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Two new subterranean ameirids (Crustacea : Copepoda : Harpacticoida) expose weaknesses in the conservation of short-range endemics threatened by mining developments in Western Australia

Tomislav Karanovic[A,C], *Stefan M. Eberhard*[B,D], *Giulia Perina*[B] *and Shae Callan*[B]

[A]Hanyang University, Department of Life Sciences, Seoul 133-791, Korea; and University of Tasmania, Institute for Marine and Antarctic Studies, Hobart, Tasmania 7001, Australia.
[B]Subterranean Ecology, Scientific Environmental Services, Suite 8, 37 Cedric Street, Stirling, WA 6021, Australia.
[C]Corresponding author taxonomy. Email: tomislav.karanovic@utas.edu.au
[D]Corresponding author conservation. Email: stefan@subterraneanecology.com.au

Abstract. The discovery of two new non-marine ameirids from the southern Yilgarn region significantly extends the geographic range for this group in Australia and exposes weaknesses in the conservation and environmental impact assessment (EIA) of subterranean species potentially threatened by mining developments. *Megastygonitocrella embe*, sp. nov. differs from seven previously described Australian congeners by the armature of the second leg endopod and absence of spinules on the somites. A key to world species of *Megastygonitocrella* is presented. Phylogenetic analysis based on 57 morphological characters and 30 species belonging to the *Stygonitocrella s.l.* group suggests that *Antistygonitocrella pardalotos*, gen. et sp. nov. has no close relatives anywhere in the world. Superficial similarities between the two new species are either plesiomorphies or homoplasies. The habitats of these new short-range endemic species are fractured-rock aquifers developed in Archaean greenstone, where the groundwater is characterised by acid conditions, high salinity and low dissolved oxygen. The population of *A. pardalotos* is threatened by a mining development. Despite the advanced level of environmental protection policy in Western Australia, our taxonomic study highlights limitations in EIA practices and discusses potential improvements which have global relevance in regions where short-range endemics coincide with extraction of mineral resources.

Additional keywords: Ameiridae, cladistics, conservation management, endemism, groundwater, micro-characters, stygofauna, taxonomy.

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Introduction

The majority of ameirids are free-living benthic or interstitial marine animals, but a few species are also found in association with flatworms (Liddell 1912), medusae (Humes 1953) or malacostracan crustaceans (Chappuis 1926; Bowman 1988). Although primarily marine, they have successfully colonised and radiated in non-marine waters and can be found today from abyssal depths (Corgosinho and Martínez Arbizu 2010) to freshwater caves (Karanovic 2000), including diverse assemblages recently described from groundwater, predominantly in Western Australia (Karanovic 2004, 2006, 2010; Karanovic and Hancock 2009). With more than 300 valid species (Boxshall and Halsey 2004), Ameiridae Monard, 1927 is the third largest harpacticoid family, after Canthocamptidae Sars, 1906 and Miraciidae Dana, 1846. They are currently classified into 46 valid genera (Walter and Boxshall 2012) and two subfamilies: Ameirinae Monard, 1927 and Stenocopiinae Lang, 1944. A sexually dimorphic basal spine on the first swimming leg is the most important synapomorphy that unites all ameirids, and this character state has (probably) been secondarily lost in only a few species (Lee and Huys 2002; Karanovic 2006; Karanovic and Hancock 2009). The primary taxonomic subdivisions in this family have traditionally been based on segmentation of the swimming legs (Lang 1948, 1965; Petkovski 1976), an approach described as simplistic and that has created many ill-defined genera (Conroy-Dalton and Huys 1996, 1998; Lee and Huys 2002; Reid *et al.* 2003; Boxshall and Halsey 2004; Karanovic 2006). Recently, Karanovic and Hancock (2009) made the first attempt to revise a freshwater branch of this family with extremely reduced endopods of the swimming legs, based on a cladistic analysis of morphological characters, and defined six new genera (three of them being Western Australian endemics).

