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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

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Abstract

The stygofaunal family of Bathynellidae, is an excellent group to study the processes that shape diversity and distribution, since they have unknown surface or marine relatives, high level of endemism, and limited dispersal abilities. Recent research on Bathynellidae in Western Australia (Pilbara) has uncovered new taxa with unexpected distributions and phylogenetic relationships, but the biogeographical processes that drive their diversification on the continent are still unclear. By exploring the diversity, distribution, and

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divergence time of Bathynellidae in a setting such as the perched and isolated aquifers of the Cleaverville Formation in the north of the De Grey River catchment (Pilbara), we aim to test the hypothesis that vicariance has shaped the distribution of this family, specifically if one or multiple vicariant events were involved. We analysed the specimens collected from perched water in different plateaus of the Cleaverville Formation, combining morphological and molecular data from mitochondrial and nuclear genes. We described two new species and genera (*Anguillanella callawaensis* gen. et sp. nov. and *Muccanella cundalinensis* gen. et sp. nov.), and two additional taxa are recognised using morphology and/or Automatic Barcode Gap Discovery and Poisson Tree Processes species delimitation methods. New genera and species result restricted to isolate perched aquifers on single plateaus and their distributions, phylogenetic relationships, and divergence time estimates support multiple vicariant events and ancient allopatric speciation.

Keywords

ABGD – *Anguillanella* gen. nov – Bathynellidae – new species – *Muccanella* gen. nov – PTP – stygofauna

Introduction

Subterranean species distributions are often restricted, and confined to particular geologies, for example isolated karst systems, limestone, fractured banded iron formations (BIFs), and porous hyporheic systems (Culver & Pipan, 2008; Harvey, 2002; Humphreys, 2017; Trontelj et al., 2009). For this reason many subterranean taxa are considered short-range endemics (SREs; *sensu* Harvey, 2002). Their small geographic ranges, and high rate of endemism make them ideal candidates to explore historical climatic and geological events. For example ice sheet coverage during the Quaternary glaciations influenced the distribution of the subterranean amphipod species of *Stygobromus* in north America (Holsinger et al., 1997) and *Niphargus virei* in France (Foulquier et al., 2008). The occurrence of the cirolanid isopod *Antrolana lira* in areas formerly submerged by the ocean, confirms the role of marine transgression in shaping cirolanid distributions (Holsinger et al., 1994), and the presence of troglobitic salamanders in Texan caves suggests the ancestor of this radiation migrated underground to survive climate aridification (Sweet, 1982).

Aquatic subterranean animals (stygobionts) often have larger distributions than terrestrial subterranean animals (troglobionts) (Barr & Holsinger, 1985; Christman & Culver, 2001; Lamoreaux, 2004). This can be due to a larger suitable continuous environment, such as the presence of groundwater in different but adjacent geological layers, or to flooding events that would increase the opportunity for dispersal (Lamoreaux, 2004). Small stygobionts are collected not only from karst areas, but also from saturated interstices in alluvial/colluvial deposits (Barr & Holsinger, 1985; Holsinger, 1988; Marmonier et al., 1993; Christman & Culver, 2001). In other cases, distributions of stygofaunal species can be restricted to small areas (Eberhard et al., 2009). Geographically close aquifers can be isolated by impermeable or semi-impermeable material such as clay, mudstone or other deposits with very low hydraulic conductivity. Accordingly, adjacent groundwater bodies can harbour different stygofaunal species. For example, calcrete aquifers in ancient palaeochannels of the Yilgarn region, in the arid zone of Western Australia, are considered "islands under the desert" and host different stygofauna communities (Cooper et al., 2002, 2008; Guzik et al., 2008; Humphreys et al., 2009; Karanovic & Cooper, 2011).

To be recognised as perched, aquifers are located above the regional water table, in the vadose zone (the unsaturated zone below the land surface). This occurs when there are layers of rocks or sediments with very low permeability such as clay, granite or mudstone, above the main water table but below the land surface (Fitts, 2012). Perched aquifers commonly form in arid, semi-arid climates (Sedghi, 2016), and can be occasionally connected to local aquifer systems, particularly during rain seasons and flooding events (Johnson & Wright, 2001). Perched aquifers within the same area in arid regions (isolated or partially isolated from regional groundwater), were probably historically connected during groundwater table fluctuations, and/or marine transgressions. For example, perched aquifers in the arid zones of Western Australia were interconnected during wetter periods when large river systems were flowing and water table was higher (Van de Graaff et al., 1977; BMR Palaeogeographic Group, 1990; González-Álvarez et al., 2016).

The Pilbara region, in the arid zone of Western Australia, exhibits many different types of aquifers, sometimes with complex hydrological connectivity. Groundwater can be contained in unconsolidated sedimentary aquifers (alluvium and colluvium in Cainozoic valley fills), chemically deposited aquifers (mainly formed by calcrete and limonite within alluvium and colluvium formations), and fractured rock aquifers, comprising Precambrian basement rock (like BIFs, dolomite and sandstone) and distinct types of aquifers can occur in the same area (Johnson & Wright, 2001). Perched aquifers are also present in the region in various geologies (Hickman et al., 1983; Johnson & Wright, 2001; WorleyParsons, 2012; Department of Water, 2016). Aquifers are recharged by rainfall and surface water, are generally fresh to brackish (Department of Water, 2010) and host a highly diverse stygofauna. Halse (2018) estimates 1329 stygofaunal species for the Pilbara alone, whose distributions reflect in part the hydrogeological complexity of the region (Finston et al., 2009, 2007; Brown et al., 2015; Perina et al., 2019).

Bathynellidae are a common component of stygofauna in different aquifers of the Pilbara region, including perched aquifers. They represent an ideal taxon to test biogeographic hypotheses as they are confined to the interstitial and subterranean environment, have no known epigean or marine relatives, and have limited dispersal abilities (Schminke, 1974; Humphreys, 2008; Coineau & Camacho, 2013). The impediments of conservative morphology and poorly resolved taxonomy, often leading to inaccurate species identifications (Camacho et al., 2018), have been alleviated in recent years, by molecular tools which have been used to integrate morphological species studies and resolve phylogenetic relationships among taxa (Camacho et al., 2002; Camacho et al., 2013a, b). Understanding of the Australian bathynellids is still limited, but a recent study from one of the major catchments in the Pilbara, the Fortescue, revealed distantly related taxa within the same catchment and even within the same aquifer (Perina et al., 2018, 2019), suggesting a complex and ancient biogeographical history. Similar results have been obtained in a study of bathynellids in south Queensland (Little et al., 2016). All investigations conducted so far in Australia, though, do not clarify the biogeographical processes that can drive Bathynellidae diversification on the continent.

In this study we aim to test vicariance in a setting that is apparently ideal; the perched aquifers of the Cleaverville formation in the north of the De Grey River in the Pilbara (fig. 1). This formation comprises ridges formed by Precambrian Banded Iron Formations (BIFs) containing perched water

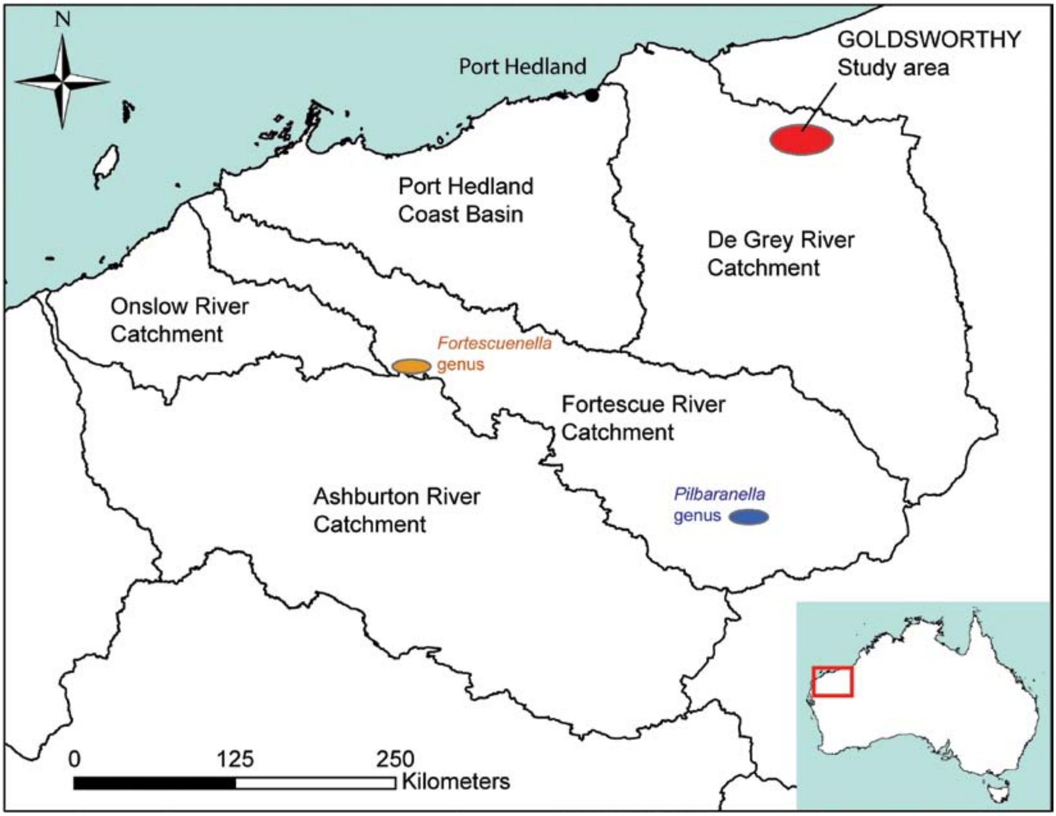


FIGURE 1 The Pilbara bioregion with the five major catchments. In red the Goldsworthy study area in the De Grey River catchment, east of Port Hedland town.

partially isolated from the regional alluvial aquifer (Hickman et al., 1983; Dames & Moore, 1992). In the past these perched aquifers were interconnected by higher water table during the Upper Cretaceous/Early Tertiary (Van de Graaff et al., 1977; BMR Palaeogeographic Group, 1990; González-Álvarez et al., 2016), and by marine transgressions which occurred in different ages (Artinksian, Oxfordian, Aptian) (Hickman et al., 1983), when dispersal between aquifers, or the origin of a marine ancestor into the system, may have been facilitated. By exploring patterns of diversity (through morphological and molecular tools), species distribution, and divergence time of Bathynellidae in the north of the De Grey River catchment, we aim to test two hypotheses. 1) a single vicariant event (for example a marine regression) isolated the perched aquifers at the same time, and therefore stygofauna, in different ridges and allopatric speciation occurred. The phylogeny expected would then show short internodes amongst the taxa identified, and low node support. 2) several vicariant events (geological events such as valley erosion and water level fluctuations) fragmented the ancestral population gradually in different ridges at different times. We would then expect multiple ancestors (nodes) at different times, with bathynellids occurring on the ridge separated by a deeper valley genetically more distinct from the others.

Material and methods

Study area and groundwater sampling methods

The study area (the former Goldsworthy mining area) is located 170 km East of Port Hedland, in the north of the De Grey River catchment (fig. 1), one of the main catchments of the Pilbara bioregion, in the arid zones of WA. It comprises low hills, plateaus alternate with sandy plains and sporadic claypans. Different ridges of about 260 m Australian Height Datum are part of the Cleaverville Iron Formation (such as Callawa, Cundaline and Yarrie ridges), fractured and weathered Archean-aged formation (Hickman et al., 1983; Williams, 2003). Small and large gaps (for example Shay and Kimberley Gap) between the ridges allow the passage of many ephemeral creeks that run through the area (Dames & Moore, 1992). Aquifers occur in the alluvial deposits, mainly in the De Grey River and Eel Creek, and in the Archean bedrock that forms the ridges in the area. There is evidence of a perched aquifer on Yarrie Ridge confined by granite and mudstone layers that underlie the BIF (see figure 3.2 in Dames & Moore, 1992). Rainfall recharges the perched aquifer through infiltration of the permeable BIF on the plateau, but the low hydraulic conductivity of the beneath layers makes the discharge into the regional water in the alluvial of the De Grey and Eel Creek very slow (Dames & Moore, 1992; Williams, 2003). All ridges of the Cleaverville Formation abut granitic rocks (Hickman et al., 1983) therefore groundwater contained in the BIF is partially isolated from the regional groundwater. In geological time frames, water tables have fluctuated much more than today, interconnecting the perched aquifers, with wetter periods of higher water levels, for example in the Cretaceous-Early Tertiary (BMR Palaeogeographic Group, 1990; González-Álvarez et al., 2016), and other periods where most of the area was submerged by marine transgressions, for example in the Artinksian, Oxfordian, and Aptian (Hickman et al., 1983). Connection and isolation of the perched aquifers probably occurred at different times, but with aridification processes started in the Mid-Miocene in the north of Western Australia (Van de Graaff et al., 1977; Macphail & Stone, 2004; Byrne et al., 2008), water tables have dropped steadily, isolating the aquifers in the area.

In 2009 BHP Billiton proposed to mine Callawa and Cundaline ridges, therefore subterranean fauna surveys were carried out to contribute to the development of advice and recommendations to the Minister of the Environment on the proposal (Environmental Protection Authority, 2007, 2009). A preliminary molecular analysis conducted on Bathynellidae identified two lineages from Cundaline and Yarrie ridges respectively (Helix Molecular Solution, 2009), but specimens from Callawa ridge were not included in the analysis, therefore uncertainties on how many taxa are present in the area, and their relationships were unresolved (Subterranean Ecology, unpubl. data).

Given the presence of Bathynellidae in different ridges of the Cleaverville Iron Formation, the water table fluctuations over geological time, the drought climate established by the Pliocene (Macphail & Stone, 2004) and the perched aquifers subsequently partially isolated from one another and from regional groundwater, we believe that this Goldsworthy area is an ideal setting to test for allopatric speciation in stygofauna.

The bathynellids collected in the Goldsworthy area during surveys between 2007 and 2009 by Subterranean Ecology is the source of the material used for this study. Stygofauna was sampled according to the EPA guidelines (Environmental Protection Authority, 2003, 2007), while specimen preservation follows Perina et al. (2018).

TABLE 1 List of markers and primers with relative sequences utilised

Marker	Primer	Sequence	Reference/Designed by	No of specimens amplified
COI	C1-J-1718 F	5’ GGAGGATTTGGAAATTGATTAGTTCC 3’	(Simon et al., 1994)	21
	C1-J-2329 R	5’ ACTGTAAATATATGATGAGCTCA 3’		
16S	16SSarL F	5’ CGCCTGTTTAACAAAAACAT 3’	(Palumbi et al., 1991)	3
	16S SbrH R	5’ CCGGTCTGAACTCAGATCACGT 3’		
	16SBathy-21F	5’ ARTAHAATCTGCCCGGTGAT 3’	(Perina et al., 2018; Whiting et al., 1997)	37
	16SBathy-53F	5' ACGGCCGCAGTAWTTTGACT 3'	(Perina et al., 2019)	5
16S	16SBathy- 453R	5’ TCCAACATCGAGGTCGHAAAC 3’	(Perina et al., 2018)	42
18Si	1F	5’ TACCTGGTTGATCCTGCCAGTAG 3’	(Giribet et al., 1996)	43
	5R	5’ CTTGGCAAATGCTTTCGC 3’		
18Sii	3F	5’ GTTCGATTCCGGAGAGGGA 3’	(Giribet et al., 1996)	43
	18Sbi	5’ GAGTCTCGTTCGTTATCGGA 3’	(Whiting et al., 1997)	
18Siii	18Sa2.0	5’ ATGGTTGCAAAGCTGAAAC 3’	(Whiting et al., 1997)	37
	9R	5’ GATCCTTCCGCAGGTTCACCTAC 3’	(Giribet et al., 1996)	
28S	28S-D1F	5’ GGGACTACCCCCTGAATTTAAGCAT 3’	(Park et al., 2000)	56
	28Sb	5’ TCGGAAGGAACCAGCTACTA 3’	(Nunn et al., 1996)	
ITS2	5.8S2	5’ GGGTCGATGAAGAACGCAGC 3’	(Rix, 2009)	33
	28S2	5’ TCCTCCGCTTATTTATATGC 3’	(Rix, 2009)	

DNA methods and data analysis

Tissue sampling, DNA extraction, PCR amplification, and DNA sequencing follow the procedures described in Perina et al. (2018). The mitochondrial Cytochrome oxidase I (COI), and 16S ribosomal RNA (16S), and the nuclear 28S, 18S ribosomal RNA, and the Internal Transcribed Spacer 2 (ITS2) markers were amplified using the primers listed in table 1.

