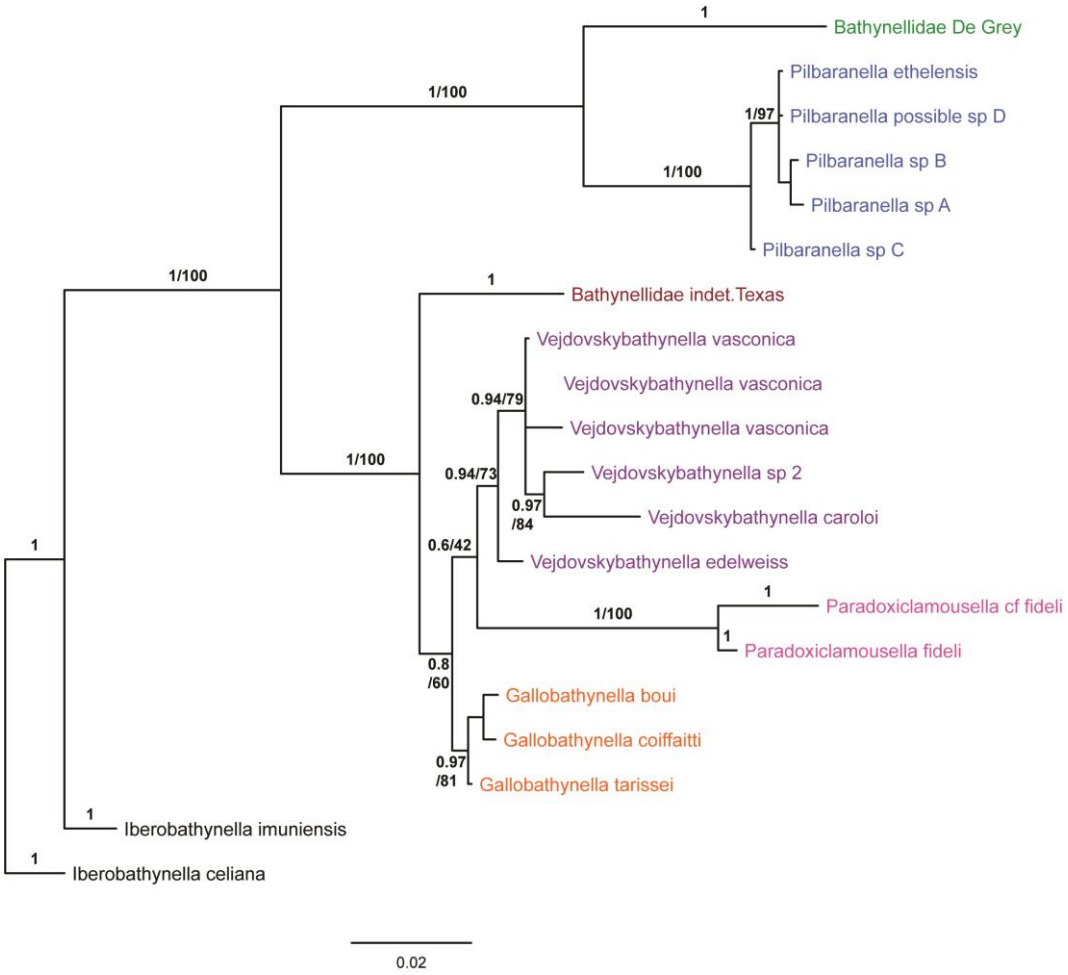

Figure 4 Bayesian consensus tree constructed using 18S data implemented in MrBayes. Representatives of Parabathynellidae (*Iberobathynella celiana, I. imuniensis*) have been chosen as outgroups. *Pilbaranella*, gen. nov. sequences and data from GenBank have been use

Systematic account

Family Bathynellidae Grobben, 1905

The Family Bathynellidae currently consists of three subfamilies: Bathynellinae Grobben, 1905, Gallobathynellinae Serban, Coineau & Delamare Deboutteville, 1971 and Austrobathynellinae Delamare Deboutteville & Serban, 1973.

The new genus shares a few characters with the Austrobathynellinae subfamily, and even fewer with the Bathynellinae and Gallobathynellinae (Table 4). Together with the genera *Austrobathynella* Delamare Deboutteville, 1960, *Transvaalthynella* Serban & Coineau, 1975 and *Transkeithynella* Serban & Coineau, 1975, which are classified as Austrobathynellinae, the new genus

Pilbaranella presents: mandible with many teeth; female thoracopod VIII reduced to one ramus; male thoracopod VIII with reduced endopod and exopod; and very small third segment of the endopod of the antennule. The paucity of data (only three genera are described entirely on the basis of morphological information) prevents us from confirming the affinity of the new genus *Pilbaranella* to Austrobathynellinae, nevertheless it appears more different from the other two subfamilies and therefore we exclude them. However more work is needed to characterize the subfamilies (morphologically and molecularly) and place the taxa discovered around the world in the correct group.

***Pilbaranella* gen. nov. Perina & Camacho**

Genus diagnosis

Antennula seven-segmented. Antenna seven-segmented, third endopodial segment very small. Paragnaths with distal claws. Labrum without sexual dimorphisms. Setae of mandibular palp similar in both sexes. Endopod of thoracopods I–VII four-segmented. Thoracopod VIII of male small and globular with only one lobe (outer lobe) on penial region (latero-external part), and the basipod in vertical position without crests; endopod small and exopod big, almost cylindrical and curved. Female thoracopod VIII reduced to: very long epipod, coxopod without setae and fused with basipod, and one small one-segmented ramus (could be the endopod or the exopod). Uropod: sympod with four large spines and endopod with two spines, one ‘special setae’ (morphology between seta and spine) and three more setae. Furcal rami: five spines.

Table 4 Differences and similarities among *Pilbaranella*, gen. nov. and the three bathynellid subfamilies

	Gallobathynellinae	Bathynellinae	Austrobathynellinae	*Pilbaranella*
Antennule:				
No. of segments	6 or 7	7	7	7
Antenna:				
No. of segments	6–8	7	7 or 8	7
Endopod segment 3	Short/long	Long	Very short	Very short
Exopod: medial seta	Present/absent	Present/absent	Absent	Absent
Mandible:				
Palp	1–3 segments	3 segments	3 segments	3 segments
Pars molaris (no. of teeth)	2 or 3	2 or 4	5 or 6	6
Sexual dimorphism	Yes/no	No	No	No
Thoracopods I–VII:				
Endopod	3 or 4 segments	4 segments	4 segments	4 segments
Thoracopod VIII, female:				
Structure	Protopod + 1 or 2 rami	Protopod + 2 rami	Protopod + 1 ramus	Protopod + 1 ramus
Rami (endopod–exopod)	1 segment	1 segment	2 segments	1 segment
Coxal seta	Present/absent	Present	Absent	Absent
Epipod	Present/absent	Present	Absent	Present
Thoracopod VIII, male:				
Penial region	1–3 lobes, frontal projection, 0–2 crests	3 lobes, frontal projection	1–4 lobes, 0–1 crest	1 lobe
Basipod	Vertical/inclined; not fused with the penial region/independent	Vertical; not fused with the penial region/independent	Vertical; partially or totally fused with the penial region	Vertical; fused with the penial region
Endopod	Small or absent	Small, 1 segment	1 or 2 segments	Small, 1 segment
Exopod	Like exopod of thoracopods	Like exopod of thoracopods	Reduced/curved backwards	Reduced/curved backwards
Uropod:				
Sympod	4 spines (maximum)	4 spines (minimum)	4 spines (maximum)	4 spines
Endopod	2–4 claws + 4 setae	3 or 4 claws + 3 or 4 setae	2 claws + 3 setae + 1 special seta	2 claws + 3 setae + 1 special seta

Type species: *Pilbaranella ethelensis* sp. nov.

***Pilbaranella ethelensis* sp. nov. Perina & Camacho**

(Figures. 5-7 Appendix 1)

Type locality. Bore W088, Ethel Gorge aquifer system (see Appendix 2 for borehole coordinates), Fortescue River, Pilbara, Western Australia.