Subterranean waters of semi-arid Western Australia are becoming known as a globally significant hotspot for subterranean biodiversity (Humphreys 2008; Eberhard *et al.*

2009; Guzik *et al*. 2011). Most of this rich diversity has only been discovered in the past 15 years in two main geographic regions – the Pilbara craton and the northern Yilgarn craton (Fig. 1). In the semi-arid northern Yilgarn, numerous (>100) isolated groundwater calcretes developed along palaeoriver systems (Morgan 1993; Mann and Horwitz 1979). Development of these calcretes was initiated during dry climatic conditions in the Oligocene and has continued during subsequent arid phases of the Late Miocene and Pliocene onwards (Morgan 1993). The calcrete aquifers have a well-developed karstic porosity and therefore provide an ideal habitat for stygofauna (aquatic subterranean fauna). Each calcrete aquifer functions as a hydrogeological 'island' (Cooper *et al*. 2002, 2008); accordingly, many species are locally endemic to a single calcrete, or a few geographically proximal calcretes (Leys and Watts 2008). Molecular phylogeographic studies have shown that the major taxonomic lineages of dytiscid beetles (Cooper *et al*. 2002; Leys *et al*. 2003), amphipods (Cooper *et al*. 2007; Bradford *et al*. 2010), isopods (Cooper *et al*. 2008), parabathynellids (Guzik *et al*. 2008) and copepods (Bradford *et al*. 2010; Karanovic and Cooper 2011, 2012) evolved within individual calcretes following independent colonisation by epigean ancestors (Cooper *et al*. 2002, 2008; Leys *et al*. 2003; Guzik *et al*. 2008; Leys and Watts 2008).

In contrast to the northern Yilgarn, the palaeochannels of the southern Yilgarn, situated in a more temperate climate zone, do not contain groundwater calcretes, which wane south of the Menzies Line (Fig. 1), a zone approximately along latitude 29°30′S, where the present (and palaeo-) climate changes from semi-arid (north) to temperate (Morgan 1993). While carbonate aquifers typically produce a habitat and water chemistry (slightly alkaline pH) favourable for stygofauna, the absence of calcretes in the southern Yilgarn, combined with mostly acid groundwater and extensive anoxic conditions resulting from reduction of sulphur possibly associated with carbonaceous sediments (Morgan 1993), led Humphreys (2008) to suggest conditions would be less suitable for stygofauna south of the Menzies Line. This has been borne out to a large extent with few stygofauna recorded in the non-carbonate aquifers of the southern Yilgarn; however, overall there has been a lower survey effort applied in this region so the apparent paucity of stygofauna in this region may also be partly a sampling artefact.

Discovery and documentation of the diverse subterranean faunas in the mineral-rich Yilgarn and Pilbara regions has coincided with increasing global demand for mineral resources that has driven the development of many new mines and underpinned Australia's economic prosperity in the past 15 years. In some cases this has led to conflict between the competing interests of resources and economic development versus Australia's commitments to biodiversity conservation. In Western Australia these coincidences have catalysed the development of a world-leading policy and regulatory framework that considers the protection of subterranean fauna during environmental impact assessments of mine projects (EPA 2003, 2007, 2012).

In Western Australia, the Environmental Protection Authority (EPA) has legislative responsibility to protect the environment and to prevent, control and abate pollution and environmental harm. The EPA fulfils these duties in part by conducting environmental impact assessments (EIA) of major projects and providing independent advice to government on whether the environmental risks and impacts can be managed. The EPA has developed a series of guidance statements for the EIA of major projects including specifically for subterranean fauna (EPA 2003, 2007). In the past two decades subterranean fauna have become prominent as a key environmental factor in around 40 major project assessments in Western Australia (EPA 2012). Most of these EIA have related to mining projects in the mineral-rich regions of the Pilbara and Yilgarn (Fig. 1). In accordance with the *Wildlife Conservation Act (1950)* and the *Environmental Protection Act (1986)*, the EPA ensures 'that proposals do not potentially threaten the viability of any subterranean species' and 'adequate protection of important habitats for these species' (EPA 2003).