We used LIMS (Laboratory Information Management Software) Biocode plug-in (http://www.mooreabiocode.org) to manage the molecular laboratory workflows. We imported the raw chromatograms into Geneious 10.2.4 software (Kearse et al., 2012), and assembled forward and reverse sequences. Each sequence was examined by eye and edited. The consensus sequences were generated and blasted against GenBank, and sequences representing contaminations were discarded from the analyses. The MAFFT (Multiple Alignment using Fast Fourier Transform) algorithm (Katoh et al., 2002) with default parameters was used to build the alignments. We used the online server GBlocks Version 0.91b (Castresana, 2000) to exclude poorly aligned sections of the 16S, ITS2, 18S and 28S alignments using the less strict parameters. Sequences of the mitochondrial COI marker were translated into amino acid chains to ensure no stop-codons were present. COI fragments presenting internal stop codons (and therefore not coding for the protein) were not considered in the analyses. COI uncorrected pairwise distance (P-distance) was calculated within and between species haplotypes from different bores (computed through Molecular Evolutionary Genetics Analysis (MEGA) 7.0 (Kumar et al., 2016) using 1000 bootstraps replications and other parameters unvaried) to compare with former analyses done on bathynellids and other groups of stygofauna.

Gene trees based on each gene alignment, and a phylogeny based on the concatenated alignment of the five genes considered (Concatenate_tree; table 2) were constructed using Bayesian and Maximum Likelihood (ML) method, implemented in RaxML_HPC_BlackBox (Randomized Axelerated Maximum Likelihood, including automatic bootstrapping stop, which calculates the optimal number of replicates to obtain stable support value using the MRE-based bootstrapping criterion (Pattengale et al., 2009), and MrBayes on XSEDE (3.2.6) in the CIPRES Science Gateway online server (Miller et al., 2010), and MrBayes 3.2.5 (Ronquist et al., 2012) respectively. *Pilbaranella ethelensis* was used as outgroup for all single gene trees, but the ITS2 tree, which was rooted at midpoint. *Pilbaranella ethelensis* is closely related to the ingroup taxa, but is a distinct genus (Perina et al., 2018). Table 2 summarises the datasets constructed, the best models of nucleotide substitution for each alignment selected by the Akaike information criterion in jModeltest 2.1.9 (Posada, 2008), and the analyses performed.

Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012) and Poisson Tree Processes (PTP) (Zhang et al., 2013) species delimitation methods were implemented, using the online websites (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html and http://sco.h-its.org/exelixis/web/software/PTP/; 13 March 2019) to evaluate hypothetical species boundaries using phylogenetic trees and alignments listed in table 2. Kimura 80 (K80) and Jukes-Cantor (JC69) distance, with default values, were used in the ABGD analysis. Trees had distant outgroups removed to improve the results, and were analysed to the PTP method, leaving other settings unvaried.

The molecular phylogeny based on the concatenate tree (Concatenate_tree; table 2) was constructed to explore the phylogenetic relationships among the new species, previously defined taxa from the Pilbara, and publicly available data from other countries to put the

TABLE 2 Datasets constructed with description, the best model selected by jModeltest, analyses done and number of sequences used

Dataset	Description	Best model selected by jModeltest (AIC/BIC)	Analysis	No of seq in analysis
COI_alignment/tree	alignment/tree of COI sequences of specimens from Callawa, Cundaline ridges and one *Pilbaranella ethelensis*	GTR+G	ABGD and PTP (results in fig. 2)	22
16S_alignment/tree	alignment/tree of 16S sequences of specimens from Callawa, Cundaline, Yarrie ridges and one *Pilbaranella ethelensis*	HKY+G	ABGD and PTP (results in fig. 2)	46
28S_alignment/tree	alignment/tree of 28S sequences of specimens from Callawa, Cundaline, Yarrie ridges and one *Pilbaranella ethelensis*	HKY+G	ABGD and PTP (results in fig. 2)	57
ITS2_alignment/tree	alignment/tree of ITS2 sequences of specimens from Callawa, Cundaline, and Yarrie ridges	GTR+I	ABGD and PTP (results in fig. 2)	33
COI_alignment_for concatenate tree	alignment of COI sequences of specimens from Callawa, Cundaline ridges, representatives of *Pilbaranella*, Central Hamersley Range, Qld, SA taxa, Gallobathynellinae and Bathynellinae, and *Iberobathynella imuniensis* (alignment trimmed at 610 bp)	GTR+G+I	–	43
16S_alignment_for concatenate tree	alignment of 16S sequences of specimens from Callawa, Cundaline, Yarrie ridges, representatives of *Pilbaranella*, Central Hamersley Range taxa, and *Bathynella* from Slovenia	HKY+G	–	54
28S_alignment_for concatenate tree	alignment of 28S sequences of specimens from Callawa, Cundaline, Yarrie ridges, representatives of *Pilbaranella*, and Central Hamersley Range taxa	GTR+G	–	63
ITS2_alignment_for concatenate tree	alignment of ITS2 sequences of specimens from Callawa, Cundaline, Yarrie ridges	GTR+I	–	33

TABLE 2 Datasets constructed with description, the best model selected by jModeltest, analyses done and number of sequences used (*cont.*)

18S_alignment_for concatenate tree	alignment of 18S sequences of specimens from Callawa, Cundaline ridges, representatives of *Pilbaranella*, Central Hamersley Range, QLD, SA taxa, Gallobathynellinae and Bathynellinae, and *Iberobathynella imuniensis*	GTR+G+I	–	68
Concatenate_tree	Tree constructed using the concatenate alignment of COI-16S-28S-ITS2-18S genes	–	RaxML and MrBays (fig. 3, supplementary material)	92
COI_8seq_for BEAST	COI alignment with representatives of species from Cundaline, Callawa ridges, *Pilbaranella* species, and *Fortescuenella serenitatis*	HKY+G	–	8
16S_10seq_for BEAST	16S alignment with representatives of species from Cundaline, Callawa, Yarrie ridges, *Pilbaranella* species, and *Fortescuenella serenitatis*	TrN+G (BEAST=TN93+G)	–	10
28S_10seq_for BEAST	26S alignment with representatives of species from Cundaline, Callawa, Yarrie ridges, *Pilbaranella* species, and *Fortescuenella serenitatis*	TrN+G (BEAST=TN93+G)	–	10
18S_10seq_for BEAST	18S alignment with representatives of species from Cundaline, Callawa, Yarrie ridges, *Pilbaranella* species, and *Fortescuenella serenitatis*	TrN (BEAST=TN93)	–	10
Concat_COI-16S-28S-18S_BEAST_10seq	Concatenate alignment of COI, 16S, 28S and 18S genes used in the BEAST analysis	–	BEAST (fig. 4)	10

Australian fauna into a worldwide context. *Iberobathynella imuniensis* (Parabathynellidae) was used as outgroup. The analysis was partitioned by genes with unlinked models. Between 200,000 and 500,000 Markov Chain Monte Carlo (MCMC) generations were run for the single gene trees, and 2×10^6 MCMC generations were run for the concatenate tree. A burnin fraction of 0.25 was chosen and the consensus tree was built from the remaining trees. The program Tracer 1.6 (Rambaut et al., 2014) was used to assess the convergence of the Bayesian analysis, making sure that the Effective Sample Size (ESS) was above 200.

The time of divergence of the Goldsworthy taxa was estimated to establish the date of the node that represents the ancestor that colonized the perched aquifers, and to establish the origins of Bathynellidae in the area. The analysis was conducted using a Bayesian evolutionary approach implemented in BEAST 2.4.8 program (Bouckaert et al., 2014) run in the CIPRES Science Gateway online server (Miller et al., 2010). The concatenated COI-16S-28S-18S alignment used in the analysis included 10 sequences with the most complete dataset. One specimen per species occurring in the study area, representatives of *Pilbaranella* species, and *Fortescuenella serenitatis* as outgroup, a distantly related genus within the same bioregion (Perina et al., 2019). In the case of incomplete data, a chimera was assembled using different specimens belonging to the same species. The data were partitioned by gene with unlinked site and clock models, linked trees and Yule model. Nucleotide substitution models selected for each gene by the Akaike information criterion in jModeltest are listed in table 2. We excluded the proportion of invariant site from the COI, 28S and 18S models to simplify the analysis and obtain convergence. A relaxed lognormal clock was set for all genes. Since no Bathynellacea fossils are available to calibrate the molecular clock, we utilized 16S and COI mean substitution rates calculated using the highest and lowest rate available, for each marker, retrieved from the literature from other crustaceans. The 16S divergence rates of 0.53% and 1.36% Ma (Stillman & Reeb, 2001) and the COI divergence rates of 1.4% (Knowlton & Weigt, 1998) and 2.76% Ma (Wares & Cunningham, 2001) were used to calculate the ucld.mean and sigma of the analysis. For the 16S ucld.mean we chose a normal distribution with mean substitution rate = 0.00473 and sigma = 0.00126 (therefore 5% Quantile = 0.00266 (corresponding to the 0.533% divergence rate) and 95% Quantile = 0.0068 (corresponding to the 1.36% divergence rate). For the COI ucld.mean we chose a normal distribution with mean substitution rate = 0.0104 and sigma = 0.00205 (therefore 5% Quantile = 0.00703 (corresponding to the 1.4% divergence rate) and 95% Quantile = 0.0138 (corresponding to the 2.76% divergence rate). The ucld.Stdev of both COI and 16S was set as exponential with mean = 1.5. 5×10^7 Monte Carlo Markov chains (MCMC) were run through BEAST 2.4.8 (Bouckaert et al., 2014) and convergence was assessed using Tracer 1.7 (Rambaut et al., 2018), ensuring the Effective Sample Size (ESS) was above 200. Results of the BEAST analysis were summarized and annotated using TreeAnnotator 2.4.7 (Bouckaert et al., 2014) in a Maximum Clade Credibility tree, setting a 10% burnin, 0.5 posterior probability limit, and mean node heights. The resulting tree was edited in Figtree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Morphological analysis

The morphological study follows the methods described in Perina & Camacho (2016) and Perina et al. (2018). The material is vouchered at the Western Australian Museum (see Appendix 2 for voucher numbers).

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

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

The results revealed the presence of two new genera and four new species. The type species of the two new genera are described in Appendix 1 (*Anguillanella callawaensis* gen. et sp. nov. and *Muccanella cundalinensis* gen. et sp. nov.) and for ease of communication, these names are used from this point forward.

Molecular results

Sixty-five specimens from Callawa, Cundaline and Yarrie ridges were sequenced and included in the phylogeny. The total number of specimens extracted and successfully amplified for each marker are summarised in table 3. Twenty-one sequences (up to 610 bp) were obtained for COI. All COI fragments were translated and revealed no stop codons. Forty-five sequences up to 375 bp for the 16S mitochondrial fragment, fifty-six sequences of about 1000 bp for 28S, thirty-three sequences up to 740 bp for ITS2, and forty-three sequences up to 1780 bp for 18S were obtained. Sequences are deposited in GenBank (Appendix 2). The ITS2 locus was sequenced for the first time for bathynellids as a supplementary locus to enable more accurate species delineation. The ITS2 primers used for *Anguillanella callawaensis* gen. et sp. nov. were also tested on several specimens of *Muccanella cundalinensis* with no success.

TABLE 3 Number of tested and successfully amplified specimens per marker, and % of success

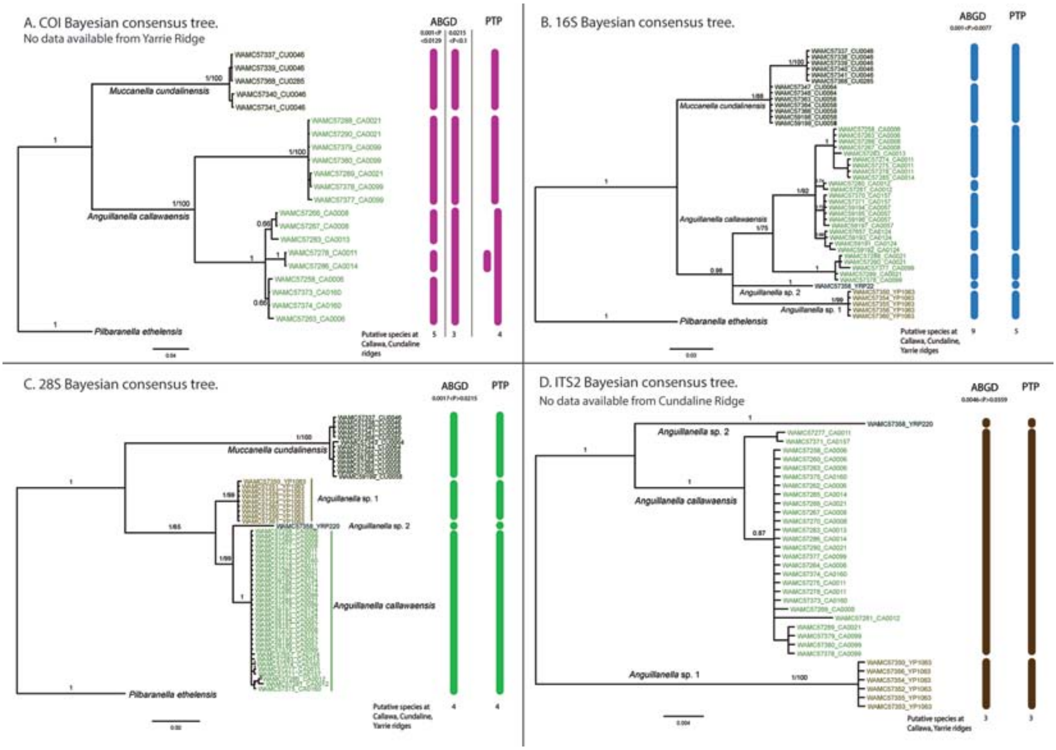
Marker	No specimens extracted	No specimens successfully amplified	% success
COI	66	21	31.8
16S	46	45	97.8
18Si	43	43	100.0
18Sii	43	43	100.0
18Siii	43	37	86.0
28S	60	56	93.3
ITS2	59	33	55.9

Species delimitation

Bayesian single gene trees for COI, 16S, 28S, and ITS2 together with the species delimitation results for ABGD (with intraspecific divergence (P) intervals) and PTP are represented in fig. 2. Specimens from Yarrie ridge failed to sequence for COI, while specimens from Cundaline failed to sequence for ITS2. *Muccanella cundalinensis* was identified by morphology and both PTP and ABGD species delimitation methods applied to COI and 28S, while ABGD method using 16S dataset returned two groups.