Material examined

Holotype. WAM C57440, male, permanent slide, bore W088, 12.iv.2012.

Allotype. WAM C57438, female, permanent slide, bore W088, 12.iv.2012.

Paratypes. WAM C54544, 1 male, permanent slide, bore OB23REG1, 9.ii.2011; WAM C54547, 3 males, 2 females, fixed in 100% ethanol, bore W088, 12.iv.2012; WAM C57292, 1 male, permanent slide, bore W088, 18.iii.2014; WAM C57294, 1 female, permanent slide, bore W088, 18.iii.2014; WAM C57295, 1 male, permanent slide, bore W088, 18.iii.2014; WAM C57269, 3 males, 3 females, fixed in 100% ethanol, bore W088, 18.iii.2014; WAM C57302, 1 female, permanent slide, bore T399, 18.iii.2014; WAM C57303, 1 male, permanent slide, bore T399, 18.iii.2014; WAM C57304, 1 male, permanent slide, bore W013, 14.xii.2013; WAM C57305, 1 male, permanent slide, bore W013, 14.xii.2013; WAM C57306, 1 male, permanent slide, bore W099, 12.iv.2012; WAM C57307, 1 female, permanent slide, bore W099, 12.iv.2012; WAM C57311, 1 female, permanent slide, bore W088, 15.xii.2013; WAM C57312, 2 males, fixed in 100% ethanol, bore W088, 15.xii.2013; WAM C5329, 1 male, permanent slide, bore OB23REG1, 9.ii.2011; WAM C5330, 1 male, permanent slide, bore OB23REG1, 9.ii.2011; WAM C57331, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57427, 1 female, permanent slide, bore W262, 15.iii.2014; WAM C57428, 1 female, permanent slide, bore W262, 15.iii.2014; WAM C57436, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57437, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57439, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57441, 1 female,

permanent slide, bore W088, 12.iv.2012; WAM C57442, 1 male, permanent slide, bore W088, 12.iv.2012; WAM C57443, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57444, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57451, 1 male, permanent slide, bore W088, 18.iii.2014; WAM C57452, 1 male, permanent slide, bore W088, 18.iii.2014; WAM C57453, 1 female, permanent slide, bore W088, 18.iii.2014; WAM C57454, 1 female, permanent slide, bore W088, 18.iii.2014; WAM C57455, 1 male, permanent slide, bore W088, 18.iii.2014; WAM C57456, 1 male, permanent slide, bore W088, 18.iii.2014; WAM C57458, 1 male, permanent slide, bore W088, 15.xii.2013; WAM C57459, 1 male, permanent slide, bore W088, 15.xii.2013; WAM C57460, 1 female, permanent slide, bore OB23REG1, 9.ii.2011; WAM C57461, 1 male, permanent slide, bore OB23REG1, 9.ii.2011; WAM C57462, 1 male, permanent slide, bore OB23REG1, 9.ii.2011; WAM C57464, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57465, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57466, 1 female, permanent slide, bore W088, 12.iv.2012; WAM C57467, 1 male, permanent slide, bore W088, 12.iv.2012; WAM C57476, 1 male, permanent slide, bore T411A, 13.iv.2012; WAM C57478, 1 female, permanent slide, bore W013, 8.ii.2012; WAM C57479, 1 male, permanent slide, bore W099, 9.ii.2012; WAM C57480, 1 female, permanent slide, bore W79D, 12.iv.2012; WAM C57482, 1 female, permanent slide, bore OB23REG1, 8.ii.2012; WAM C57483, 1 male, permanent slide, bore W013, 12.iv.2012; WAM C57484, 1 male, permanent slide, bore W088, 9.ii.2012; WAM C57485, sex not identifiable, permanent slide, bore W262, 8.ii.2012; WAM C57487, sex not identifiable, permanent slide, bore W79D, 8.ii.2012; WAM C57489, 1 female, permanent slide, bore HEC0303, 14.xii.2013; WAM C57500, 1 female, permanent slide, bore W088, 19.iii.2015; WAM C57612, 1 female, fixed in 100% ethanol, bore W088, 12.iv.2012; WAM C57613, 1 male, permanent slide, bore W088, 15.xii.2013; WAM C59083, 1 male and 1 female, fixed in 100% ethanol, bore W088, 19.iii.2015; WAM C60393, sex not identifiable, fixed in 100% ethanol, bore W088, 19.iii.2015; eight whole specimens were used for DNA extraction: WAM C57293, 1 female, bore W088, 18.iii.2014; WAM C57310, 1 juvenile, bore W088, 15.xii.2013; WAM C57332, 1 female, bore W088,

12.iv.2012; WAM C57333, 1 female, bore W088, 12.iv.2012; WAM C57457, 1 male, bore W088, 15.xii.2013; WAM C57477, 1 female, bore W013, 8.ii.2012; WAM C57614, sex not identifiable, bore OB23REG1, 9.ii.2011; WAM C57615, sex not identifiable, bore OB23REG1, 9.ii.2011.

Description (based on adults/subadults)

Body. Total length: holotype 0.93 mm; allotype 1.12 mm. Body length 0.64-0.99 mm (males), and 0.5-1.12 mm (females). Body not conspicuously elongated; almost cylindrical, approximately six times as long as wide; segments slightly widening posteriorly. Head as long as wide. Pleotelson with one long barbed dorsal seta on both sides. All drawings represent the holotype and allotype except for: the labrum in Figure 5K and the masticatory part of the mandible in Figure

Antennule (Figure 5A). Seven-segmented; length of first three segments slightly longer than other four segments; first segment is longest; second to fifth segments similar in length; sixth and seventh segments slightly longer than the previous four; inner flagellum trapezoidal, almost square; setation as in Figure 5A; one aesthetascs on the sixth segment and two on the seventh segment.

Antenna (Figure 5B). Seven-segmented; much shorter than A.I (2/3 of the length); first four segments almost as long as sixth and seventh; fifth (third of endopod) very small, without setae; terminal segment, the longest, slightly longer than the sixth; setal formula = 0/1+exp/2+0/1+0/0/2+1/5; exopod as long as fourth segment, with two terminal setae, one of which is a bifurcated sensory seta; ventromedial seta absent.

Labrum (Figure 5C-K). Almost trapezoidal, with smooth free edge and a median cleft.

Paragnath (Figure 5D-F). Almost rectangular, distal part with a very long and strong claw and thick setation.

Mandible (Figure 5E-G-H-L). Palp with three segments, terminal segment with two long and strong barbed claws (Figure 5E-G), more or less cylindrical without expansions. Masticatory part (Figure 5G-H-L): incisor process with three teeth and *pars molaris* with six smaller teeth.

Maxillule (Figure 5I). Proximal endite with four setae; distal endite with six teeth (four with denticles and two setae-like), three plumose setae, and a tuft of long setules on outer margin.

Maxilla (Figure 5J). Four segments; setal formula = 7, 5, 8, 5.

Thoracopods I-VII (Figure 6A-G). Epipod present on Th I–VII. Th I coxa with long and strong plumose seta; basipod with two smooth setae. Exopod of Th I–VII one-segmented, with five barbed setae: two terminal, one dorsal and two ventral (Th I with four setae), shorter than endopod, and bearing tuft of setules on ventral margin (on dorsal margin too on Th I); exopod of Th III–VII similar in size to first three segments of endopod. Endopod four-segmented in all thoracopods, setal formulae (number of setae on basipod in parentheses at start):
Th I: (2) 4+0/2+1/2+0/3
Th II: (1) 2+0/2+1/1+0/3
Th III-IV: (1) 2+0/1+1/0+0/3
Th V: (1) 1+0/1+1/0+0/3
Th VI-VII: (1) 0+0/0+1/0+0/2(1)

Male thoracopod VIII (Figure 7A-B). Small and globular with only one reduced lobe (outer lobe) on penial region (latero-external part); basipod, merged in the penial region, with vertical position and bilobed distal end; endopod small with one seta; exopod big, almost cylindrical and curved, with simplified morphology and two distal setae.