The Spotted Quoll nickel deposit, located near Forrestania in the southern Yilgarn region, was discovered in mid-2007, and the mining company Western Areas NL applied to develop an open-cut mine 150 m deep. Due to the shallow depth of groundwater the mine pit would require dewatering of the local aquifer thus triggering subterranean fauna as a factor for consideration in EIA. Initial field surveys confirmed the presence of an ameirid copepod species collected from a single groundwater bore (WWMB4) within the dewatering impact area (Rockwater 2009*a*). A program of broader geographic sampling for stygofauna was then undertaken in the Forrestania area to attempt to locate this species in similar groundwater habitats outside the mine impact area (EPA 2009). These surveys succeeded in detecting a population of ameirids from another groundwater bore (MB08) at the Cosmic Boy deposit situated 12.5 km away and outside the Spotted Quoll impact area (Rockwater 2009*b*). Several para-taxonomists from private environmental consulting companies were engaged by Western Areas NL, with widely different opinions on the conspecificity of these two populations. The rapid timelines imposed on approval of the mine project precluded more detailed morphological or molecular examination of the material to properly characterise the extent of variability in the populations and determine if they represented the same or a different species. The mine proposal and consultants' technical reports were submitted for assessment to the EPA, who accepted the technical opinions which favoured regional distribution of stygofauna and a single species at both localities. On this basis environmental approval for the Spotted Quoll mine was granted 24 months later in mid-2009 (EPA 2009).

Shortly after the mine project was approved specimens were examined by the first author who concluded that the populations at Spotted Quoll and Cosmic Boy not only belonged to two different species but to two different genera. Their superficial similarity was a consequence of typical convergent morphology exhibited by subterranean fauna. These two species are described here as new to science, and their phylogenetic relationship studied using cladistic methods. The habitat and environmental tolerances of both new species are characterised in relation to aquifer type, groundwater salinity, pH and dissolved oxygen. Their observed distribution is interpreted in relation to the local (hydro-) geology and regional biogeography. Despite the advanced level of environmental protection policy in Western Australia, our taxonomic study highlights limitations in EIA practices and discusses potential improvements which have