Anguillanella callawaensis was recognised by morphology and by both methods applied to 28S and ITS2. However, ABGD of COI alignment split the species into four ($0.001 < P < 0.0129$) and two ($0.0215 < P < 0.19$) taxa, ABGD of 16S identified five groups ($0.001 < P < 0.0077$, with P = prior intraspecific divergence), and PTP defined three and two groups respectively for COI and 16S (fig. 2A-B). *Anguillanella* sp. 1 was consistently identified by morphological and molecular data; *Anguillanella* sp. 2 is represented by one specimen only and both methods and all markers identified it as a distinct species, although a morphological comparison was not possible since the only individual was a juvenile.

Results of the COI uncorrected P-distance are shown in table 4. P-distance among haplotypes from different bores (within the same ridge) ranged between 0 to 16.3%, and within same bore holes ranged between 0 to 0.9% in *Anguillanella callawaensis* gen. et sp. nov. COI P-distance between and within haplotypes



A. COI Bayesian consensus tree.
No data available from Yarrie Ridge

B. 16S Bayesian consensus tree.

C. 28S Bayesian consensus tree.

D. ITS2 Bayesian consensus tree.
No data available from Cundaline Ridge

FIGURE 2 Bayesian consensus single gene trees for COI, 16S, 28S and ITS2. Numbers on branches represent Bayesian posterior probabilities followed by maximum likelihood bootstrap percentage. ABGD and PTP results are reported next to the trees. ABGD method: major partitions are showed; PTP: partitions with the highest support for each group are represented.

from the same and different bore holes in *Muccanella cundalinensis* gen. et sp. nov was 0.1%. Supplementary table S1 provides COI P-distances among all sequences obtained from specimens occurring in Callawa and Cundaline ridges. Ten bathynellids from Yarrie ridge were amplified for COI but resulted in contaminations. The mean pairwise distance within *Anguillanella callawaensis* and *Muccanella cundalinensis* is respectively 8.9% and 0.1%, while the mean distance between the two taxa was 20.1%.

Molecular phylogeny

Maximum likelihood and Bayesian analyses of the concatenated sequences produced slightly different topologies, but both defined by three major monophyletic and well supported groups: Gallobathynellinae and Bathynellinae subfamilies, and the Australian bathynellids (fig. 3, supplementary fig. S1). The Australian clade is formed by: two taxa from South Australia and two from Queensland, *Fortescuenella serenitatis* and the other three lineages from the Central Hamersley Range (nr *Fortescuenella* sp., Bathynellidae indet. from Mount Florence and SV0138), five taxa of *Pilbaranella* genus, *Anguillanella callawaensis* gen. et sp. nov., *Muccanella cundalinensis* gen. et sp. nov., *Anguillanella* sp. 1 and *Anguillanella* sp. 2. *Muccanella cundalinensis*, *Anguillanella callawaensis* and *Anguillanella*

TABLE 4 COI Estimates of Evolutionary Divergence over Sequence Pairs between haplotypes from the same bore hole. Diagonally in bold: within bore P-distance with standard error. Above the diagonal: standard error

	CA0006	CA0160	CA0008	CA0013	CA0011	CA0014	CA0021	CA0099	CU0046	CU0285
CA0006	**0.009 (SE = 0.005)**	0.002	0.005	0.005	0.007	0.007	0.015	0.015	0.017	0.017
CA0160	0.004	**0.000**	0.005	0.005	0.006	0.006	0.015	0.015	0.017	0.017
CA0008	0.018	0.014	**0.000**	0.004	0.007	0.007	0.015	0.015	0.017	0.017
CA0013	0.020	0.015	0.012	–	0.007	0.007	0.015	0.015	0.017	0.017
CA0011	0.030	0.026	0.033	0.034	–	0.000	0.015	0.015	0.017	0.018
CA0014	0.030	0.026	0.033	0.034	0.000	–	0.015	0.015	0.017	0.018
CA0021	0.161	0.159	0.155	0.162	0.157	0.157	**0.001 (SE = 0.001)**	0.001	0.017	0.017
CA0099	0.162	0.159	0.156	0.163	0.158	0.158	0.002	**0.003 (SE = 0.002)**	0.017	0.017
CU0046	0.196	0.193	0.193	0.198	0.204	0.204	0.206	0.206	**0.001 (SE = 0.001)**	0.001
CU0285	0.197	0.194	0.194	0.199	0.204	0.204	0.207	0.207	0.001	–

sp. 1 form monophyletic and well-supported lineages (Posterior Probability (PP) comprised between 0.96 and 1, and bootstrap (BS) comprised between 95 and 100). The two species collected from Yarrie Ridge form a non-supported clade (PP = 0.72) sister to *Anguillanella callawaensis*, while in the RaxML analysis *Anguillanella* sp. 1 results sister to *Anguillanella callawaensis*, but again the node is not supported (BS = 46, see supplementary fig. S1). *Anguillanella* genus results sister group with *Muccanella cundalinensis* (PP/BS = 1/100).

The clades formed by: Gallobathynellinae, Bathynellinae, *Fortescuenella serenitatis* and nr *Fortescuenella* sp., Bathynellidae from SA, and Bathynellidae from QLD are well supported (BS > 96, PP = 1), but BS and PP are quite low at deeper nodes.

Molecular clock analysis

Figure 4 represents the Maximum Clade Credibility (MCC) tree resulting from the molecular dating analysis. Node bars are represented by height 95% Higher Posterior Density (HPD). Node ages are indicated above the bars while posterior probability (PP) is below. A chimera was assembled for *Pilbaranella* sp. C using sequences from two specimens (WAMC57501-WAMC60391). The age of the most recent common ancestor (MRCA) between *Anguillanella* and *Muccanella* genera is estimated about 64.8 Ma. The mean divergence time of *Anguillanella* species is 22.6 Ma ago, while *Anguillanella callawaensis* and *Anguillanella* sp. 1 result sister species with node age of 15.2 Ma. The MRCA of all *Pilbaranella* species is dated at 34.2 Ma ago, while the most recent possible speciation event between *P. ethelensis* and *Pilbaranella* possible sp. D dates back to 11.3 Ma. The divergence time between *Pilbaranella* species and the Goldsworthy taxa is estimated around 96.7 Ma.

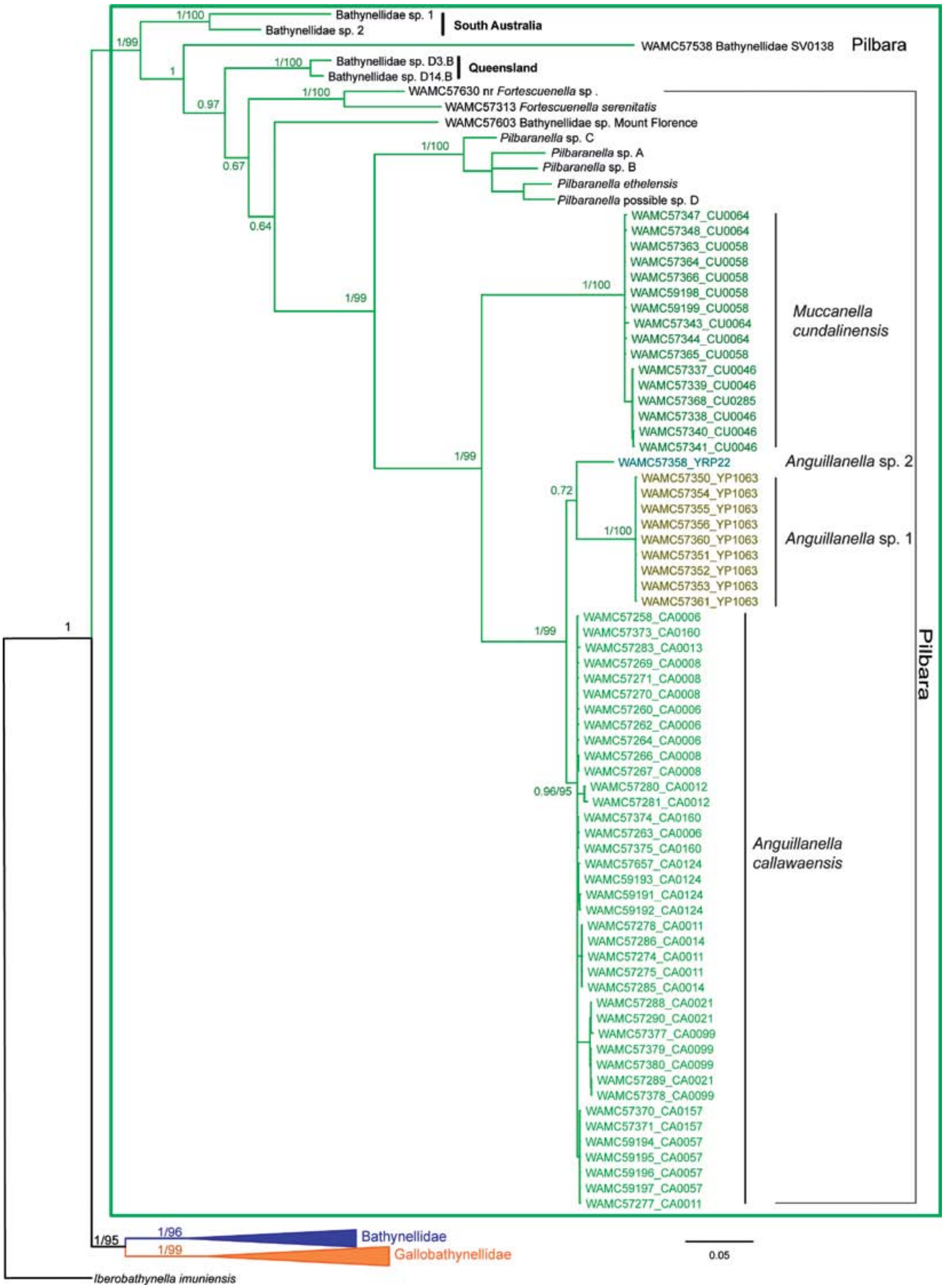


FIGURE 3 Bayesian consensus tree representing the known Bathynellidae taxa constructed using COI, 16S, 28S, ITS2 and 18S alignments and model partitioning implemented in MrBayes. Numbers on branches represent Bayesian posterior probabilities followed by maximum likelihood bootstrap percentage. Bathynellinae and Gallobathynellinae clades are collapsed for easier interpretation.

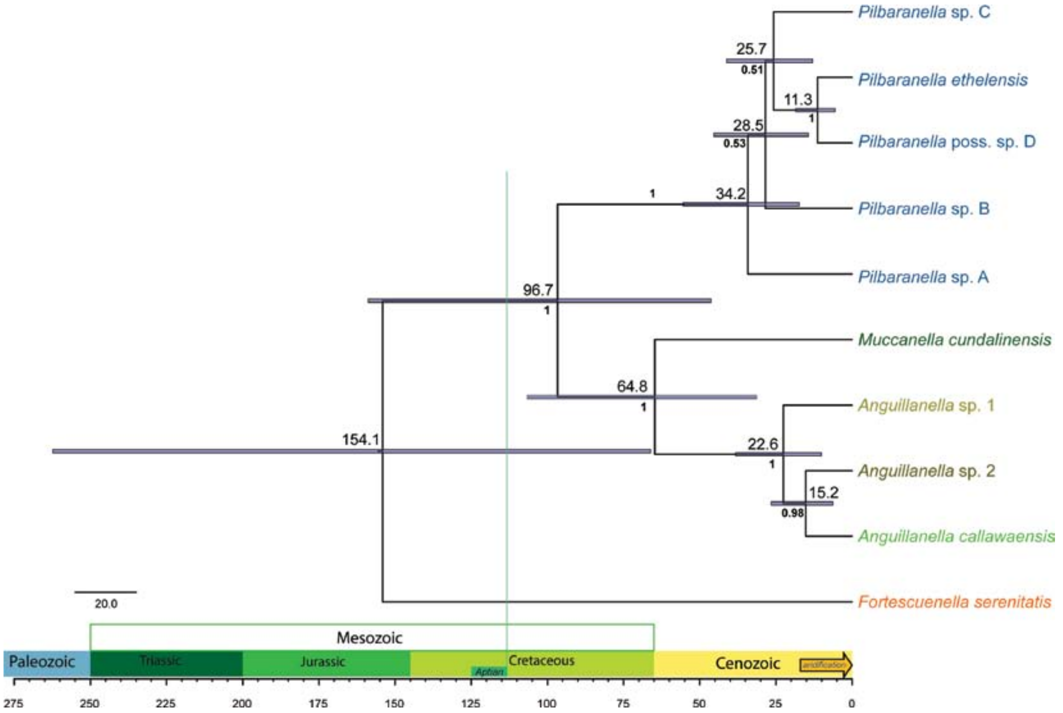


FIGURE 4 Maximum Clade Credibility Tree inferred using a concatenate COI, 16S, 28S and 18S alignment using BEAST. Node bars are 95% Higher Posterior Density, scale bar is in million years ago (Ma), starting from present 0. Numbers above bars = node age; numbers below bars (bold) = posterior probability of the node.

Discussion

De Grey River Bathynellidae diversity

An abundance of material previously collected during several environmental surveys allowed us to examine the morphological and molecular inter- and intra-specific variability, and describe two new genera, *Muccanella cundalinensis* gen. et sp. nov. and *Anguillanella callawaensis* gen. et sp. nov. Although there is a slow discharge of the perched aquifer into the regional one (Dames & Moore, 1992), the results show different lineages on different ridges, which indicates that stygofauna are isolated in each perched aquifer.

The results of the species delimitation methods adopted here (fig. 2) are supported by morphological analyses, although ABGD and PTP applied to mitochondrial markers recognise different species distributed in different bores (fig. 2A-B). We considered the mitochondrial variability to represent intraspecific population structure (Perina et al., 2018, 2019), and we used the morphological species concept integrated with the above mentioned species delimitation methods. The tree in fig. 3 (Concatenate_tree; table 2) presents the phylogenetic relationships among representatives of known genera and lineages of Bathynellidae and specimens belonging to the new genera. Each genus and species identified is reciprocally monophyletic supporting morphology and species delimitation methods.