First pleopod (Figure 7D-E). Two segments, first with one very long seta; second with six setae (two distal with different length, and two subdistal on each side).

Female allotype Th VIII (Figure 7C). Coxa without setae and fused with basipod; basipod rectangular; one-segmented ramus very small, partially fused with the basipod, and bearing two setae (one long, one short); very large epipod, 2.5 times longer than basipod.

Female allotype Th I to VII. Number of segments of endopods and exopods like in male holotype. Number of setae on segments of endopod and basipod differs from that of male holotype. Setal formulae of allotype (number of setae of basipod in parentheses at the start):
Th I: (2) 3+0/2+1/2+0/3
Th II: (1) 2+0/2+1/1+0/3
Th III: (1) 2+0/1+1/0+0/3
Th IV: (1) 2+0/2+1/0+0/3
Th V: (1) 1+0/1+1/0+0/2
Th VI- VII: (1) 0+0/0+1/0+0/2(1)

Uropods (Figure 7E). Sympod 1.4 times longer than endopod, rectangular, 2 times longer than wide, with four long equal distal spines, almost as long as the endopod; endopod twice as long as exopod, with two strong claws ("uropodial claws" *sensu* Delamare Deboutteville and Serban (1973), the most distal one almost twice as long as the proximal one). Endopod with one "special seta-claw" (ornamentation with characters between seta and claw: thinner than a claw but with same setation pattern), one terminal seta very long, one shorter subterminal ventrally located setae, and one ventral plumose seta near base; exopod with four setae, two terminal and two medial. Endopod with spinous projection on distal outer corner.

Pleotelson (Figure 7G). With one long, barbed dorsal seta on either side near base of furca.

Furcal rami (Figure 7F-H). Small, almost square, bearing five spines; dorsal spine slightly longer than the fourth one. Second and third spines similar in length and about 1.5 times longer than the dorsal one. First spine twice length of second and third ones. Spines length similar in the allotype (Figure 7H)

Variability

Observed variability affects the number of setae (which can be more or less numerous) on different segments of endopod and/or exopod of thoracopods I to VII on males and females, left or right side. Some exopods of thoracopos I to VII seem to have a “duplication” of the setae that can involve one or both sides, so it is possible to find specimens with 6 or 7 setae on exopods. Also the number of setae on the second segment of the pleopod can vary between 5 and 7. Most of the time, fewer setae on exopods and pleopod is associated with sub-adult specimens.

Etymology

The genus name, *Pilbaranella*, comes from the name of the region where the material was collected: Pilbara, which derives from the Aboriginal word *bilybara*, meaning 'dry' in the Nyamal and Banyjima languages (Sharp & Thieberger, 1992). The specific epithet, *ethelensis*, derives from the name of the Ethel Gorge aquifer.

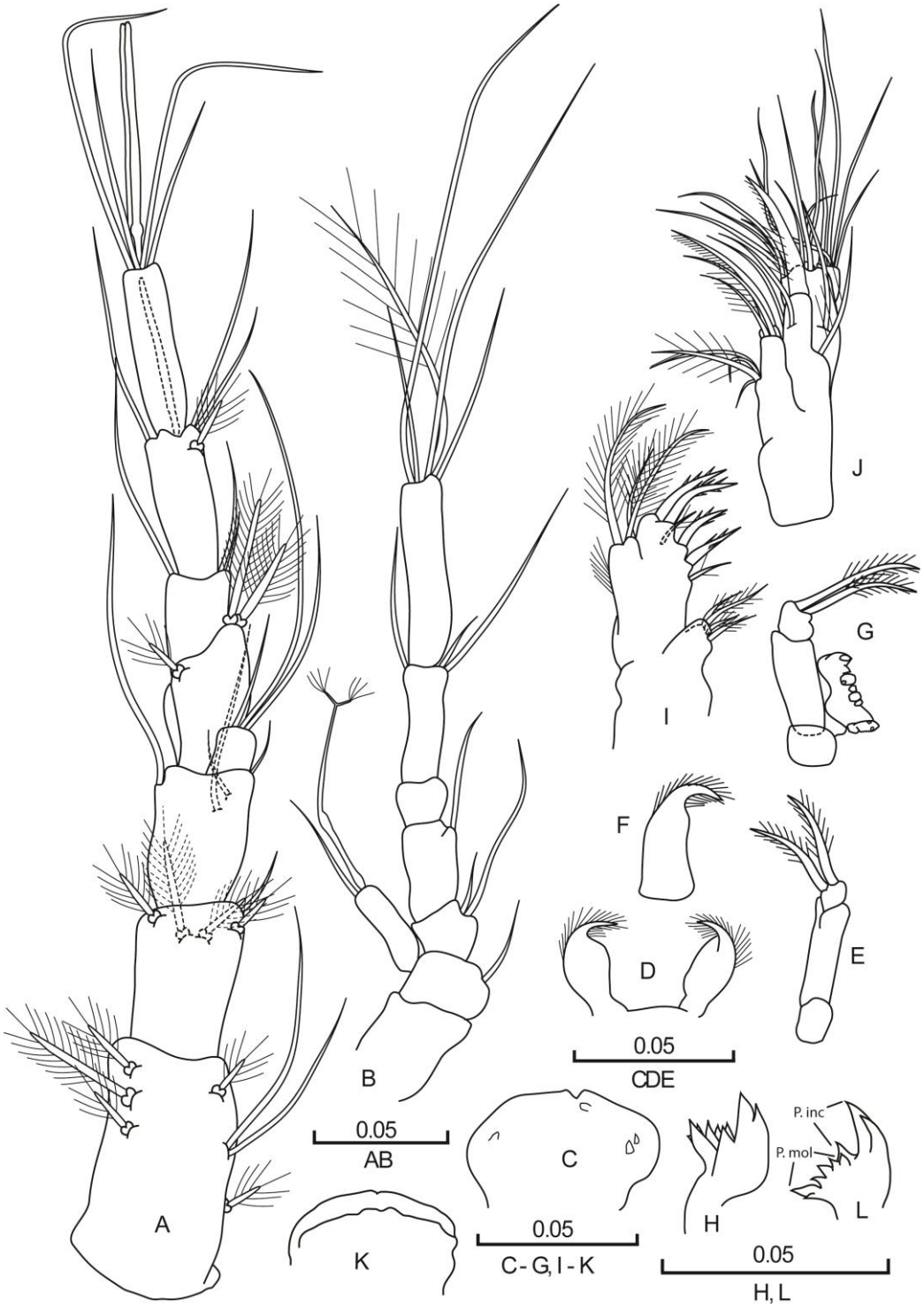

Figure 5 *Pilbaranella ethelensis* gen. et sp. nov. (A) Antennule (dorsal view), male holotype; (B) antenna (dorsal view), male holotype; (C) Labrum, female allotype; (D) paragnath, female allotype; (E) palp, female allotype, mandible; (F) paragnath, male holotype; (G) mandible, male holotype; (H) mandible, female allotype, masticatory part; (I) maxillule, male holotype; (J) maxilla (dorsal view), male holotype; (K) labrum, male paratype (WAMC57452); (L) mandible, male paratype (WAMC57479), masticatory part. Scale bar is in millimetres.

Figure 6 *Pilbaranella ethelensis*, gen. et sp. nov., male holotype. (A) Thoracopod I; (B) thoracopod II; (C) thoracopod III; (D) thoracopod IV; (E) thoracopod V; (F) thoracopod VI; (G) thoracopod VII. Scale bar is in millimetres.