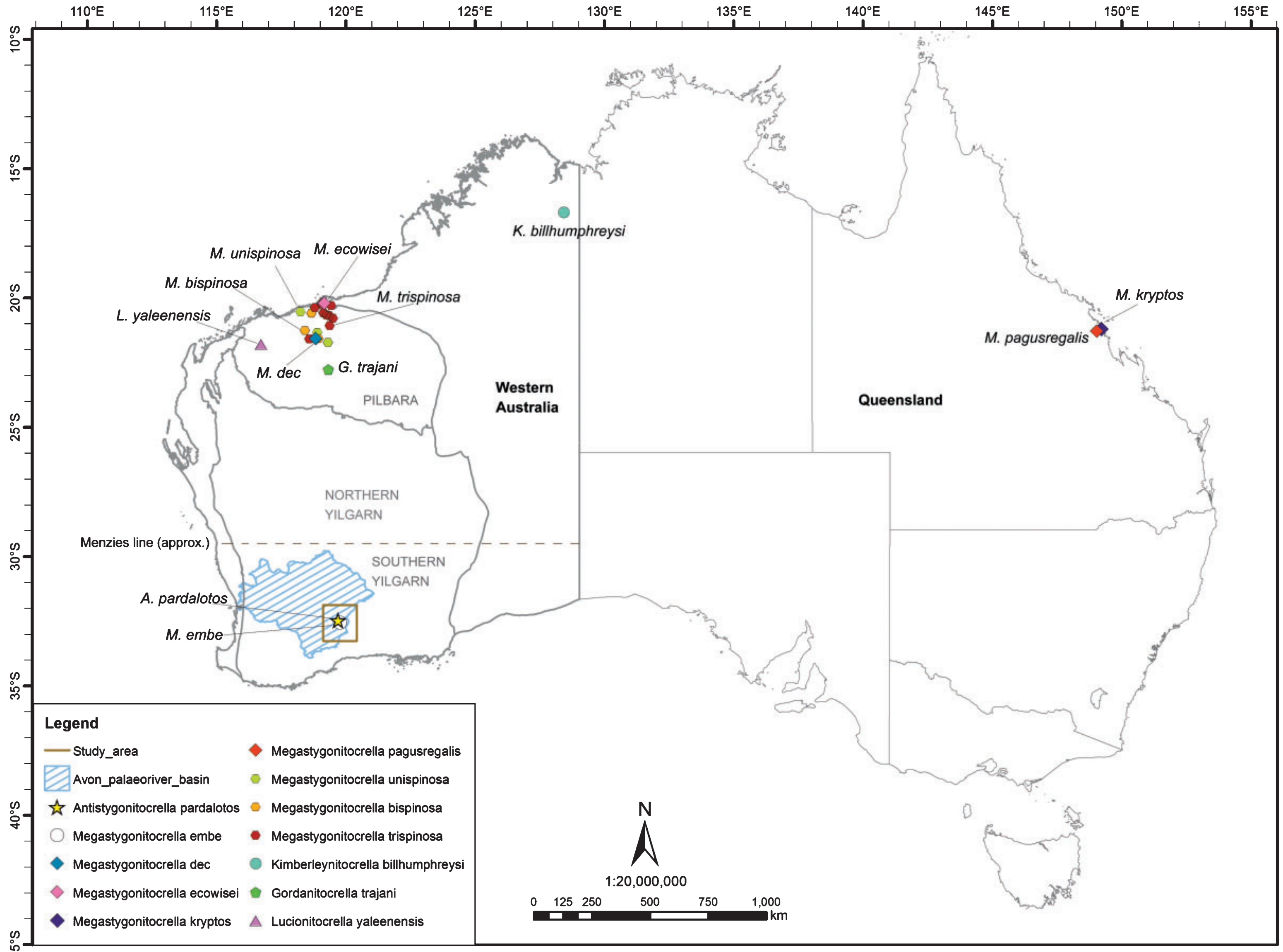

Fig. 1. Records of *Stygonitocrella s.l.*-group in Australia showing the Forrestania study area in the Avon palaeoriver basin in the southern Yilgarn region, the northern Yilgarn and Pilbara stygo-regions.

global relevance in regions where short-range endemic species coincide with extraction of mineral resources.

Material and methods

The Yilgarn craton is composed of Archaean rocks, dominantly granitoids with narrow northerly trending fold belts of older greenstone rocks (Morgan 1993) (Fig. 1). The greenstone that contains the nickel deposits of mining interest within our study area comprises a succession of metasedimentary, ultramafic and metabasalt rocks, which are faulted and fractured creating secondary porosity and productive aquifers, whereas the granitioid rocks generally have very low porosity except where weathered near the surface (Rockwater 2008) (Fig. 2). The groundwater is generally saline, with salinities ranging from 30 000 to 104 000 mg L^{-1} total dissolved solids (TDS).

The study area occupies ~400 km^2 near Forrestania within the Avon Palaeoriver system in the southern Yilgarn (Fig. 1). The topography is very subdued, with a greenstone belt forming a hydrographic divide separating the Lake Hurlstone palaeodrainage tributary on the west from the Barker Lake palaeodrainage tributary on the east. Regionally, the direction of groundwater flow at Forrestania is presumed to mimic the ground-surface topography; west to north-westerly on the western side of the greenstone divide (Lake Hurlstone palaeodrainage), and north to north-easterly on the eastern side (Barker Lake palaeodrainage) (Rockwater 2008).

Sixty groundwater bores were sampled, some on multiple occasions, during five field visits undertaken between August 2008 and May 2009 by Rockwater Pty Ltd (Rockwater 2009*a*, 2009*b*). Most bores were distributed within the north–south trending belt of greenstone rocks ~40 km in length and 10 km wide. Initial sampling efforts were focused on 16 bores located within the Spotted Quoll deposit, the area of interest for mine development. Subsequent sampling efforts extended to bores located in other mine tenements, borefields and farm wells in the Forrestania area (Fig. 2).