Anguillanella callawaensis occurs in the north-eastern part of Callawa Ridge (fig. 5). The population structure highlighted by the mitochondrial loci is comparable to that found for *Pilbaranella ethelensis*, with different haplotypes in different bores and similar ones within the same bore. *Fortescuenella serenitatis*, instead, seems to have a more complex population structure with highly divergent haplotypes (mean COI uncorrected pairwise distance up to 9% (Perina et al., 2019)) within some bores and the same haplotypes occurring in different bores (see "4 genera networks" in supplementary fig. S2). Despite subtle morphological differences among specimens of *Anguillanella callawaensis*, and ABGD and PTP methods applied to 28S and ITS2, nuclear markers do not recognize different species, the COI uncorrected pairwise distance (P-distance) between specimens occurring in bores CA0021 and CA0099 (western side of the ridge) and the ones collected from bores located in the eastern side (CA0006, CA0008, CA0011 CA0013, CA0014 and CA0160) (fig. 5) is quite high (between 15.6 and 16.5%, see supplementary fig. S2). This "gap" in COI divergences could be due to sampling (the south-western part of the ridge was not sampled, Subterranean Ecology unpublished data) and hence uncertainties remain regarding the distribution of this species, or the two lineages are diverging (perhaps due to hydrogeological discontinuities), but not long enough to be detected by nuclear markers and clear morphological differences. We chose to be conservative and

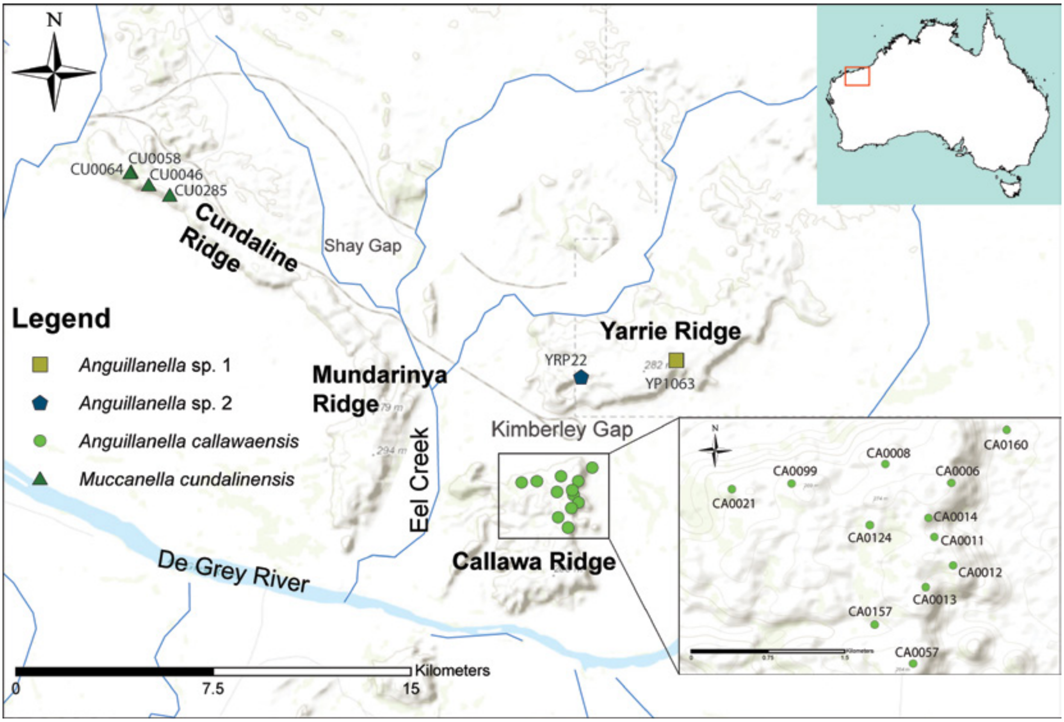


FIGURE 5 Bathynellidae species distribution in the Goldsworthy area (Callawa, Cundaline, Yarrie ridges).

consider one species, and acknowledge that more study is needed, especially on the hydrogeological connectivity, to better understand the biodiversity and distribution on Callawa Ridge.

Anguillanella sp. 1 and *Anguillanella* sp. 2 were only found in bore YP1063 and YRP22 respectively at Yarrie Ridge (fig. 5), although other bores in the surrounding area were sampled, excluding the eastern side of the ridge (Subterranean Ecology unpublished data). According to molecular data, these lineages are more closely related to *Anguillanella* genus, supporting the morphological analysis of *Anguillanella* sp. 1 (taxon not formally described in this paper), while morphological data for *Anguillanella* sp. 2 are not available. The presence of multiple species on Yarrie Ridge suggests the possible existence of isolated aquifers within the same plateau.

Muccanella cundalinensis occurs only in the northern part of the Cundaline Ridge (fig. 5), despite broad sampling across the whole plateau (Subterranean Ecology unpublished data). Accordingly the habitat suitable for this group seems to be quite restricted, but additional sampling could reveal a different distribution. Its population structure appears less complicated than *Anguillanella callawaensis*, probably due to the smaller area where it occurs (less than 2 km between bores, fig. 5).

As with *Pilbaranella ethelensis* and *Fortescuenella serenitatis* in the Fortescue catchment, the four species occurring in the De Grey River have localised distributions, and they can be considered Short Range Endemics, even though distributions might change with more extensive sampling. The 'patchy' nature of sampling for these taxa is demonstrated by *Muccanella cundalinensis* which was only collected in 2009 surveys and not in previous ones (2007–2008, Subterranean Ecology, unpubl. data) suggesting a possible interannual variability, perhaps connected to flooding events, as storms and rainfalls can change water levels and chemistry and therefore food inflow (Goater, 2009). Similar seasonal dynamics have been observed for some copepod species from the Yilgarn region (Karanovic & Cooper, 2012). This demonstrates the difficulty and the time frame needed to discover true subterranean biodiversity, and that an absence of taxa resulting from a survey does not necessary mean absence of subterranean fauna.

Once again, previously unstudied aquifers have revealed new taxa of Bathynellidae, confirming the trend of diversification observed for many other stygofaunal groups in the Pilbara, including the Bathynellidae sister family Parabathynellidae (Cho et al., 2005; Hong & Cho, 2009; Cho & Humphreys, 2010; Abrams et al., 2012), the Tainisopidae and Phreatoicidae isopods (Knott & Halse, 1999; Wilson, 2003; Keable & Wilson, 2006; Finston et al., 2009), oligochaetes (Pinder, 2008; Brown et al., 2015) and the Paramelitidae amphipods (Finston et al., 2007, 2008, 2011).

Allopatric speciation and the origins of bathynellid taxa in the Goldsworthy area

The aim of this paper was to test the hypothesis that vicariance has shaped the distribution of Bathynellidae in the Goldsworthy area: specifically we wanted to test if one or multiple vicariant events were involved in the diversification of the group. The molecular phylogeny in fig. 3 and clock analysis in fig. 4 show the relationships amongst the taxa occurring in the area (and other lineages) and their divergence times.

All taxa identified in the area are related, forming a monophyletic clade and sharing a common ancestor at 64.8 Ma ago, but different divergence times were obtained for *Anguillanella* species. On this basis we are able to exclude the hypothesis of a single vicariant event responsible for the fragmentation of the common ancestor's area. The date for the most recent common ancestor of the two new genera (at 64.8 Ma), also allows us to exclude an influence of marine transgression/regression events, since the latest occurred in the area in the Aptian (125–113 Ma) (see fig. 4). The data suggest that multiple vicariant events fragmented the ancestral population at different times.

Anguillanella and *Muccanella* origins (64.8 Ma ago)

In the Upper Cretaceous, a large river system crossing the current Office and Canning Basins was flowing towards the Indian Ocean (BMR Palaeogeographic Group, 1990), and hence we can assume that different rivers were also flowing in the north of the De Grey River. Likewise, from Late Cretaceous to mid Miocene the climate was much wetter (between 70 and 50 Ma ago the average annual rainfall of the Pilbara region was over 200 cm (González-Álvarez et al., 2016)) with widespread temperate rainforest (Byrne et al., 2008). The valleys between the plateaus were probably filled (or partially filled) with several meters of deposits (probably accumulated during the last marine transgression in the Aptian, such as the Parda Formation (Hickman et al., 1983)), eroded later by flowing water. Examples of extensive erosion can be found in Western Australia in the Yilgarn Craton, where the minimum denudation rate between the end of the Cretaceous and Early Tertiary was about 2.0 m Ma^{-1} (Kohn et al., 2002; Humphreys, 2017). The MRCA between *Anguillanella* and *Muccanella* suggests that the Eel Creek eroded the deposits faster than other putative creeks between other ridges, isolating the Cundaline population around the end of the Cretaceous, while bathynellid ancestors were still able to move between the perched water on Callawa and Yarrie ridges. The depth of the Eel Creek valley is 13 to 24 m deeper than the Kimberley Gap between Callawa and Yarrie (depths obtained from Google Earth Pro software), which supports the hypothesis of a faster erosion and therefore an earlier isolation of Cundaline stygofauna.

Anguillanella species origins (22.6–15.2 Ma ago)

The relationships among taxa of this genus are not well resolved. The nodes representing the ancestors of *Anguillanella* are well supported in the BEAST analysis (PP = 1 and 0.98, fig. 4), but the error bars largely overlap. The tree in fig. 3 shows a different topology, with *Anguillanella* sp. 1 and *Anguillanella* sp. 2 sister species, but with a low node support (PP = 0.72). The single-gene trees also represent different topologies where nuclear markers support the relationship between *Anguillanella callawaensis* and *Anguillanella* sp. 2, while the 16S results in a trichotomy (fig. 2). These relationships could represent a polytomy, which implies that the hydrological connection (higher water table) that allowed the colonization of the perched water on Callawa and Yarrie ridges from a common ancestor, stopped between 22.6 and 15.2 Ma ago, isolating the three *Anguillanella* species at similar time (one vicariant event, such as water table drop, perhaps coinciding with the start of the aridification processes (Byrne et al., 2008)).

Two scenarios could, instead, explain the data if *Anguillanella callawaensis* and *Anguillanella* sp. 2 are "true" sister species (as shown in fig. 4). The first explanation is one of vicariance: the connection with *Anguillanella* sp. 1 ceased 22.6 Ma ago, while the hydrological connectivity between the western side of Yarrie and Callawa ridges persisted longer. The presence of faults within the Yarrie Ridge (Dames & Moore, 1992) could explain the interruption of the connectivity in the early Miocene (22.6 Ma). The MRCA between *Anguillanella callawaensis* and *Anguillanella* sp. 2 is dated at 15.2 Ma ago, when aridification processes started in the north of Western Australia (Bowler, 1976; Byrne et al., 2008) and the water table dropped steadily, separating the two species, and successively maintaining the isolation of bathynellid populations. The second explanation draws on complex dispersal processes of Bathynellidae ancestors within Yarrie and between Yarrie and Callawa ridges to explain current distributions. Better understanding of the hydrogeology of the area, past climatic and geological events, additional material and molecular markers will help to resolve the relationships among lineages and clarify the drivers of diversification. Additionally, relationships from other subterranean taxa collected from different ridges in the Goldsworthy area may be instructive, for example two species of *Atopobathynella* (Parabathynellidae) (Abrams et al., 2012), two troglofauna species of *Trinemura* silverfish (*T. callawae*, *T. cundalinae*) (Smith et al., 2012), and two genera of subterranean carabids (Giachino, Eberhard, Perina in preparation). Molecular clock analyses of these groups could help to clarify the diversification processes involved in the area and support our findings.

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Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.8863475

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APPENDIX 1

Systematic account

Thirty-nine specimens of *Anguillanella callawaensis* gen. et sp. nov. were used for DNA extraction and are part of the type series. The extractions were obtained from: 18 whole specimens, and 21 selected body parts (few body segments where no morphological characters are present and/or a piece of the specimen's upper part of pereionites and pleonites). Sixteen specimens of *Muccanella cundalinensis* gen. et sp. nov. have been used for DNA extraction and are part of the type series. The extractions were obtained from eight whole specimens and eight selected body parts. Selected body parts of nine individuals of *Anguillanella* sp. 1 gen. nov., and one whole specimen of *Anguillanella* sp. 2 have been sequenced successfully and used in the molecular study.

Family Bathynellidae Grobben, 1905

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

Anguillanella Perina and Camacho gen. nov.

Generic diagnosis

Zoobank: urn:lsid:zoobank.org:act:17F7F477-6B59-458C-9A43-E3496A17B317

Antennula seven-segmented. Antenna seven-segmented, third endopodial segment very small. Paragnaths with distal strong claws. Mandibular palp three segmented with two long and strong equal barbed claws, and without sexual dimorphism. *Pars incisiva* with two teeth; *processus incisivus accessorius* with one tooth and one seta-like tooth; *pars molaris* with two dentate structures, parallel to main axis of teeth: first structure (closest to *processus incisivus accessorius*) with four teeth, and second structure formed by a bulb with three to five small denticles. Thoracopods I to VII with epipod and endopod four-segmented. Thoracopod VIII male rectangular and compact, with only one well developed lobe (outer lobe) on penial region (latero external part) and two projections (frontal and posterior); basipod in vertical position with large distal crest; endopod small and exopod large, perpendicular to basipod and curved outward, with simplified morphology and two distal setae present. Female thoracopod VIII simplified: coxopod, without setae; epipod very long; basipod large; one-segmented small ramus (endopod or exopod). Uropod: sympod with four inhomonomous spines and endopod with two spines, one special seta (seta "X") and three more setae (one very short). Furcal rami with five spines.

Type species: *Anguillanella callawaensis* gen. nov., sp. nov

Anguillanella callawaensis Perina and Camacho sp. nov. (figs. 6,7,8–9)

Zoobank: urn:lsid:zoobank.org:act:2399400E-4AA2-4697-80D6-0925B1DAD43E

Type locality. Bore CA0008, Callawa Ridge, De Grey River Catchment, Pilbara, Western Australia (see Appendix 3 for borehole coordinates).

Material examined

Holotype. WAMC57418, male, permanent slide, bore CA0008, 24 July 2009, Bell (stygo net haul).
Allotype. WAMC57370, female, permanent slide, bore CA0157, 10 July 2009, Bell (stygo net haul).
Paratypes. WAMC57258, female, permanent slide, Western Australia, Pilbara, bore CA0006, 13 June 2009, Bell & Ridley (stygo net haul); WAMC57260, female, 100% ethanol, Western Australia, Pilbara, bore CA0006, 13 June 2009, Bell & Ridley (stygo net haul); WAMC57262, female, 100% ethanol, Western Australia, Pilbara, bore CA0006, 13 June 2009, Bell & Ridley (stygo net haul); WAMC57263, sex not recorded, whole specimen for DNA, Western Australia, Pilbara, bore CA0006, 26 April 2008, Bell