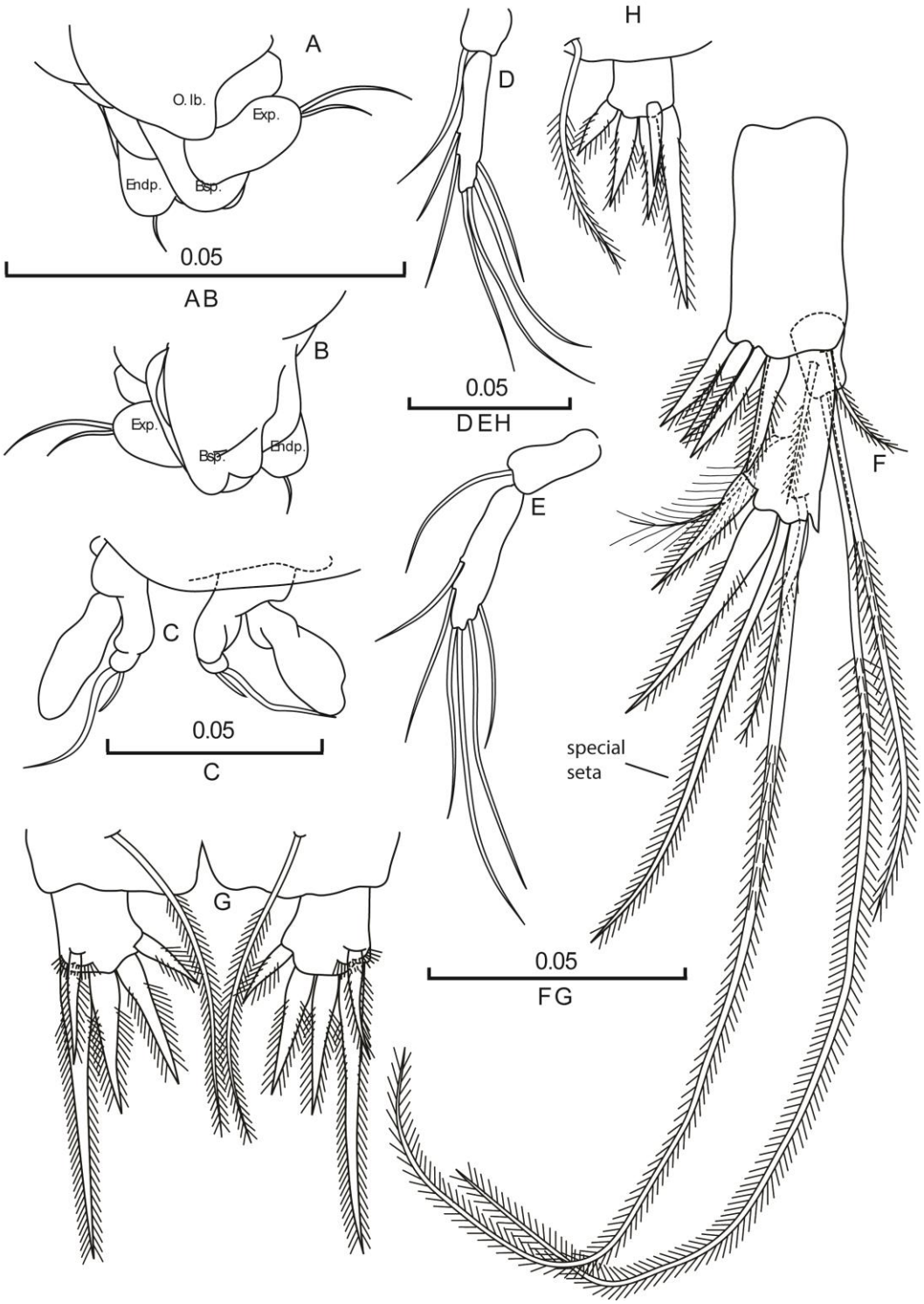

Figure 7 *Pilbaranella ethelensis*, gen. et sp. nov. (A) Thoracopod VIII (latero-external view), male holotype; (B) thoracopod VIII (latero-internal view), male holotype; (C) thoracopod VIII, female allotype (frontal view); (D) first pleopod, male holotype; (E) first pleopod, allotype female; (F) uropod (ventral view), male holotype; (G) furcal rami (dorsal view), male holotype; (H) furcal rami, allotype female. Scale bar is in millimetres. Abbreviations: O. lb, outer lobe; Bsp, basipod; Endp, endopod; Exp, exopod.

DISCUSSION

Morphology and Phylogeny

Pilbaranella ethelensis is the first genus and species of Bathynellidae described for Western Australia using abundant material and molecular data to support the morphology, while Schminke's description in 1973 was based on the morphology of one specimen only. The differences between *P. ethelensis* and *Bathynella primaustraliensis* are not only in the setation of the endopod of the thoracopods and pleopod, but also in the mandible, female thoracopod VIII, and length of spines on furca, uropod and pleotelson seta.

Although Schminke included the species found in Victoria in *Bathynella*, the female thoracopod VIII he described is uniramus and not biramus as per Serban's characterisation of *Bathynella* (Serban, 1966b). The conservative morphology of Bathynellidae, and the lack of material and additional supporting data (e.g. molecular) probably made the generic identification of the only Australian specimen problematic in 1973. Figure 8 presents the mandible, and male/female thoracopod VIII of the type genera and species of the three subfamilies so far described. *Bathynella natans*, the type species for Bathynellinae, is poorly described and not useful for the comparison. We chose instead *Bathynella paranatans* Serban, 1971, since it is a well described species belonging to Bathynellinae and it clearly belongs to '*Bathynella*'. We excluded the affinity of *Pilbaranella* to Bathynellinae and Gallobathynellinae based on the morphology of both thoracopods VIII of male and female presented by Serban (1966a, 1966b), and the number of teeth on the mandible (Figure 8). *Pilbaranella* is instead more similar morphologically to *Austrobathynella patagonica* Delamare Deboutteville & Roland, 1963, which is the type species of Austrobathynellinae based on the many teeth on the *pars molaris* (six) (Figure 8C); the small third segment of the endopod of the antenna; the endopod of the uropod with two spines and one 'special seta' (Delamare Deboutteville & Serban, 1973); the sympod with four spines; the simplified female thoracopod VIII (Figure 8P) with one ramus (however the epipod is absent, while it is present in *Pilbaranella*); the globose male thoracopod VIII (Figure 8G-K) with its basipod integrated in the penial

region, the reduced endopod and exopod (although the endopod is very small in the new species while it is similar in size to the exopod in *A. patagonica*), and the developed outer lobe (large in *Austrobathynella* and smaller in *Pilbaranella*). For the other two subfamilies *Bathynella paranatans* and *Gallobathynella coiffaiti* (Delamare Deboutteville, 1961) present: only four and three teeth on *pars molaris* respectively (Figure 8A-B); female thoracopod VIII biramus (Figure 8M) (although in some Gallobathynellinae the female thoracopod VIII can present only one ramus formed by the basipod (Figure 8N), like in *Vandelibathynella vandeli* (Delamare Deboutteville and Chappuis, 1954) ; and male thoracopod VIII unfolded with more developed endopod and exopod (Figure 8E-F-I-J).

Although *Pilbaranella* shows similarities to members of Austrobathynellinae, the data for this subfamily are too limited and patchy to draw further conclusions regarding the affinity of the new genus. We excluded *Bathynella* (in contrast to the classification of the first species described for Australia) on the basis of the morphological differences mentioned above, and the low likelihood that a genus of this family could have a worldwide distribution (Serban, 2000), when all known species of Bathynellacea occur in the subterranean/interstitial environment, usually with restricted distributions, and with genera seemingly confined to a single continent, as proposed by (Abrams, 2012). Uncertainties in the morphology point to the need to use molecular techniques to reconstruct the 'true' phylogeny of Bathynellidae. The 18S rRNA conserved region has been used to compare the new genus with the sequenced genera (and lineages) known for the rest of the world. From the phylogeny represented in Figure 4 it is clear that the species occurring in Ethel Gorge do not belong to any of the European/Texan lineages (the latter form a defined clade with members of the Gallobathynellinae); it forms instead a well supported clade with the specimen collected from the De Grey River catchment (in the Pilbara region). Unfortunately, sequences of species belonging to Bathynellinae are not yet available to support the morphological data.