Field collection was carried out in accordance with the sampling methods and EIA guidelines issued by the EPA (2003, 2007). Groundwater bores were sampled by Rockwater Pty Ltd (Rockwater 2009*a*, 2009*b*) using a combination of net-hauling and pumping methods. The net-hauling method involved using a plankton net of an appropriate diameter (80 mm) to match the bore size. The net (50 and 150 µm mesh), with a weighted vial attached, was lowered into the bore and then hauled through the water column. Each bore was sampled with at least six hauls (three hauls with each mesh size). Bores were pump sampled from approximately one metre above the bottom of the bore, or at the extent of the pump hose (length 55 m). A range of pumps was used due to the range in bore diameters and bore volumes. These included Grundfos MP1 and SQ3–55 pumps (Grundfos, Bjerringbro, Denmark) in addition to a 12 V mega-monsoon pump (bore BRC29 only). At least three times the water volume of each bore was pumped and the water passed through a 50-µm plankton net. Each net-haul and pump sample was transferred to a labelled vial and preserved in 100% ethanol then transported to the laboratory for sorting. To avoid contamination between sites the sampling nets were thoroughly washed with a decontaminant solution and then rinsed with distilled water. In each bore, water physico-chemistry (salinity, conductivity, pH, dissolved oxygen and temperature) was measured using a HydrolabMiniSonde multiparameter probe (Hach Company, Loveland, CO), from either a pumped sample or by lowering the probe to one meter below the water level in the bore.

Samples were sorted in a laboratory under a Leica M205C dissecting microscope (Leica Microsystems, Wetzlar, Germany). Copepod specimens were dissected and mounted on microscope slides in Faure's medium, which was prepared following the procedure discussed by Stock and von Vaupel Klein (1996), and dissected appendages were then covered by a coverslip. For the urosome or the entire animal two human hairs were mounted between the slide and coverslip so the parts would not be compressed. By manipulating the coverslip carefully by hand, the whole animal or a particular appendage could be positioned in different aspects, allowing the observation of morphological details. During the examination, the water slowly evaporated and appendages eventually remained in completely dry Faure's medium, ready for long-term depositing. All drawings were prepared using a drawing tube attached to a Leica MB2500 phase-interference compound microscope, with N-PLAN (5×, 10×, 20×, 40× and 63× dry) or PL FLUOTAR (100× oil) objectives. Intact specimens were either mounted in toto on slides (also in Faure's medium) or returned to 99.5% ethanol. Type specimens are deposited in the Western Australian Museum (WAM), Perth.

Morphological terminology follows Huys and Boxshall (1991), except for the caudal ramus setae numbering and small differences in the spelling of some appendages (antennula, mandibula, maxillula instead of antennule, mandible, maxillule), as an attempt to standardise the terminology for homologous appendages in different crustacean groups. Description of the second species was shortened by making it comparative, and only the first species is described here in full. Sensilla on all somites (body segments) were given Arabic numerals, to aid recognition of homologous structures in different taxa; they are not intended as novel terminology. Speleological terminology follows Humphreys (2000).

The cladistic analysis was performed on all 30 species of Ameiridae with one-segmented endopods of the fourth leg (the *Stygonitocrella*-group) from around the world (the ingroup), including the two new species described below. *Biameiropsis barrowensis* Karanovic, 2006 was chosen as an outgroup. This species was described from anchialine waters of Barrow Island (NW Western Australia) and it is among the most primitive members of the family Ameiridae (see Karanovic 2006). In total, 57 morphological characters were used in the analysis, and the characters (and character states) are the same as in Karanovic and Hancock (2009: table 1). Characters were coded, optimised and weighted using the computer program WinClada, version 1.00.08 (Nixon 2002), and then analysed using NONA, version 2 (Goloboff 1999). Standard coding was used in the analysis: '0' representing a presumably plesiomorphic character state, and '1' a presumed apomorphy. Unknown or missing values were coded '-' and polymorphic characters were marked in the matrix with an asterisk (*). Characters of the fifth and sixth legs (47–56) were weighted 0.75, because many show intraspecific variability and are proven to differ often between