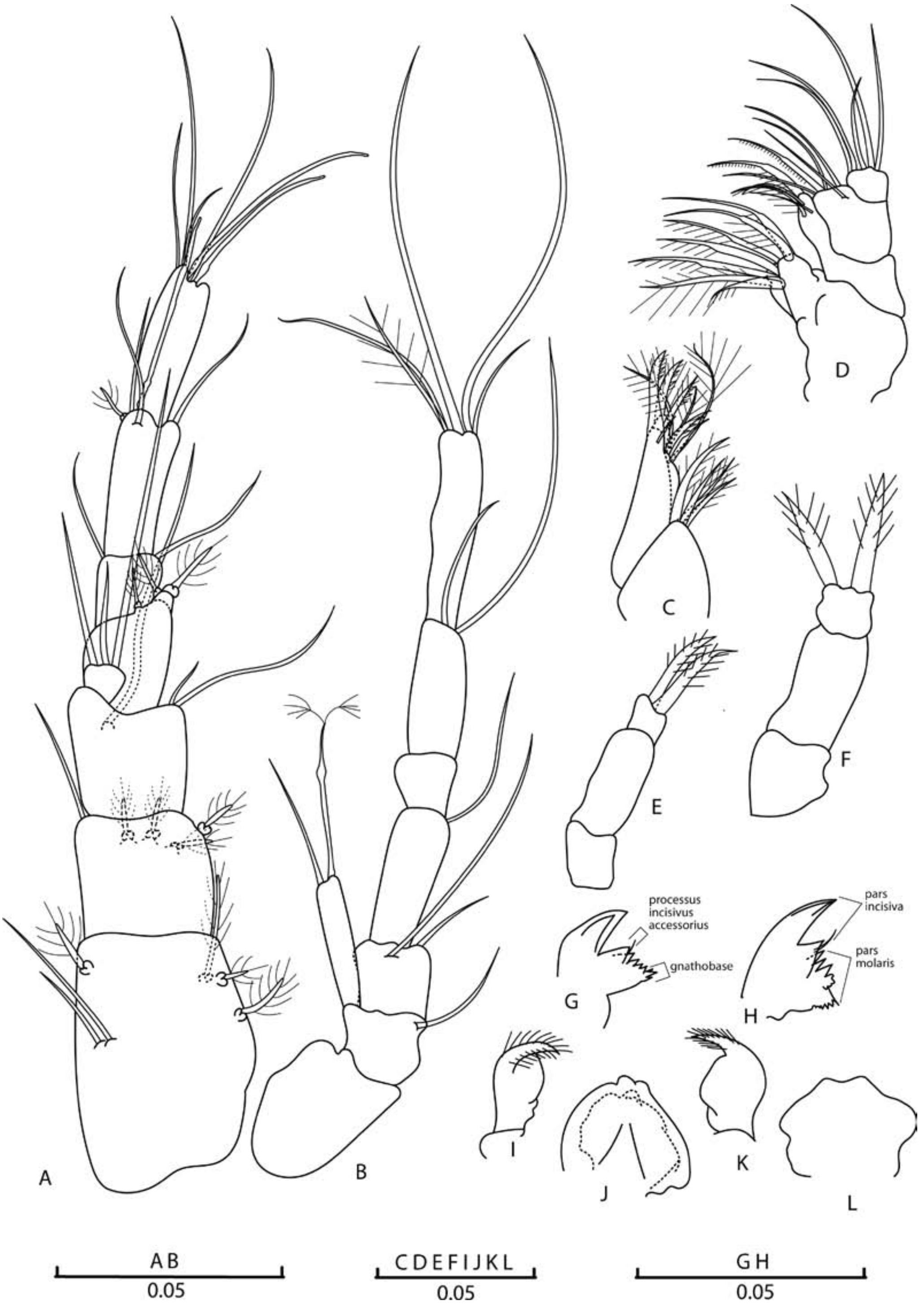

FIGURE 6 *Anguillanella callawaensis* gen. et sp. nov., male holotype (A–E, G); female allotype (F, H, K, L); male paratype (I, J). (A) Antennula (dorsal view); (B) antenna (dorsal view); (C) max Maxilla; (D) maxillula; (E) mandibular palp male holotype; (F) palp female allotype; (G) mandible male holotype; (H) mandible female allotype; (I) paragnath male WAMC57657 (J) labrum male WAMC57423 (ventral view); (K) Paragnath female allotype; (L) labrum female allotype (dorsal view). Scale bar in mm.

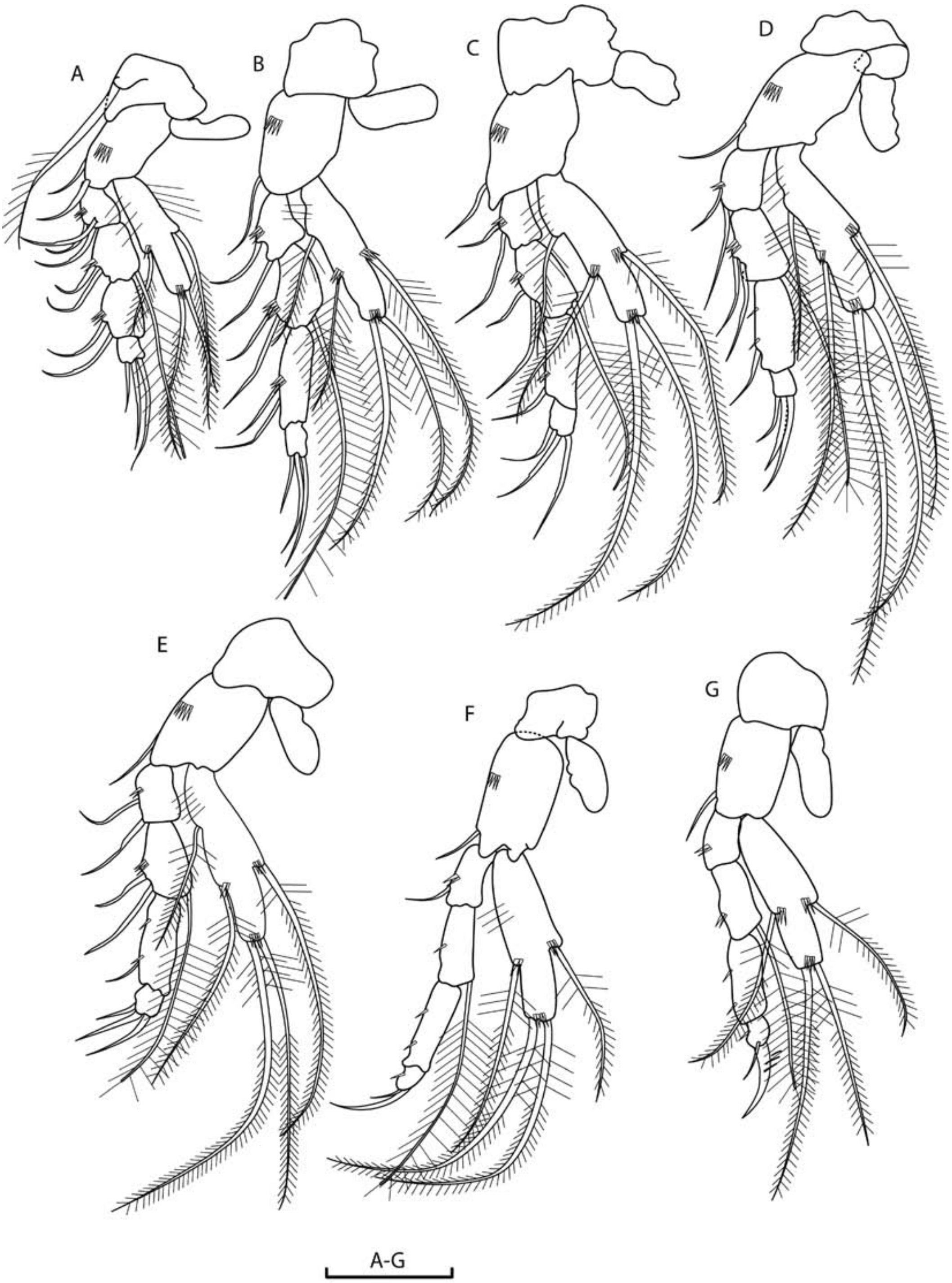

FIGURE 7 *Anguillanella callawaensis* gen. et sp. nov., male holotype. (A) Thoracopod I; (B) thoracopod II; (C) thoracopod III; (D) thoracopod IV; (E) thoracopod V; (F) Thoracopod VI; (G) thoracopod VII. Scale bar in mm.

FIGURE 8 *Anguillanella callawaensis* gen. et sp. nov., (A–D, F, G, H) male holotype. (A, B) thoracopod VIII (posterior view); (C, D) thoracopod VIII (frontal view); (E) thoracopod VIII female allotype (frontal view); (F) first pleopod; (G) furcal rami and dorsal seta (dorsal view); (H) uropod (latero-internal view). Scale bar in mm. Abbreviations: O. lb, outer lobe; Bsp, basipod; Endp, endopod; Exp, exopod; P.pr, posterior projection; Fr.pr, frontal projection.

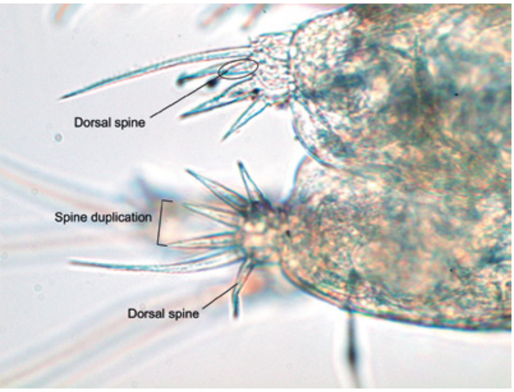

FIGURE 9 *Anguillanella callawaensis* furca variability (WAMC57370 photo).

& Eberhard (stygo net haul); WAMC57264, sex not recorded, whole specimen for DNA, Western Australia, Pilbara, bore CA0006, 26 April 2008, Bell & Eberhard (stygo net haul); WAMC57266, male, permanent slide, Western Australia, Pilbara, bore CA0008, 09 July 2009, Bell (stygo net haul); WAMC57267, male, permanent slide, Western Australia, Pilbara, bore CA0008, 09 July 2009, Bell (stygo net haul); WAMC57269, female, 100% ethanol, Western Australia, Pilbara, bore CA0008, 22 July 2009, Bell (stygo net haul); WAMC57270, female, 100% ethanol, Western Australia, Pilbara, bore CA0008, 22 July 2009, Bell (stygo net haul); WAMC57271, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0008, 22 July 2009, Bell (stygo net haul); WAMC57273, female, permanent slide, Western Australia, Pilbara, bore CA0011, 23 July 2009, Bell (stygo net haul); WAMC57274, sex not recorded, whole specimen for DNA, Western Australia, Pilbara, bore CA0011, 23 July 2009, Bell (stygo net haul); WAMC57275, male, permanent slide, Western Australia, Pilbara, bore CA0011, 23 July 2009, Bell (stygo net haul); WAMC57277, female, 100% ethanol, Western Australia, Pilbara, bore CA0011, 12 June 2009, Bell & Ridley (stygo net haul); WAMC57278, male, whole specimen for DNA, Western Australia, Pilbara, bore CA0011, 12 June 2009, Bell & Ridley (stygo net haul); WAMC57280, female, permanent slide, Western Australia, Pilbara, bore CA0012, 31 May 2009, Bell & Barnet (stygo net haul); WAMC57281, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0012, 31 May 2009, Bell & Barnet (stygo net haul); WAMC57283, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0013, 09 July 2009, Bell (stygo net haul); WAMC57285, male, permanent slide, Western Australia, Pilbara, bore CA0014, 09 July 2009, Bell (stygo net haul); WAMC57286, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0014, 09 July 2009, Bell (stygo net haul);WAMC57288, female, permanent slide, Western Australia, Pilbara, bore CA0021, 31 May 2009, Bell & Barnet (stygo net haul); WAMC57289, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0021, 31 May 2009, Bell & Barnet (stygo net haul); WAMC57290, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0021, 31 May 2009, Bell & Barnet (stygo net haul); WAMC57371, female, 100% ethanol, Western Australia, Pilbara, bore CA0157, 10 July 2009, Bell (stygo net haul); WAMC57373, female, 100% ethanol, Western Australia, Pilbara, bore CA0160, 09 July 2009, Bell (stygo net haul); WAMC57374, juvenile, whole specimen for DNA, Western Australia, Pilbara, bore CA0160, 09 July 2009, Bell (stygo net haul); WAMC57375, juvenile, whole specimen for DNA, Western Australia, Pilbara, bore CA0160, 09 July 2009, Bell (stygo net haul); WAMC57377, female, 100% ethanol, Western Australia, Pilbara, bore CA0099, 13 June 2009, Bell & Ridley (stygo net haul); WAMC57378, female, 100% ethanol, Western Australia, Pilbara, bore CA0099, 13 June 2009, Bell & Ridley (stygo net haul); WAMC57379, male, permanent slide Western Australia, Pilbara, bore CA0099, 13 June 2009, Bell & Ridley (stygo net haul); WAMC57380, female, permanent slide Western Australia, Pilbara, bore CA0099, 13 June 2009, Bell & Ridley (stygo net haul); WAMC57414, female, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57415, female, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul);

WAMC57416, sex not identifiable, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57417, female, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57419, female, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57420, male, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57421, female, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57422, female, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57423, male, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57424, female, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57425, male, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57426, juvenile, permanent slide Western Australia, Pilbara, bore CA0008 09 July 2009, Bell (stygo net haul); WAMC57657, male, permanent slide, Western Australia, Pilbara, bore CA0124, 14 June 2009, Bell & Ridley (stygo net haul); WAMC59191, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0124, 14 June 2009, Bell & Ridley (stygo net haul); WAMC59192, sex not recorded, whole specimen for DNA, Western Australia, Pilbara, bore CA0124, 14 June 2009, Bell & Ridley (stygo net haul); WAMC59193, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0124, 14 June 2009, Bell & Ridley (stygo net haul); WAMC59194, male, permanent slide, Western Australia, Pilbara, bore CA0057, 23 July 2009, Bell (stygo net haul); WAMC59195, male, permanent slide, Western Australia, Pilbara, bore CA0057, 23 July 2009, Bell (stygo net haul); WAMC59196, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0057, 23 July 2009, Bell (stygo net haul); WAMC59197, female, whole specimen for DNA, Western Australia, Pilbara, bore CA0057, 23 July 2009, Bell (stygo net haul);

Abbreviations used: Th, thoracopod; A.I, antennule; A.II, antenna; Md, mandible, Mx.I, maxillule and Mx.II maxilla.

Description (*based on adults*)

Body. Total length of holotype and allotype 1 mm. Total length of males 0.7–1 mm, and females 0.8–1. mm. Body elongated; almost cylindrical, nearly eight times as long as wide; segments slightly widening towards posterior end. Head slightly longer than wide. Pleotelson with one plumose dorsal seta on either side. All drawings are of the holotype and allotype except: paragnath in fig. 6I, and labrum in fig. 6J.

Antennule (fig. 6A). Seven-segmented; length of first three segments slightly longer than the last four; first segment is the longest, then sixth and seventh, as well as second and third, of similar length; fourth small and fifth very small; inner flagellum small and trapezoidal; setation as in fig. 6A; one and two aesthetascs on segments sixth and seventh respectively.

Antenna (fig. 6B). Seven-segmented; as long as the first six segments of A.I; first four segments almost as long as the last three ones; fifth (third of endopod) very small, without setae; terminal segment is the longest; first, fourth and sixth segments similar in length and about 0.8 times as long as seventh segment; setal formula: 0/1+exp/2+0/1+0/0/2+0/5; exopod about twice as long as second segment, with two terminal setae, one smooth and one bifurcated sensory seta; ventromedial seta absent.

Labrum (fig. 6J, L). Almost square, with smooth free edge, and with two small lobes, more or less developed, on the distal part.

Paragnath (fig. 6I, K). Almost rectangular, strong long claw on distal part with thick setation, distal ventral section slightly dilated.

Mandible (fig. 6E–H). Palp with three segments, terminal segment with two long and strong equal barbed claws, more or less cylindrical without expansions (fig. 6E, F). Masticatory part (fig. 6G, H): *pars incisiva* (incisor process) with two teeth;

processus incisivus accessorius with one tooth and one seta-like tooth; *pars molaris* with two dentate structures, parallel to main axis of teeth: first structure (closest to *processus incisivus accessorius*) with four teeth, and second structure bulbous with three to five small denticles.

Maxillule (fig. 6C). Proximal endite with four setae; distal endite with six teeth, four with denticles and two setae-like, and three plumose setae on the outer margin of endite.

Maxilla (fig. 6D). Four-segmented; setal formula 7, 4, 5, 5.