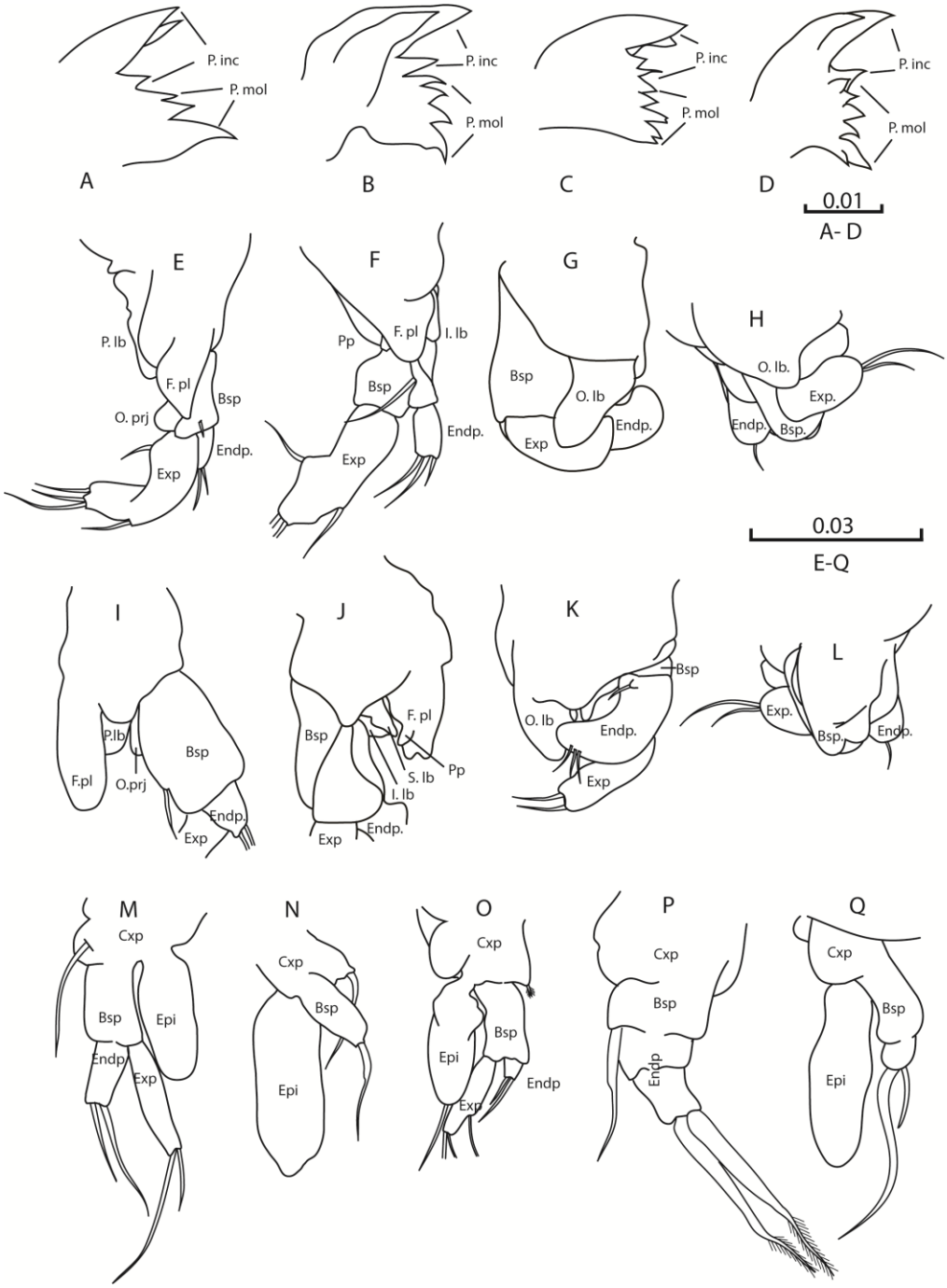

Figure 8 Comparison of mandibles and male and female thoracopods VIII of species from Bathynellinae, Gallobathynellinae, Austrobathynellinae and *Pilbaranella*, gen. nov. A–D: mandible. (A) *Gallobathynella coiffaiti*; (B) *Bathynella paranatans*; (C) *Austrobathynella patagonica*; (D) *Pilbaranella ethelensis*, gen. et sp. nov. E–L: male thoracopods VIII. (E, I) *Gallobathynella coiffaiti*; (F, J) *Bathynella paranatans*; (G, K) *Austrobathynella patagonica*; (H, L) *Pilbaranella ethelensis* gen. et sp. nov. M–Q: female thoracopods VIII. (M) *Gallobathynella coiffaiti*; (N) *Vandelibathynella vandeli*; (O) *Bathynella paranatans*; (P) *Austrobathynella patagonica*; (Q) *Pilbaranella ethelensis*, gen. et sp. nov.

Species delimitation

Due to convergent evolution and extreme progenetic development of Bathynellidae (Schminke, 1981), which leads to subadults with developed genitalia but reduced size and incomplete setation, the characters available for taxonomic identification are hard to determine, and often very few and subtle. An integrative approach to define the boundaries of new (and old) species is summarised in Figure 2, and the results show that there are at least four species. The morphology supports the molecular data, showing subtle but consistent characters to distinguish the four species (although the quality and quantity of the material for some of these taxa was insufficient for a formal morphological description). Molecular data confirm the presence of a fifth species, '*Pilbaranella* possible sp. D', with morphological characters closer to *P. ethelensis*, but only juveniles were collected, which makes identification almost impossible. More material is needed to confirm the status of this lineage. Two species, *Pilbaranella* spp. B and C, were consistently identified by means of all markers and methods applied, while for *P. ethelensis* and *Pilbaranella* sp. A different markers and methods produced different partitions. The mtDNA genes split these two lineages into multiple genetic species, according to the boreholes where the haplotypes originated (Figure 2). This 'over splitting' by the mitochondrial genes is probably due to population structure, which is common in fragmented habitat, especially in subterranean groups (Cook et al., 2012; Guzik, Cooper, Humphreys, & Austin, 2009). Figure 9 shows the distribution of *P. ethelensis*, while COI divergences between *P. ethelensis* haplotypes from different bore holes are shown in Table 5 and range between 0.3 and 7.4%. Most diverse haplotypes occur in boreholes north and north-west of the Ophthalmia Dam (HEC0303, W79D, OB23REG1) with COI divergences among haplotypes from other bores of 5.5–7.4%. This COI variability probably reflects the complexity of the Ethel Gorge aquifer system. Four main surface tributaries of the Fortescue River occur in the area; aquifers are contained in the banded iron formation, alluvium and calcrete deposits (Johnson & Wright, 2001), where the latter can be isolated in some places by layers of clay; and local perched aquifers can develop when the alluvium is saturated by flooding events (Johnson & Wright, 2001). The whole aquifer system is complex but

appears to be interconnected: other stygofauna species collected during the monitoring surveys (identified morphologically and molecularly) have similar distributions to that of *P. ethelensis* (Helix Molecular Solution, 2011a, 2011b; Subterranean Ecology, 2012, 2013, 2014) (Figure 10).

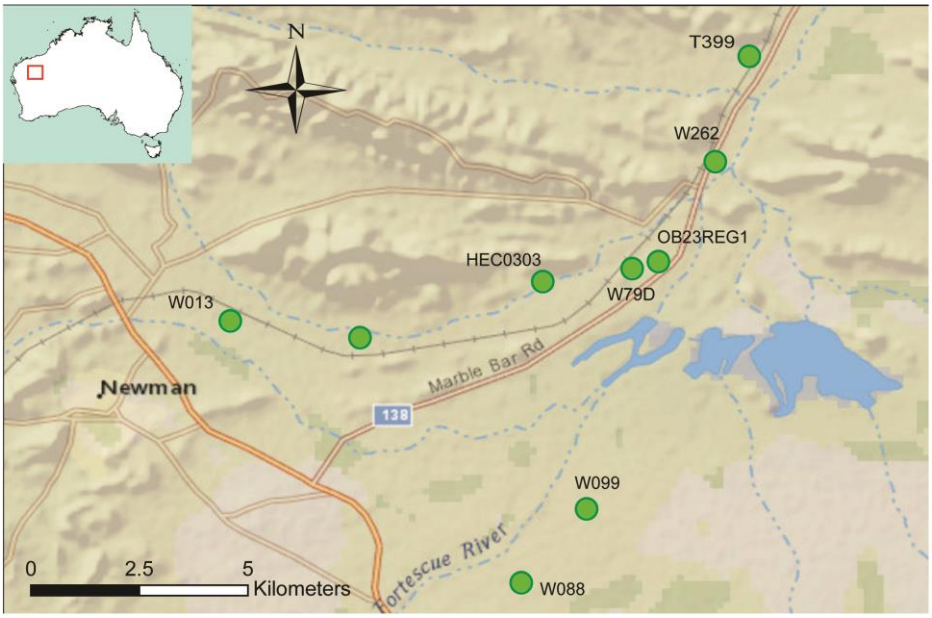

Figure 9 Distribution of *Pilbaranella ethelensis*, gen. et sp. nov.