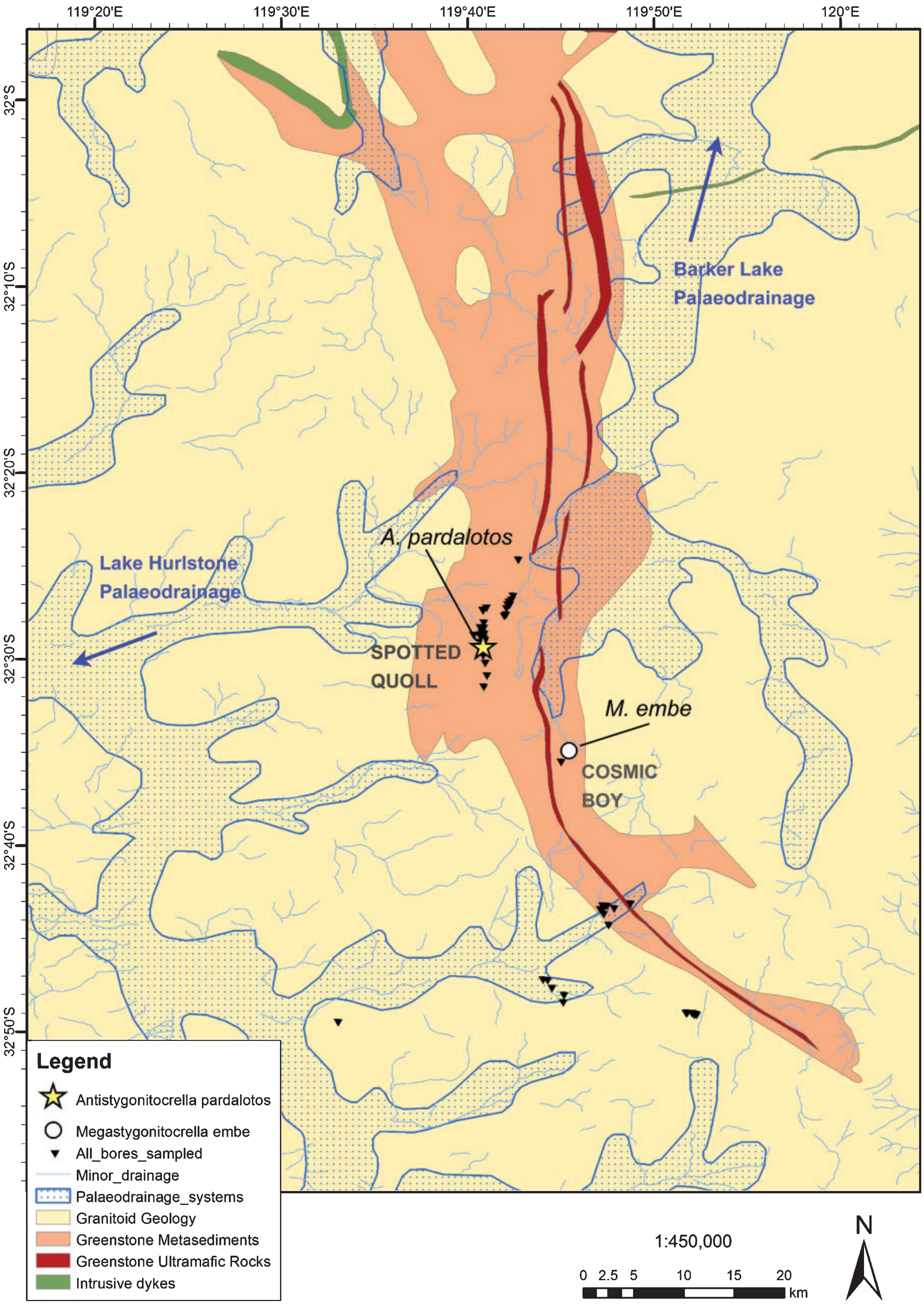

Fig. 2. Forrestania study area surface geology and palaeodrainage showing all bores sampled and collection records for *Megastygonitocrella embe*, sp. nov. at Cosmic Boy mine and *Antistygonitocrella pardalotos*, gen. et sp. nov. at Spotted Quoll mine.

closely related species. Characters 10–15 were weighted 0.5, because they are unknown (undescribed) in many species. All other characters were weighted 1. Analyses were also performed with unweighted characters. One data matrix was created (Table 1) and characters analysed using Rachet Island Hopper searches with the following WinClada parameters: 1000 replications; 5 trees to hold; 10 characters to sample; 10 random constraint level; and 'amb-poly =' (amb- collapses a branch if the ancestor and descendant have different states under same resolutions of multistate characters or of '-'; 'poly =' treats trees as collapsed). Rachet is a method that searches tree space very effectively by reducing the search effort spent on generating new starting points and retaining more information from existing results of tree searches (Nixon 1999). Bootstrap values were also calculated using WinClada with default parameters: 100 replications, 10 searches, 1 starting tree per replication, 0 random seed, 100 max trees.