Thoracopods I-VII (fig. 7A–G). Epipod present on Th I to VII. Th I coxa with a long and strong plumose seta; basipod with two smooth setae. One-segmented exopod, shorter than endopod on all thoracopods; exopod reaches about the middle of the third segment of endopod in all thoracopods; it bears four barbed setae on Th I, Th VI and Th VII, two terminal, one dorsal and one ventral; and five barbed setae on Th III to V. Exopod of thoracopos I to VI with tuft of setules on ventral margin (on dorsal margin too on thoracopod I). Endopod with four segments in all thoracopods, setal formulae (number of setae on basipod in brackets):

Th I: (2) 3+0/2+1/2+0/3
Th II: (1) 2+0/2+1/2+0/3
Th III-IV-V: (1) 2+0/2+1/1+0/3
Th VI: (1) 1+0/0+1/0+0/2(1)
Th VII: (1) 0+0/0+1/0+0/2(1)

Male thoracopod VIII (fig. 8A–D). Small, rectangular and compact, with one lobe (outer lobe) on penial region (latero external part), almost trapezoidal very well developed and larger than the small triangular posterior projection (P. pr.). Small frontal projection (Fr. pr) integrated in penial region does not exceed the outer lobe and the basipod. Basipod vertical with big medial crest on distal end (bigger than O. lb.); endopod small, rectangular, with one short seta; exopod big, with simplified morphology and dilated on distal end, with two terminal setae, and with relatively square base and distal part curved outwardly, almost perpendicular to the main axis of basipod. The latero-internal view (fig. 8A–B) shows the relationship between basipod, exopod and endopod: distal end of basipod bears the endopod, concealed by the crest, and proximal end of basipod embedded near the base of the small penial region.

First pleopod (fig. 8F). Two segmented, first segment with one long seta; second segment with five setae, two distal of similar length and three subdistal unequal setae.

Female allotype thoracopod VIII (fig. 8E). Coxa without setae; basipod almost square; one small ramus, half of the size of basipod, with two setae (one thick and very long); very large epipod, about 2.5 times length of basipod.

Female allotype Th I-VII. Number of segments of endopods and exopods as in male holotype. Number of setae on segments of endopod and basipod differs from male holotype. Setal formulae of allotype (number of setae of basipod in brackets at the start):

Th I: (2) 3+0/2+1/2+0/3
Th II: (1) 2+0/2+1/2+0/3
Th III: (1) 1+0/2+1/1+0/3
Th IV-V: (1) 2+0/2+1/1+0/3
Th VI- VII: (1) 0+0/0+1/0+0/2(1)

Uropods (fig. 8H). Sympod 1.8 times longer than endopod, rectangular, about 1.5 times longer than wide, with four spines: first two distal spines smallest, almost half the length of endopod; third one as long as endopod; and fourth (proximal spine) two-third the length of third spine, but longer than first two distal ones. Endopod with spinous projection on distal outer corner, twice as long as exopod, bearing two strong claws ("uropodial claws" *sensu* Delamare Deboutteville & Serban, 1973) (distal claw 1.5 times longer than proximal claw); one "special seta" ("X", which would correspond to the first claw on *Bathynella* according to Delamare Deboutteville & Serban, 1973); one plumose seta near the base on the ventral edge; one long terminal and one short subterminal seta, ventrally located. Exopod with four setae: two medial setae and two terminal (one very long and thick and the other one short and thinner).

Pleotelson (fig. 8G). With one long, plumose dorsal seta on either side.

Furcal rami (fig. 8G, H). Small, almost square, bearing five spines; dorsal spine as long as second and fourth, third longer than second and first twice the length of second spine.

Variability

The number of setae on segments of thoracopod endopod and second segment of pleopod vary; pleopod can have five to eight setae on second segment and the same specimen can have left setal formula different from right side. Spines on furca can have different size compare to the holotype; in some specimens the dorsal spine is half of the size of the second spine, and spine duplication can result in six spines on furca (fig. 9).

Etymology

The genus name comes from the Latin translation of Eel creek (Eel = Anguilla), which separates Callawa from Cundaline ridge. Substantive in opposition. The species name derives from the Callawa ridge where the species was collected.

Muccanella Perina and Camacho gen. nov.

Generic diagnosis

Zoobank: urn:lsid:zoobank.org:act:24FC9F35-6C73-4B99-AD19-C953E813EB7F

Antennula seven-segmented. Antenna seven-segmented; third endopodial segment very small. Paragnaths with distal strong claws. Mandibular palp without sexual dimorphisms. Setae of mandibular palp similar in both sexes. Endopod of thoracopods I to VII four-segmented. Male thoracopod VIII compact and trapezoidal with only one small lobe (outer lobe) on penial region (latero external part) and two small projections (frontal and posterior); large rectangular basipod in vertical position with small distal crest; endopod small with one short seta; exopod perpendicular to principal axis of basipod, bearing two distal setae of similar length. Female thoracopod VIII simplified: coxopod without setae; long epipod; basipod slightly longer than coxopod bearing one seta; two-segmented ramus as long as basipod, with two distal setae: one short and one long. Uropod: sympod with four strong and big subequal spines, endopod with two spines, one “special” seta and three more setae. Furcal rami with five spines.

Type species: *Muccanella cundalinensis* gen. nov., sp. nov

Muccanella cundalinensis Perina and Camacho sp. nov. (figs. 10–12)

Zoobank: urn:lsid:zoobank.org:act:42CAEBC3-AE62-4346-9C20-E0E0AE64DCFC

Type locality. Bore CU0046, Cundaline Ridge, De Grey River Catchment, Pilbara, Western Australia (see appendix 3 for borehole coordinates).

Material examined

Holotype. WAMC57340, male, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet.

Allotype. WAMC57337, female, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet.

Paratypes. WAMC57338, female, whole specimen for DNA, bore CU0046, 29 May 2009, Bell & Barnet; WAMC57339, female, whole specimen for DNA, bore CU0046, 29 May 2009, Bell & Barnet; WAMC57341, female, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet; WAMC57343, male, permanent slide, bore CU0064, 21 July 2009, Bell; WAMC57344, female, whole specimen for DNA, bore CU0064, 21 July 2009, Bell; WAMC57347, female, permanent slide, bore CU0064, 21 July 2009, Bell; WAMC57348, male, whole specimen for DNA, bore CU0064, 21 July 2009, Bell; WAMC57363, female, permanent slide, bore CU0058, 29 May 2009, Bell & Barnet; WAMC57364, male, permanent slide, bore CU0058, 29 May

FIGURE 10 *Muccanella cundalinensis* gen. et sp. nov., male holotype (A, B, D, F, H, I); female allotype (E, G); male paratype (C). (A) Antennula (dorsal view); (B) antenna (dorsal view); (C) Paragnath male WAMC57343; (D) labrum; (E) labrum female WAMC57341; (F) palp and mandible male holotype; (G) palp and mandible female allotype; (H) maxillule; (I) maxilla. Scale bar in mm.

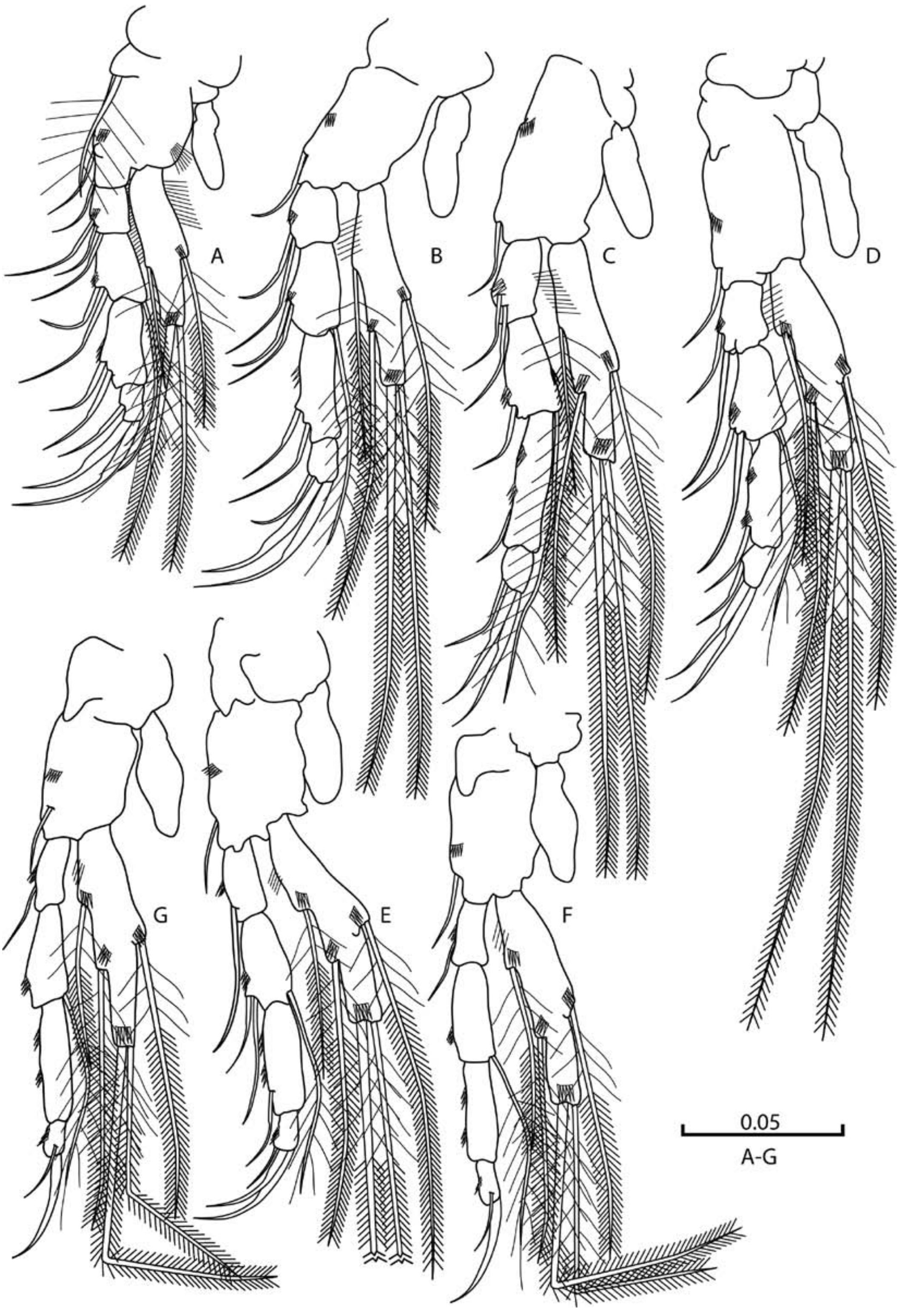

FIGURE 11 *Muccanella cundalinensis* gen. et sp. nov., male holotype. (A) Thoracopod I; (B) thoracopod II; (C) thoracopod III; (D) thoracopod IV; (E) thoracopod V; (F) Thoracopod VI; (G) thoracopod VII. Scale bar in mm.

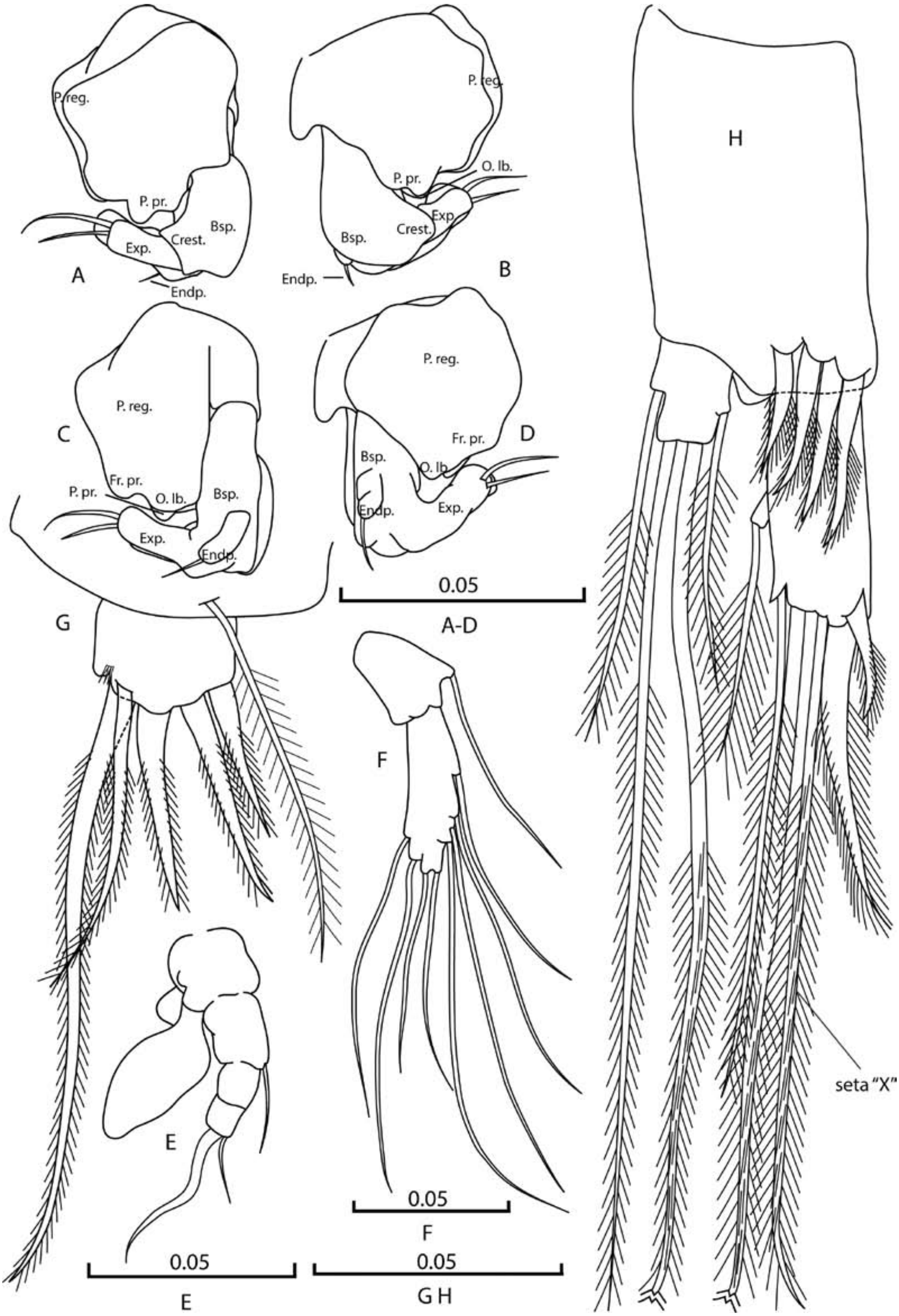

FIGURE 12 *Muccanella cundalinensis* gen. et sp. nov., (A–D, F–H) male holotype; female allotype (E). (A, B) thoracopod VIII (posterior view); (C, D) thoracopod VIII (frontal view); (E) thoracopod VIII female allotype (frontal view); (F) first pleopod; (G) furcal ramus and dorsal seta (dorsal view); (H) uropod (dorsal view). Scale bar in mm. Abbreviations: O. lb, outer lobe; Bsp, basipod; Endp, endopod; Exp, exopod; P.pr, posterior projection; Fr.pr, frontal projection.

2009, Bell & Barnet; WAMC57365, female, whole specimen for DNA, bore CU0058, 29 May 2009, Bell & Barnet; WAMC57366, juvenile, whole specimen for DNA, bore CU0058, 29 May 2009, Bell & Barnet; WAMC57368, female, permanent slide, bore CU0285, 30 May 2009, Bell & Barnet; WAMC59198, male, permanent slide, bore CU0058, 10 June 2009, Bell & Ridley; WAMC59199, sex not recorded, permanent slide, bore CU0058, 10 June 2009, Bell & Ridley; WAMC59205, female, permanent slide, bore CU0058, 29 May 2009, Bell & Barnet; WAMC59206, male, permanent slide, bore CU0064, 21 July 2009, Bell; WAMC59207, male, permanent slide, bore CU0064, 21 July 2009, Bell; WAMC59208, male, permanent slide, bore CU0064, 21 July 2009, Bell; WAMC59209, male, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet; WAMC59210, female, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet; WAMC59211, male, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet; WAMC59212, male, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet; WAMC59213, female, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet; WAMC59214, male, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet; WAMC59215, male, permanent slide, bore CU0046, 29 May 2009, Bell & Barnet;

Abbreviations used: Th, thoracopod; A.I, antennule; A.II, antenna; Md, mandible, Mx.I, maxillule and Mx.II maxilla.