Table 5 Estimates of evolutionary divergence over sequence pairs between groups (= haplotypes from the same bore hole) of *Pilbaranella ethelensis*. Standard error estimate(s) are shown above the diagonal. Diagonally in bold: estimates of average evolutionary divergence over sequence pairs within groups with standard error. Analyses were conducted in MEGA 7.

	OBREG1	HEC0303	W79D	W088	W099	T399	W013	T411A	W262
OBREG1	**0.006 (s.e. = 0.002)**	0.003	0.005	0.009	0.010	0.010	0.009	0.010	0.010
HEC0303	0.007	-	0.005	0.009	0.009	0.010	0.009	0.010	0.010
W79D	0.016	0.016	-	0.010	0.010	0.010	0.010	0.010	0.010
W088	0.063	0.057	0.069	**0.002 (s.e. = 0.001)**	0.002	0.006	0.007	0.008	0.008
W099	0.063	0.057	0.069	0.003	**0.0 (s.e. = 0.0)**	0.006	0.007	0.008	0.008
T399	0.068	0.062	0.074	0.023	0.021	-	0.008	0.009	0.008
W013	0.058	0.055	0.064	0.036	0.033	0.043	**0.0 (s.e. = 0.0)**	0.004	0.008
T411A	0.063	0.061	0.066	0.041	0.038	0.048	0.012	-.	0.008
W262	0.066	0.064	0.066	0.044	0.042	0.045	0.036	0.042	-

The high variability of the mtDNA results supports the necessity of an integrated and multigene approach to define species, with nuclear markers informative at species level. A thorough morphological study can clarify uncertainties in the molecular data and vice versa, but gathering both kinds of data from small organisms, such as many of the subterranean ones, is often difficult. DNA degradation is accelerated by hydrolytic and oxidative processes (Dessauer, Cole, & Hafner, 1995). Tissue fixation and storage in 100% ethanol or other chemical or physical treatments (e.g. cryopreservation) can delay DNA degeneration (Dawson, Raskoff, & Jacobs, 1998). This is particularly important for small aquatic specimens, such as stygofauna, where water concentration in tissues and storage temperature play an important role in maintaining nucleic acids. Subsampling of small organisms, such as the subterranean ones, decreases the chance of retrieving enough DNA for PCR, but allows morphological studies. This study confirms the possibility of obtaining both molecular and morphological information from the same specimen, even for minute and delicate organisms such as Bathynellidae, as long as the material is fixed and preserved in 100% ethanol and refrigerated. Success in DNA extractions and amplifications was achieved for individuals collected up to five years earlier that had been refrigerated.

Biodiversity and Distribution

Ethel Gorge is an ideal system for assessing the level of biodiversity in a complex aquifer system. As a threatened ecological community (TEC), the stygofauna of this area have been monitored regularly for several years, as per ministerial compliance (Minister for the Environment; Employment and Training, 1998). Many species have been named (Cho & Humphreys, 2010; Finston et al., 2004; Finston et al., 2011; I. Karanovic, 2006, 2007; T. Karanovic, 2006; Keable & Wilson, 2006), and many more are awaiting formal description (Halse et al., 2014). The reason for such richness is likely the variety of habitats that the area offers, especially the presence of a thick layer of saturated calcrete all around the gorge (Middlemis, 2006) that provides an ideal habitat for stygofauna (Humphreys, 1999). The most abundant bathynellid species of Bathynellidae collected in the study area seems to be *P. ethelensis*, followed by *Pilbaranella* sp.

A, and despite the intensive sampling effort, only few specimens of the *Pilbaranella* spp. B and C have been collected so far. These species might have a more extended distribution beyond the sampled area, or they could be "rare" species, like many of the subterranean taxa (Eberhard et al., 2009). Consequently, we expect that the Bathynellidae community in an aquifer could comprise a few abundant species and possibly more "rare" ones, for which an extensive sampling effort will be needed to detect them.

Pilbaranella sp. A seems to occur mostly around Homestead Creek and the further western side of the Ophthalmia Range (Figure 3). The only two specimens collected from the western side present significant mitochondrial divergence (7.7–8.4%, see COI sequence divergences in the Excel supplementary file, which probably reflects, in this case, the geographical distance (~35 km west of Ethel Gorge). Other stygofauna collected during the monitoring surveys support the connection of Ethel Gorge with the western Ophthalmia Range *(Helix Molecular Solution, 2011a, 2011b; Subterranean Ecology, 2012, 2013, 2014)* (Figure 10). No samples were collected in the area between, so we may not have a complete dataset of the variability of this group. Nevertheless, the surveys conducted in the past few years in Ethel Gorge and surrounds were designed for monitoring purposes and used pre-established bore-holes; the aims were not to explore specific distribution, therefore the actual species boundaries are difficult to assess. However, material under examination by the authors and collected 50 km north-west and 120 km west of the gorge do not belong to *Pilbaranella*. We can infer that bathynellid taxa have quite restricted distributions and probably can be considered Short Range Endemic (SRE) taxa, according to the definition of maximum SRE distribution being less than 10 000 km^2 (Harvey, 2002).

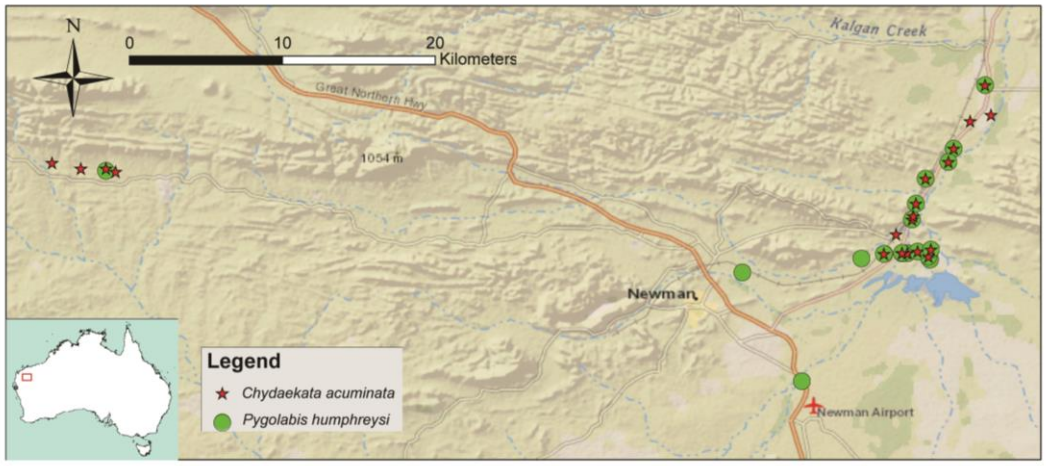

Figure 10 Distribution of other stygofauna species in the Ethel Gorge and surrounding: circles, *Pygolabis humphreysi*; stars, *Chydaekata acuminata*.

In this preliminary work, the Bathynellidae appears to be quite diverse in the Pilbara region, with few taxa defined morphologically or molecularly, which are genetically distinct from Australian lineages previously deposited in GenBank. Therefore we can assume that the Australian biodiversity of this family, as for the sister family Parabathynellidae, will be quite diverse.