Description (*based on adults*)

Body. Total length of holotype about 0.95 mm and allotype 0.94 mm. Total length of males 0.82–1.08 mm, of females 0.92–1.07 mm. Body almost cylindrical, nearly six times as long as wide; segments slightly widening towards posterior end. Head as long as wide. Pleotelson with one plumose dorsal seta on either side. All drawings are of the holotype and allotype except for fig. 10C, E.

Antennule (fig. 10A). Seven-segmented; length of first three segments as long as the last three; first and last segments are the longest, then sixth, second and third of similar length and fourth slightly shorter, fifth very small; inner flagellum small and longer than wide; setation as in fig. 10A; two aesthetascs on both sixth and seventh segments.

Antenna (fig. 10B). Seven-segmented; nearly as long as A.I; first four segments almost as long as the last three combined; fifth (third of endopod) very small, without setae; terminal segment is the longest, fourth and sixth segments similar in length and about three-fourth the length of seventh segment; setal formula: 0/1+exp/2+0/1+0/0/2+0/5; exopod 0.8 times the length of the first segment, with two terminal setae, one smooth and one bifurcated sensory seta; ventromedial seta absent.

Labrum (fig. 10D, E). Almost square, with smooth free edge, and with two small lobes on the distal part.

Paragnath (fig. 10C). Almost rectangular, strong long claw on distal part with thick setation.

Mandible (fig. 10F, G). Palp with three segments, terminal segment with two long and strong barbed claws of similar length, more or less cylindrical without expansions. Masticatory part: *pars incisiva* with two teeth; *processus incisivus accessorius* with one tooth and one seta-like tooth; *pars molaris* with three teeth close to *processus incisivus accessorius* and one distal strong tooth with one denticle on each side.

Maxillule (fig. 10H). Proximal endite with four setae; distal endite with six teeth, four with denticles and two setae-like, and three plumose setae in outer margin of endite.

Maxilla (fig. 10I). Four-segmented; setal formula 7, 3, 7, 5.

Thoracopods I-VII (fig. 11A–G). Epipod present on Th I to VII. Th I coxa with a long and strong plumose seta and an external bundle of thin hair; basipod with two smooth setae. One-segmented exopod, shorter than endopod on all thoracopods; exopod reaches about the middle of the third segment of endopod in all thoracopods; it bears four barbed setae on Th I, two terminal, one dorsal and one ventral; and five barbed setae on Th II to VII. Exopod of thoracopos I to VI with tuft of setules on ventral margin (on dorsal margin too on thoracopod I). Endopod with four segments in all thoracopods, setal formulae (number of setae on basipod in brackets):

Th I: (2) 3+0/2+1/2+0/3
Th II: (1) 2+0/2+1/2+0/3
Th III: (1) 2+0/1-2+1/1+0/3
Th IV-V: (1) 2+0/2+1/1+0/3
Th VI- VII: (1) 1+0/0+1/0+0/2(1)

Male thoracopod VIII (fig. 12A–D). Compact and trapezoidal, with one small lobe (outer lobe) on penial region (latero external part). Small frontal and posterior projection (Fr. pr; P. pr.) integrated in penial region. Basipod vertical with small crest on distal end; endopod small, rectangular, with one short seta; exopod with simplified morphology, curved outward, almost perpendicular to the main axis of basipod and bearing two setae of slightly unequal length on distal part. Penial region well developed.

First pleopod (fig. 12F). Two segmented, first segment enlarged with one long seta; second segment with eight smooth setae, two distal of similar length and shorter than all the others.

Female allotype thoracopod VIII (fig. 12E). Coxa without setae; basipod rectangular bearing one inner seta; one ramus two-segmented, same size of basipod, with two setae of different length (one thick and very long) on distal end; very large epipod, about twice the length of basipod.

Female allotype Th I-VII. Number of segments of endopods and exopods as in male holotype. Number of setae on segments of endopod and basipod differs from male holotype. Setal formulae of allotype (number of setae of basipod in brackets at the start):

Th I: (2) 3+0/2+1/2+0/3
Th II: (1) 2+0/2+1/2+0/3
Th III: (1) 2+0/2+1/1+0/3
Th IV-V: (1) 2+0/2+1/1+0/3
Th VI- VII: (1) 1+0/0+1/0+0/2(1)

Uropods (fig. 12H). Sympod 1.5 times the length of endopod, rectangular, about 1.5 times longer than wide, with four spines of similar length. Endopod with spinous projection on distal outer corner, 2.5 times longer than exopod, with two strong claws ("uropodial claws" *sensu* Delamare Deboutteville & Serban, 1973) (distal one 2.5 times longer than proximal one); one "special seta" ("X", which correspond to the first claw on *Bathynella* according to Delamare Deboutteville & Serban, 1973); one plumose seta near the base on the ventral edge; one long terminal and one short subterminal seta, longer than distal claw and ventrally located. Exopod with four setae: two medial setae and two terminal (one very long and thick, and one short and thinner).

Pleotelson (fig. 12G). With one long, plumose dorsal seta on either side near the base of furca.

Furcal rami (fig. 12G). Small, almost square, bearing five spines; first spine is the longest, about three times the length of the second one; second and third spines of similar length and 2/3 shorter than dorsal one; fourth spine shortest.

Variability
The number of setae on segments of endopod of thoracopods and second segment of pleopod can vary (usually one more or one less seta).

Etymology
The genus name comes from the name of the station near the type locality: Muccan Station). The species name derives from the Cundaline ridge where the species was collected.

Morphological remarks

Anguillanella and *Muccanella* genera share few characters: mandibular palp, mandible, labrum and paragnaths are very similar and also similar to *Pilbaranella* and *Fortescuenella* described from the Fortescue catchment (although the mandibular palp of *Fortescuenella* has claws of slightly different length). All thoracopods of *Pilbaranella, Anguillanella* and *Muccanella* have exopod bearing five setae (excluding thoracopod I that has four setae, and thoracopod VI and VII of *Anguillanella* that also have four). *Fortescuenella serenitatis* differs from these genera having all exopods of thoracopos bearing four setae, and lacking epipod on thoracopod I.

The structure of the male thoracopod VIII is quite diverse in all four genera described for

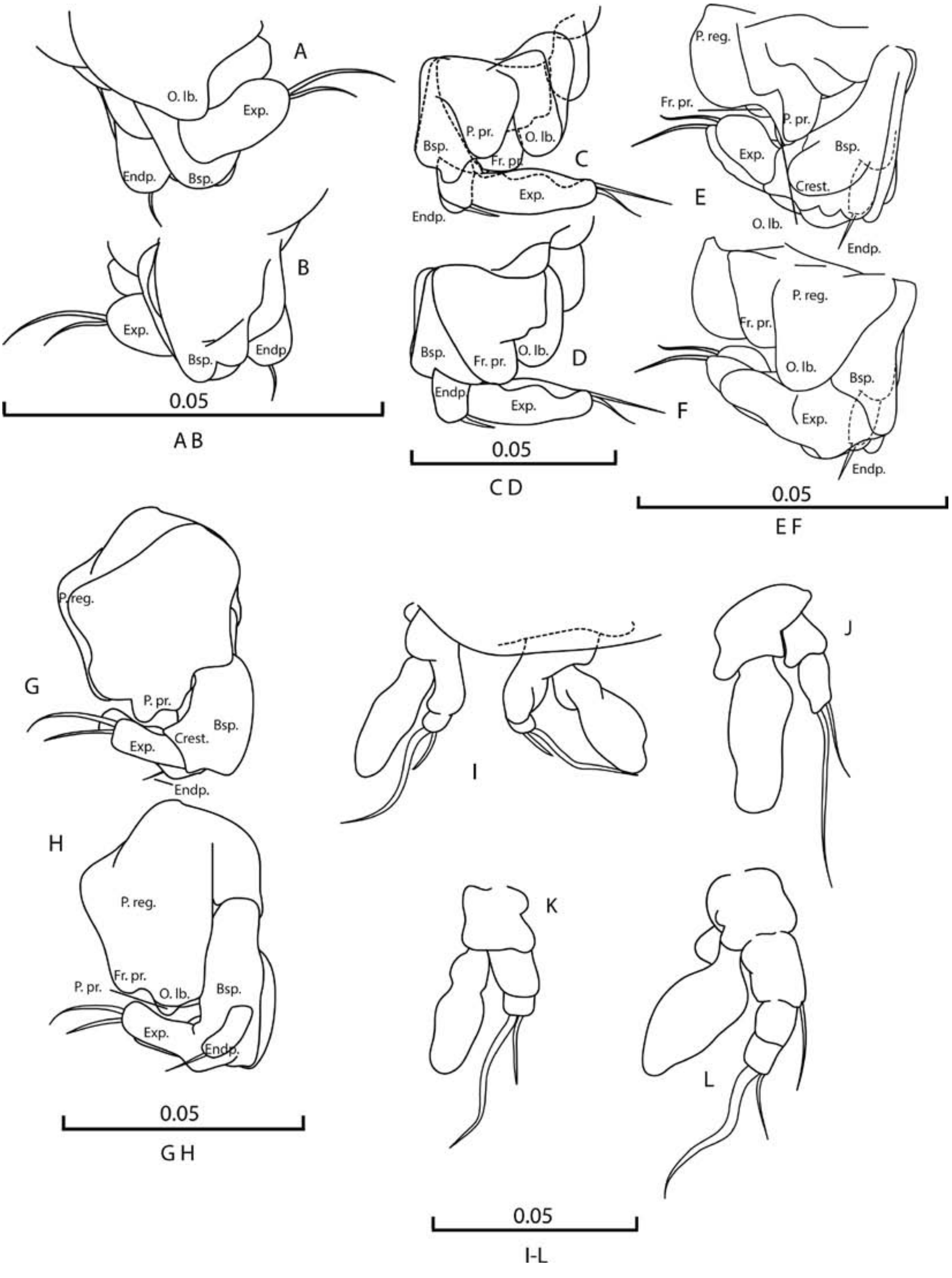

FIGURE 13 Male and female thoracopods VIII of the four genera described for WA. (A, B) male ThVIII of *Pilbaranella ethelensis*; (C, D) male ThVIII of *Fortescuenella serenitatis*; (E, F) male ThVIII of *Anguillanella callawaensis*; (G, H) male ThVIII of *Muccanella cundalinensis*; (I) female ThVIII of *Pilbaranella ethelensis;* (J) female ThVIII of *Fortescuenella serenitatis;* (K) female ThVIII of *Anguillanella callawaensis;* (L) female ThVIII of *Muccanella cundalinensis.* Scale bar in mm

WA (fig. 13) but all have endopod and exopod reduced, and the latter curved backwards like Austrobathynellinae, while Bathynellinae and Gallobathynellinae have unfolded and more developed rami. In *Pilbaranella* the male thoracopod VIII is simplified and reduced; in *Fortescuenella* it is more complex with a frontal and posterior projection and a well-developed outer lobe; in *Muccanella* and *Anguillanella* we have the same structures present in *Fortescuenella* (frontal-posterior projection and outer lobe), but in *Muccanella* they are reduced while they are well-developed in *Anguillanella,* and both taxa have basipod with crest. Setation on endopod and exopod is the same in all four genera (one short seta on endopod and two setae on exopod).

Female thoracopod VIII (fig. 13) is uniramus like in *Austrobathynella patagonica* Delamare Deboutteville & Roland, 1963; it is very simple in *Pilbraranella* with most of the segments fused; in *Fortescuenella* and *Anguillanella* it is formed by: coxopod, basipod and one-segmented ramus, while in *Muccanella* the ramus is two-segmented. All genera so far described for WA present epipod, contrary to *Austrobathynella patagonica.*

The pleopod I differs in all taxa by the number of setae, which are all quite elongated in *Pilbraranella, Anguillanella* and *Muccanella,* while *Fortescuenella* presents one very short apical seta. Another character that distinguishes *Fortescuenella* is the absence of the "special seta X" (Delamare Deboutteville & Serban, 1973), which is present in the other three genera. All four taxa have four spines on sympod (but different length), two on endopod of uropod and five on furca with distinct ration length.

Specimens from bore YP1063 show affinity to *Anguillanella callawaensis,* but also distinctive morphological characters. The only specimen sequenced from bore YRP22 on Yarrie Ridge was a juvenile wholly used for DNA extraction, so no morphological characters are available, but the molecular data place it within the "*Anguillanella* group" therefore we included it in this genus. Specimens from bores CA0021 and CA0099 have slightly different morphology from the rest of *A. callawaensis*. Differences could represent intraspecific variation or perhaps the population from these bores is diverging, but not long enough to show clear morphological differences.

Bathynella primaustraliensis (Schminke, 1973), the only bathynellid species described for Australia before 2018 and collected from the Hughes creek in Victoria, differs from the new genera in mandible and female thoracopod VIII. A comparison of the male thoracopod VIII (penis) is not possible since the description is based on one female (Schminke, 1973), but we justify the erection of two new genera based on molecular evidence and that bathynellid species and genera of the Pilbara bioregion seem to be restricted to well-defined parts of the catchments where they occur (Perina et al., 2018, 2019).

As *Pilbaranella* and *Fortescuenella*, we exclude the new genera from Bathynellinae and Gallobathynellinae subfamilies based on: number of teeth on pars molaris and structure of male and female thoracopod VIII, supporting the molecular data.

The new genera exhibit some common characters with members of the Austrobathynellinae subfamily, but they are quite distinguished from members of Bathynellinae and Gallobathynellinae (table 5). With *Austrobathynella* Delamare Deboutteville, 1960, *Transvaalthynella* Serban & Coineau, 1975 and *Transkeithynella* Serban & Coineau, 1975, genera belonging to Austrobathynellinae, *Anguillanella* and *Muccanella* present: many teeth on *pars molaris* of the mandible; female thoracopod VIII reduced to one ramus; male thoracopod VIII with reduced endopod and exopod; and third segment of antennule's endopod very small (short). The paucity of data (only three genera are described based solely on morphological data) prevents us from corroborating the affinity of the new genera *Anguillanella* and *Muccanella* to Austrobathynellinae, nevertheless they are more dissimilar from Bathynellinae and Gallobathynellinae and therefore we exclude them. Further morphological and molecular data are needed to outline Bathynellidae subfamilies, resolve taxonomic uncertainties, and systematically organise the taxa discovered in different continents.