Currently, there are no fossil data for the Bathynellidae (Camacho, Rey, Dorda, Machordom, & Valdecasas, 2002), but their Pangaean distribution suggests that their ancestors were already present in the Carboniferous-Permian period. They were possibly living in the warm seas in the Boreal Hemisphere (Brooks, 1962); they adapted to the surface or interstitial life towards the late Palaeozoic and early Mesozoic, and subsequently invaded subterranean fresh water (Coineau & Camacho, 2013). Consequently, the Bathynellidae, given their old evolutionary history and their limited dispersal ability due to their confined environment (Humphreys, 2008; Schminke, 1974), could reveal interesting connections among aquifers and river catchments, and contribute to an understanding of the hydrogeological history of the Pilbara region.

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AUTHOR'S CONFLICT OF INTERESTS

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Appendix 1. *Pilbaranella ethelensis* WAM C57612 from bore W088 female habitus (photograph).

Appendix 2. Coordinates of bore holes

Bore code	Latitude	Longitude
EMP0139	23°18′03.20″S	119°43′44.10″E
EXR0908	23°16′39.94″S	119°22′02.03″E
EXR0916	23°16′41.51″S	119°23′12.00″E
F3NR	23°20′51.66″S	119°50′13.83″E
HEC0303	23°19′51.81″S	119°49′32.63″E
HHS0032	23°17′43.30″S	119°43′23.20″E
HIST0723R	23°18′5.58″S	119° 44′52.68″E
HST0062R	23°18′23.23″S	119°45′17.62″E
HST0186R	23°18′40.50″S	119°45′38.70″E
HST0723R	23°18′5.58″S	119°44′52.68″E
HST0907R	23º18′40.68″S	119°45′35.22″E
OB23REG1	23°19′36.88″S	119°50′59.14″E
T399	23°17′03.36″S	119°52′07.06″E
T411A	23°20′33.83″S	119°47′15.96″E
W013	23°20′21.35″S	119°45′39.04″E
W088	23°23′37.13″S	119°49′16.56″E
W099	23°22′41.83″S	119°50′05.41″E
W262	23°18′22.19″S	119°51′41.61″E
W79D	23°19′42.18″S	119°50′39.38″E
WP14S	23°18′56.59″S	119°51′08.10″E
WP56	23°18′29.52″S	119°51′38.69″E

Appendix 3. GenBank accession numbers for *Pilbaranella*, gen. nov.

[A]Pending correction in GenBank (A. I. Camacho, personal comment).

Species/lineage	Bore Code	Country	WAMC/MNCN REGNO	GenBank Accession Number			
				CO1	18S	16S	28S
Iberobathynella celiana		Spain	MNCN/ADN29452	HQ659862	KC469527	–	–
Iberobathynella imuniensis		Spain	MNCN/ADN29166	HQ659850	KC469528	–	–
Paradoxiclamousella fideli[A] (in GenBank: *Clamousella* sp. 3)		Spain	MNCN/ADN29735	JX121252	KC469523	–	–
Paradoxiclamousella cf *fideli*[A] (in GenBank: *Bathynellidae* gen. sp. 1)		Spain	MNCN/ADN29594	JX121249	JX121235	–	–
Vejdovskybathynella edelweiss		Spain	MNCN/ADN29440	HQ596571	KC469513	–	–
Vejdovskybathynella caroloi		Spain	MNCN/ADN29877	KC469538	KC469525	–	–
Vejdovskybathynella sp. 2		Spain	MNCN/ADN29523	HQ596573	KC469515	–	–
Vejdovskybathynella vasconica[A] (in GenBank: *Vejdovskybathynella* sp. 3)		Spain	MNCN/ADN29646	KC469535	KC469521	–	–
Vejdovskybathynella vasconica[A] (in GenBank: *Bathynellidae* sp. 1)		Spain	MNCN/ADN29623	–	KC469516	–	–
Vejdovskybathynella vasconica[A] (in GenBank: *Bathynellidae sp. 2*)		Spain	MNCN/ADN29627	–	KC469517	–	–
Gallobathynella boui		Spain	MNCN/ADN54600	KP974146	KP999757	–	–
Gallobathynella coiffaitti		Spain	MNCN/ADN54602	–	KP999759	–	–
Gallobathynella tarissei		Spain	MNCN/ADN54592	–	KP999752	–	–
Pilbaranella ethelensis	OB23REG1	Australia	WAMC54544	MF074335	–	MF044215	MF044294
Pilbaranella sp. B	W262	Australia	WAMC54545	MF074336	–	MF044216	–
Pilbaranella sp. B	W262	Australia	WAMC54546	–	–	MF044217	–

				MF0	MF0	MF0	MF0
Bathynellidae	CA00	Aust	WAMC5	7433	4220	4221	4229
DeGrey	06	ralia	7258	7	9	8	5
				MF0		MF0	
Pilbaranella		Aust	WAMC5	7433		4221	
ethelensis	W088	ralia	7292	8	–	9	–
				MF0		MF0	
Pilbaranella		Aust	WAMC5	7433		4222	
ethelensis	W088	ralia	7293	9	–	0	–
				MF0		MF0	
Pilbaranella		Aust	WAMC5	7434		4222	
ethelensis	W088	ralia	7294	0	–	1	–
				MF0		MF0	
Pilbaranella		Aust	WAMC5	7434		4222	
ethelensis	W088	ralia	7295	1	–	2	–
				MF0		MF0	MF0
		Aust	WAMC5	7434		4222	4229
Pilbaranella sp. A	W262	ralia	7297	2	–	3	6
				MF0		MF0	MF0
		Aust	WAMC5	7434		4222	4229
Pilbaranella sp. A	W262	ralia	7298	3	–	4	7
				MF0		MF0	MF0
		Aust	WAMC5	7434		4222	4229
Pilbaranella sp. A	W262	ralia	7299	4	–	5	8
						MF0	MF0
		Aust	WAMC5			4222	4229
Pilbaranella sp. A	W262	ralia	7300	–	–	6	9
				MF0		MF0	MF0
Pilbaranella		Aust	WAMC5	7434		4222	4230
ethelensis	T399	ralia	7302	5	–	7	0
				MF0		MF0	MF0
Pilbaranella		Aust	WAMC5	7434		4222	4230
ethelensis	W013	ralia	7304	6	–	8	1
				MF0		MF0	MF0
Pilbaranella		Aust	WAMC5	7434		4222	4230
ethelensis	W099	ralia	7306	7	–	9	2
				MF0		MF0	MF0
		Aust	WAMC5	7434		4223	4230
Pilbaranella sp. B	WP56	ralia	7308	8	–	0	3
				MF0		MF0	MF0
Pilbaranella		Aust	WAMC5	7434		4223	4230
ethelensis	W088	ralia	7310	9	–	1	4
				MF0		MF0	MF0
Pilbaranella		Aust	WAMC5	7435		4223	4230
ethelensis	W088	ralia	7311	0	–	2	5

				MF07435		MF04223	MF04230
Pilbaranella ethelensis	OB23 REG1	Australia	WAMC57329	1	–	3	6
Pilbaranella ethelensis	OB23 REG1	Australia	WAMC57330	–	–	MF04223 4	–
Pilbaranella ethelensis	W088	Australia	WAMC57331	MF07435 2	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57332	MF07435 3	–	–	MF04230 7
Pilbaranella ethelensis	W088	Australia	WAMC57333	MF07435 4	–	–	MF04230 8
Pilbaranella sp. B	W262	Australia	WAMC57334	–	–	MF04223 5	–
Pilbaranella sp. B	W262	Australia	WAMC57335	MF07435 5	–	MF04223 6	MF04230 9
Pilbaranella sp. B	W262	Australia	WAMC57336	MF07435 6	–	MF04223 7	–
Pilbaranella sp. A	W013	Australia	WAMC57448	–	–	MF04223 8	–
Pilbaranella sp. B	W262	Australia	WAMC57450	–	–	MF04223 9	MF04231 0
Pilbaranella ethelensis	W088	Australia	WAMC57451	MF07435 7	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57452	MF07435 8	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57453	MF07435 9	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57454	MF07436 0	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57455	MF07436 1	–	–	–

Pilbaranella ethelensis	W088	Australia	WAMC57456	MF07436 2	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57457	MF07436 3	–	–	MF04231 1
Pilbaranella ethelensis	W088	Australia	WAMC57458	MF07436 4	–	MF04224 0	MF04231 2
Pilbaranella ethelensis	W088	Australia	WAMC57459	MF07436 5	–	MF04224 1	MF04231 3
Pilbaranella ethelensis	OB23 REG1	Australia	WAMC57460	MF07436 6	MF04221 0	MF04224 2	MF04231 4
Pilbaranella ethelensis	OB23 REG1	Australia	WAMC57461	–	–	MF04224 3	–
Pilbaranella ethelensis	OB23 REG1	Australia	WAMC57462	MF07436 7	–	MF04224 4	–
Pilbaranella sp. B	W262	Australia	WAMC57463	–	–	MF04224 5	–
Pilbaranella ethelensis	W088	Australia	WAMC57464	MF07436 8	–	MF04224 6	–
Pilbaranella ethelensis	W088	Australia	WAMC57465	–	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57466	MF07436 9	–	–	–
Pilbaranella ethelensis	W088	Australia	WAMC57467	MF07437 0	–	–	–
Pilbaranella sp. A	HST0186R	Australia	WAMC57468	MF07437 1	–	MF04224 7	–
Pilbaranella sp. A	HST0186R	Australia	WAMC57469	–	–	MF04224 8	–
Pilbaranella sp. A	HST0186R	Australia	WAMC57470	MF07437 2	–	MF04224 9	MF04231 5

						MF04225	MF04231
Pilbaranella sp. A	HST0186R	Australia	WAMC57471	–	–	0	6
				MF07437		MF04225	MF04231
Pilbaranella sp. A	HST0186R	Australia	WAMC57472	3	–	1	7
				MF07437		MF04225	MF04231
Pilbaranella sp. A	HST0186R	Australia	WAMC57473	4	–	2	8
				MF07437		MF04225	MF04231
Pilbaranella poss. sp. D	HHS0032	Australia	WAMC57474	5	–	3	9
				MF07437	MF04221	MF04225	MF04232
Pilbaranella poss. sp. D	HHS0032	Australia	WAMC57475	6	1	4	0
				MF07437		MF04225	MF04232
Pilbaranella ethelensis	T411A	Australia	WAMC57476	7	–	5	1
				MF07437			MF04232
Pilbaranella ethelensis	W013	Australia	WAMC57477	8	–	–	2
				MF07437		MF04225	
Pilbaranella ethelensis	W013	Australia	WAMC57478	9	–	6	–
				MF07438			
Pilbaranella ethelensis	W099	Australia	WAMC57479	0	–	–	–
						MF04225	
Pilbaranella ethelensis	W79D	Australia	WAMC57480	–	–	7	–
				MF07438	MF04221	MF04225	MF04232
Pilbaranella sp. B	WP14S	Australia	WAMC57481	1	2	8	3
				MF07438		MF04225	MF04232
Pilbaranella ethelensis	OB23REG1	Australia	WAMC57482	2	–	9	4
				MF07438		MF04226	MF04232
Pilbaranella ethelensis	W013	Australia	WAMC57483	3	–	0	5
				MF07438		MF04226	
Pilbaranella ethelensis	W088	Australia	WAMC57484	4	–	1	–
				MF07438		MF04226	
Pilbaranella ethelensis	W262	Australia	WAMC57485	5	–	2	–

				MF07438		MF04226	
Pilbaranella sp. B	W262	Australia	WAMC57486	6	–	3	–
				MF07438		MF04226	MF04232
Pilbaranella ethelensis	W79D	Australia	WAMC57487	7	–	4	6
						MF04226	MF04232
Pilbaranella sp. B	F3NR	Australia	WAMC57488	–	–	5	7
				MF07438			MF04232
Pilbaranella ethelensis	HEC0303	Australia	WAMC57489	8	–	–	8
				MF07438			MF04232
Pilbaranella sp. B	W013	Australia	WAMC57490	9	–	–	9
				MF07439		MF04226	
Pilbaranella sp. A	HST0186R	Australia	WAMC57491	0	–	6	–
							MF04233
Pilbaranella sp. A	HST0186R	Australia	WAMC57493	–	–	–	0
				MF07439		MF04226	MF04233
Pilbaranella sp. A	HST0062R	Australia	WAMC57494	1	–	7	1
Pilbaranella sp. A	EMP0139	Australia	WAMC57495	–	–	–	–
Pilbaranella sp. A	EMP0139	Australia	WAMC57496	–	–	–	–
						MF04226	MF04233
Pilbaranella sp. A	EMP0139	Australia	WAMC57497	–	–	8	2
				MF07439		MF04226	MF04233
Pilbaranella sp. A	EXR0916	Australia	WAMC57498	2	–	9	3
						MF04227	
Pilbaranella sp. A	EXR0908	Australia	WAMC57499	–	–	0	–
				MF07439		MF04227	
Pilbaranella ethelensis	W088	Australia	WAMC57500	3	–	1	–
	HIST0723				MF04221	MF04227	MF04233
Pilbaranella sp. C	R	Australia	WAMC57501	–	3	2	4

				MF0		MF0	
Pilbaranella		Aust	WAMC5	7439		4227	
ethelensis	W088	ralia	7613	4	–	3	–
				MF0		MF0	
Pilbaranella	OB23	Aust	WAMC5	7439		4227	
ethelensis	REG1	ralia	7614	5	–	4	–
				MF0		MF0	MF0
Pilbaranella	OB23	Aust	WAMC5	7439		4227	4233
ethelensis	REG1	ralia	7615	6	–	5	5
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7439		4227	4233
Pilbaranella sp. A	186R	ralia	7617	7	–	6	6
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7439		4227	4233
Pilbaranella sp. A	186R	ralia	7618	8	–	7	7
				MF0	MF0	MF0	MF0
	HST0	Aust	WAMC5	7439	4221	4227	4233
Pilbaranella sp. A	186R	ralia	7619	9	4	8	8
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7440		4227	4233
Pilbaranella sp. A	186R	ralia	7620	0	–	9	9
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7440		4228	4234
Pilbaranella sp. A	186R	ralia	7621	1	–	0	0
				MF0		MF0	
	HST0	Aust	WAMC5	7440		4228	
Pilbaranella sp. A	186R	ralia	7622	2	–	1	–
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7440		4228	4234
Pilbaranella sp. A	186R	ralia	7623	3	–	2	1
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7440		4228	4234
Pilbaranella sp. A	186R	ralia	7624	4	–	3	2
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7440		4228	4234
Pilbaranella sp. A	186R	ralia	7625	5	–	4	3
						MF0	
	HST0	Aust	WAMC5			4228	
Pilbaranella sp. A	186R	ralia	7626	–	–	5	–
				MF0		MF0	
	HST0	Aust	WAMC5	7440		4228	
Pilbaranella sp. A	186R	ralia	7627	6	–	6	–
						MF0	MF0
	HEC0	Aust	WAMC5			4228	4234
Pilbaranella sp. C	303	ralia	9080	–	–	7	4

	HIST 0723	Aust	WAMC5			MF0 4228	MF0 4234
Pilbaranella sp. C	R	ralia	9081	–	–	8	5
				MF0		MF0	MF0
	HST0	Aust	WAMC5	7440		4228	4234
Pilbaranella sp. A	907R	ralia	9082	7	–	9	6
				MF0		MF0	MF0
	HEC0	Aust	WAMC6	7440		4229	4234
Pilbaranella sp. C	303	ralia	0390	8	–	0	7
	HIST			MF0		MF0	MF0
	0723	Aust	WAMC6	7440		4229	4234
Pilbaranella sp. C	R	ralia	0391	9	–	1	8
				MF0		MF0	MF0
	HST0	Aust	WAMC6	7441		4229	4234
Pilbaranella sp. A	907R	ralia	0392	0	–	2	9
						MF0	
Pilbaranella		Aust	WAMC6			4229	
ethelensis	W088	ralia	0393	–	–	3	–