TABLE 5 Differences and similarities among Central Hamersley Range species and lineages, *Pilbaranella* genus and the three Bathynellidae subfamilies. Austrobathynellinae*: three species described, two species represented by male only. AI = antenna I; AII = antenna II; Th = thoracopod, N/A = not available

	Gallobathynellinae	**Bathynellinae**	**Austrobathynellinae***	***Pilbaranella***	***Fortescuenella***	**Bathynellidae sp SVW**	**Bathynellidae sp FLO**	***Anguillanella***	***Muccanella***
AI: number of segments	6/7	7	7	7	7	7	N/A	7	7
AII: number of segments	6/7/8	7	7/8	7	7	7	N/A	7	7
endopod segment 3	short/long	long	very short	very short	short	very short	N/A	very short	very short
exopod: medial seta	present/absent	present/absent	absent	absent	absent	N/A	N/A	absent	absent
Mandible:									
palp	1 to 3 segments	3 segments	3 segments	3 segments	3 segments	N/A	N/A	3 segments	3 segments
pars molaris (number of teeth)	2/3	2/4	5/6	6	7	N/A	N/A	7 to 9	6
sexual dimorphism	yes/no	no	no	no	no	N/A	N/A	no	no
Th I-Th VII: endopod	3/4 or 4 segments	4 segments	4 segments	4 segments	4 segments	N/A	N/A	4 segments	4 segments
Th VIII female:	protopod + 1/2 rami	protopod + 2 rami	protopod + 1 ramus	protopod + 1 ramus	protopod + 1 ramus	N/A	N/A	protopod + 1 ramus	protopod + 1 ramus
rami (endop-exop)	1 segment	1 segment	2 segments	1 segment	2 segments	N/A	N/A	1 segment	2 segments
coxal seta	present/absent	present	absent	absent	absent	N/A	N/A	absent	absent
epipod	present/absent	present	absent	present	present	N/A	N/A	present	present

Th VIII male:									
penial region	1 to 3 lobes, frontal projection, 0 to 2 crests	3 lobes, frontal projection	1 to 4 lobes, 0-1 crest	1 lobe	1 lobe, frontal/posterior projections	N/A	N/A	1 lobe, frontal/posterior projections	1 lobe, frontal/posterior projections
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	vertical; fused with the penial region	N/A	N/A	vertical; not fused with the penial region/independent	vertical; not fused with the penial region/independent
endopod	small or absent	small-1 segment	1-2 segment	small, 1 segment	small, 1 segment	N/A	N/A	small, 1 segment	small, 1 segment
exopod	like exopod of thoracopods	like exopod of thoracopods	reduced/curve backwards	reduced/curve backwards	reduced/curve backwards	N/A	N/A	reduced/curve backwards	reduced/curve backwards
Uropod:									
sympod	4 spines (maximum)	4 spines (minimum)	4 spines (maximum)	4 spines	4 spines	5 spines	4 spines	4 spines	4 spines
endopod	2-4 claws + 4 setae	3-4 claws + 3-4 setae	2 claws + 3 setae + 1 special seta	2 claws + 3 setae + 1 special seta	2 claws + 4 setae	3 claws + 5 setae	2 claws + 3 setae + 1 special seta	2 claws + 3 setae + 1 special seta	2 claws + 3 setae + 1 special seta

APPENDIX 2

GenBank accession numbers (WA: Western Australia; QLD: Queensland; SA: South Australia; UK: United Kingdom)

sp ID	Bore code/Location	Country	Museum Voucher #	GenBank Accession Numbers				
				COI	18S	16S	28S	ITS2 Acc
Iberobathynella imuniensis	Torca de los Morteros	Spain	MNCN:ADN:29166	HQ659850	KC469528	–	–	–
Bathynella sp.	Kamniska Cave	Slovenia	MNCN:ADN:54668	MF114309	MF094716	–	–	–
Bathynella sp.	Trubarjeva Cave	Slovenia	MNCN:ADN:54718	MF114308	MF094715	–	–	–
Bathynellidae sp.1	Lubra Water	South Australia	N/A	JN817409	–	–	–	–
Bathynellidae sp.2	Lubra Water	South Australia	N/A	JN817410	JQ446079	–	–	–
Bathynellidae sp. D3.B	Dawson Catchment	Queensland	N/A	KX022544	KX022517	–	–	–
Bathynellidae sp. D14.B	Dawson Catchment	Queensland	N/A	KX022541	KX022514	–	–	–
Antrobathynella stammeri	Ogof Cave	United Kingdonm	MNCN:ADN:54711	MF114307	MF094714	–	–	–
Gallobathynella buoi	La Devèze Cave	France	MNCN/ADN54600	KP974146	KP999757	–	–	–
Gallobathynella coiffatti	Falgas Cave	France	MNCN/ADN54602	–	KP999759	–	–	–
Gallobathynella tarissei	Limousis Cave	France	MNCN/ADN54592	–	KP999752	–	–	–
Paradoxiclamousella sp.	CO.209 Cave	Spain	MNCN:DNA:29594	JX121252	JX121235	–	–	–
Paradoxiclamousella fideli	Fuente del Carnero Cave	Spain	MNCN:ADN:29735	JX121249	KC469523	–	–	–
Vejdovskybathynella caroloi	Gándara Cave	Spain	MNCN:ADN:29877	–	KC469525	–	–	–
Vejdovskybathynella edelweiss	Ojo Guareña Cave	Spain	MNCN:ADN:29440	HQ596571	KC469513	–	–	–
Vejdovskybathynella vasconica	Goikoetxe Cave	Spain	MNCN:ADN:29623	–	KC469516	–	–	–
Hobbsinella edwardensis	Edward Aquifer	Texas (USA)	MNCN:ADN:29943	–	KP999685	–	–	–
Pacificabathynella yupik	Kwethluk River	Alaska (USA)	MNCN:ADN:29963	KF974126	–	–	–	–
Pilbaranella ethelensis	W088	Western Australia	WAMC57310	MF074349	MN149123	MF042231	MF042304	–
Pilbaranella sp. A	HST0186R	Western Australia	WAMC57617	MF074397	MN149139	MF042276	MF042336	–
Pilbaranella sp. B	WP14S	Western Australia	WAMC57481	MF074381	MF042212	MF042258	MF042323	–
Pilbaranella sp. C	HST0723R	Western Australia	WAMC60391	MF074409	MN149150	MF042291	MF042348	–
Pilbaranella sp. poss D	HHS0032	Western Australia	WAMC57475	MF074376	MF042211	MF042254	MF042320	–
Fortescuenella serenitatis	SOM049	Western Australia	WAMC57313	MK134990	–	MK134902	MK134948	–
Bathynellidae Mount Florence	Deep Well	Western Australia	WAMC57603	MK135031	MK134944	MK134931	MK134981	–
Bathynellidae nr *Fortescuenella*	SM5422	Western Australia	WAMC57630	MK135040	MK134946	–	–	–
Bathynellidae Sheila Valley West	SV0138	Western Australia	WAMC57538	MK135021	MK134943	MK134920	–	–
Anguillanella callawaensis	CA0006	Western Australia	WAMC57258	MF074337	MF042209	MF042218	MF042295	MN149073
Anguillanella callawaensis	CA0006	Western Australia	WAMC57260	–	–	–	–	MN149074
Anguillanella callawaensis	CA0006	Western Australia	WAMC57262	–	–	–	–	MN149075
Anguillanella callawaensis	CA0006	Western Australia	WAMC57263	MN136071	MN149117	MN149277	–	MN149076

Anguillanella callawaensis	CA0006	Western Australia	WAMC57264	–	–	–	–	MN149077
Anguillanella callawaensis	CA0008	Western Australia	WAMC57266	MN136072	MN149118	MN149278	–	–
Anguillanella callawaensis	CA0008	Western Australia	WAMC57267	MN136073	MN149119	MN149279	–	MN149078
Anguillanella callawaensis	CA0008	Western Australia	WAMC57269	–	–	–	MN149230	MN149079
Anguillanella callawaensis	CA0008	Western Australia	WAMC57270	–	–	–	MN149231	MN149080
Anguillanella callawaensis	CA0008	Western Australia	WAMC57271	–	–	–	MN149232	–
Anguillanella callawaensis	CA0011	Western Australia	WAMC57274	–	–	MN149280	MN149233	–
Anguillanella callawaensis	CA0011	Western Australia	WAMC57275	–	–	MN149281	MN149234	MN149081
Anguillanella callawaensis	CA0011	Western Australia	WAMC57277	–	–	–	MN149235	MN149082
Anguillanella callawaensis	CA0011	Western Australia	WAMC57278	MN136074	–	MN149282	MN149236	MN149083
Anguillanella callawaensis	CA0012	Western Australia	WAMC57280	–	–	MN149283	MN149237	–
Anguillanella callawaensis	CA0012	Western Australia	WAMC57281	–	–	MN149284	MN149238	MN149084
Anguillanella callawaensis	CA0013	Western Australia	WAMC57283	MN136075	MN149120	MN149285	MN149239	MN149085
Anguillanella callawaensis	CA0014	Western Australia	WAMC57285	–	–	MN149286	MN149240	MN149086
Anguillanella callawaensis	CA0014	Western Australia	WAMC57286	MN136076	–	–	MN149241	MN149087
Anguillanella callawaensis	CA0021	Western Australia	WAMC57288	MN136077	MN149106	MN149287	MN149242	MN149088
Anguillanella callawaensis	CA0021	Western Australia	WAMC57289	MN136078	MN149121	MN149288	MN149243	MN149089
Anguillanella callawaensis	CA0021	Western Australia	WAMC57290	MN136079	MN149122	MN149289	MN149221	MN149090
Muccanella cundalinensis	CU0046	Western Australia	WAMC57337	MN136080	MN149113	MN149290	MN149228	–
Muccanella cundalinensis	CU0046	Western Australia	WAMC57338	–	–	MN149291	MN149244	–
Muccanella cundalinensis	CU0046	Western Australia	WAMC57339	MN136081	MN149124	MN149292	–	–
Muccanella cundalinensis	CU0046	Western Australia	WAMC57340	MN136082	MN149125	MN149293	MN149245	–
Muccanella cundalinensis	CU0046	Western Australia	WAMC57341	MN136083	MN149126	MN149294	MN149246	–
Muccanella cundalinensis	CU0064	Western Australia	WAMC57343	–	–	–	MN149222	–
Muccanella cundalinensis	CU0064	Western Australia	WAMC57344	–	–	–	MN149229	–
Muccanella cundalinensis	CU0064	Western Australia	WAMC57347	–	–	MN149295	–	–
Muccanella cundalinensis	CU0064	Western Australia	WAMC57348	–	–	MN149296	–	–
Anguillanella sp. 1	YP1063	Western Australia	WAMC57350	–	MN149127	MN149297	MN149247	MN149091
Anguillanella sp. 1	YP1063	Western Australia	WAMC57351	–	MN149107	–	MN149248	–
Anguillanella sp. 1	YP1063	Western Australia	WAMC57352	–	MN149128	–	MN149249	MN149092
Anguillanella sp. 1	YP1063	Western Australia	WAMC57353	–	MN149108	–	MN149250	MN149093
Anguillanella sp. 1	YP1063	Western Australia	WAMC57354	–	MN149129	MN149298	MN149251	MN149094
Anguillanella sp. 1	YP1063	Western Australia	WAMC57355	–	MN149130	MN149299	MN149252	MN149095
Anguillanella sp. 1	YP1063	Western Australia	WAMC57356	–	MN149109	MN149300	MN149253	MN149096

GenBank accession numbers (WA: Western Australia; QLD: Queensland; SA: South Australia; UK: United Kingdom) (*cont.*)

sp ID	Bore code/Location	Country	Museum Voucher #	GenBank Accession Numbers				
				COI	18S	16S	28S	ITS2 Acc
Anguillanella sp. 2	YRP22	Western Australia	WAMC57358	–	MN149131	MN149301	MN149254	MN149097
Anguillanella sp. 1	YP1063	Western Australia	WAMC57360	–	MN149132	MN149302	MN149255	–
Anguillanella sp. 1	YP1063	Western Australia	WAMC57361	–	MN149110	–	MN149256	–
Muccanella cundalinensis	CU0058	Western Australia	WAMC57363	–	MN149133	MN149303	MN149223	–
Muccanella cundalinensis	CU0058	Western Australia	WAMC57364	–	MN149111	MN149304	MN149224	–
Muccanella cundalinensis	CU0058	Western Australia	WAMC57365	–	MN149134	–	MN149225	–
Muccanella cundalinensis	CU0058	Western Australia	WAMC57366	–	MN149135	MN149305	MN149226	–
Muccanella cundalinensis	CU0285	Western Australia	WAMC57368	MN136084	MN149112	MN149306	MN149257	–
Anguillanella callawaensis	CA0157	Western Australia	WAMC57370	–	–	MN149307	MN149258	–
Anguillanella callawaensis	CA0157	Western Australia	WAMC57371	–	–	MN149308	MN149259	MN149098
Anguillanella callawaensis	CA0160	Western Australia	WAMC57373	MN136085	MN149114	–	MN149260	MN149099
Anguillanella callawaensis	CA0160	Western Australia	WAMC57374	MN136086	MN149115	–	MN149261	MN149100
Anguillanella callawaensis	CA0160	Western Australia	WAMC57375	–	–	–	MN149262	MN149101
Anguillanella callawaensis	CA0099	Western Australia	WAMC57377	MN136087	MN149116	MN149309	MN149263	MN149102
Anguillanella callawaensis	CA0099	Western Australia	WAMC57378	MN136088	MN149136	MN149310	MN149264	MN149103
Anguillanella callawaensis	CA0099	Western Australia	WAMC57379	MN136089	MN149137	–	MN149265	MN149104
Anguillanella callawaensis	CA0099	Western Australia	WAMC57380	MN136090	MN149138	–	MN149266	MN149105
Anguillanella callawaensis	CA0124	Western Australia	WAMC57657	–	MN149140	MN149311	MN149267	–
Anguillanella callawaensis	CA0124	Western Australia	WAMC59191	–	MN149141	MN149312	MN149268	–
Anguillanella callawaensis	CA0124	Western Australia	WAMC59192	–	MN149142	MN149313	MN149269	–
Anguillanella callawaensis	CA0124	Western Australia	WAMC59193	–	MN149143	MN149314	MN149270	–
Anguillanella callawaensis	CA0057	Western Australia	WAMC59194	–	MN149144	MN149315	MN149271	–
Anguillanella callawaensis	CA0058	Western Australia	WAMC59195	–	MN149145	MN149316	MN149272	–
Anguillanella callawaensis	CA0059	Western Australia	WAMC59196	–	MN149146	MN149317	MN149273	–
Anguillanella callawaensis	CA0057	Western Australia	WAMC59197	–	MN149147	MN149318	MN149274	–
Muccanella cundalinensis	CU0058	Western Australia	WAMC59198	–	MN149148	MN149319	MN149275	–
Muccanella cundalinensis	CU0058	Western Australia	WAMC59199	–	MN149149	MN149320	MN149227	–

APPENDIX 3

Bore holes coordinates

Bore	**Latitude**	**Longitude**
CA0006	20°38’40.02”S	120°18’22.57”E
CA0008	20°38’33”S	120°18’2”E
CA0011	20°38’57”S	120°18’17”E
CA0012	20°39’6”S	120°18’23”E
CA0013	20° 39’ 12.96”S	120° 18’ 14.47”E
CA0014	20° 38’ 51.10”S	120° 18’ 15.33”E
CA0021	20° 38’ 41.92”S	120° 17’ 13.23”E
CA0057	20°39’37”S	120°18’10”E
CA0099	20°38’40”S	120°17’32”E
CA0124	20°38’54”S	120°17’57”E
CA0157	20°39’25”S	120°17’58”E
CA0160	20°38’23”S	120°18’40”E
CU0046	20°32’36.46”S	120°09’35.42”E
CU0058	20°32’21.8”S	120°09’12.3”E
CU0064	20°32’20.61”S	120°09’13.5”E
CU0285	20°32’49.38”S	120°10’00.98”E
YP1063	20°36’11.74”S	120°20’23.46”E
YRP22	20°36’32.22”S	120°18’26.42”E