Except for the two new species, all characters were taken from literature, from original descriptions and any subsequent redescriptions (see Chappuis 1937; Petkovski 1959; Miura 1962; Noodt 1965; Borutzky 1967, 1972, 1978; Borutzky and Michailova-Neikova 1970; Sterba 1973; Pesce 1985; Rouch 1985, 1992; Lee and Huys 2002; Reid *et al*. 2003; Suárez-Morales and Iliffe 2005; Karanovic 2006; Karanovic and Hancock 2009). Here follows the list of unabbreviated names of species analysed, in alphabetical order and with their current generic names (after Karanovic and Hancock 2009): *Antistygonitocrella pardalotos*, gen. et sp. nov.; *Biameiropsis barrowensis* Karanovic, 2006; *Eduardonitocrella mexicana* (Suárez-Morales & Iliffe, 2005); *Gordanitocrella trajani* Karanovic & Hancock, 2009; *Inermipes humphreysi* Lee & Huys, 2002; *Kimberleynitocrella billhumphreysi* Karanovic & Hancock, 2009; *Lucionitocrella yalleenensis* Karanovic & Hancock, 2009; *Megastygonitocrella bispinosa* (Karanovic, 2006); *M. colchica* (Borutzky & Michailova-Neikova, 1970); *M. dec* Karanovic & Hancock, 2009; *M. ecowisei* Karanovic & Hancock, 2009; *M. embe*, sp. nov.; *M. karamani* (Petkovski, 1959); *M. kryptos* Karanovic & Hancock, 2009; *M. ljovuschkini* (Borutzky, 1967); *M. pagusregalis* Karanovic & Hancock, 2009; *M. petkovskii* (Pesce, 1985); *M. trispinosa* (Karanovic, 2006); *M. unispinosa* (Karanovic, 2006); *Neonitocrella insularis* (Miura, 1962); *Psammonitocrella boultoni* Rouch, 1992; *P. longifurcata* Rouch, 1992; *Reidnitocrella borutzkyi* Karanovic & Hancock, 2009; *R. djirgalanica* (Borutzky,

Table 1. Character matrix for the phylogenetic analysis of freshwater ameirids with endopod of the fourth leg one-segmented, and *Biameiropsis barrowensis* Karanovic, 2006 as an outgroup (all 57 morphological characters adopted from Karanovic and Hancock 2009)

Note that *Stygonitocrella orghidani* (Petkovski, 1973) was excluded because of its incomplete description. Symbols used: 0, presumed plesiomorphic character state; 1, presumed apomorphy; –, unknown value;*, dimorphic character. See text for more details and non-abbreviated species names

Taxa	Characters					
	0000000000 0123456789	1111111111 0123456789	2222222222 0123456789	3333333333 0123456789	4444444444 0123456789	5555555 0123456
A. pardalotos	1111010101	0010000001	00011101*1	10001101*1	1111110000	0*00100
B. barrowensis	0011000000	0000000000	0000000000	0000000000	0000000000	0000000
E. mexicana	0111001101	1011110100	1-01110011	1000110001	1110010001	1111---
G. trajani	0011010101	1010000000	0001010101	1000010100	1101011001	0111100
I. humphreysi	1011011111	1110111101	0011101111	1000100111	1100111011	1111111
K. billhumphreysi	0011010001	1110000000	000100010*	100000010*	1001111000	0111110
L. yalleenensis	0011010111	1010000001	0000110101	1000110010	1101010000	1111111
M. bispinosa	0010010001	1010000101	0001101111	1000100111	1000111000	00101*0
M. colchica	0-1101-00-	------0101	0001101111	1000100111	1100111000	0000---
M. dec	1111010101	1010000101	0001101111	1000100111	11*0111000	0111100
M. ecowisei	0011010001	1010000101	0001101111	1000100111	1100111000	0001100
M. embe	0111010101	0010000001	0001100111	1000100111	1110111000	0*00100
M. karamani	0111010000	------0101	0001100111	1000100111	1110111000	0010*00
M. kryptos	0-11010001	1010000101	0001101111	1000100111	1100111100	0111010
M. ljovuschkini	001101-00-	------0101	0001101111	1000100111	1110111000	0000100
M. pagusregalis	0111010001	1010000101	0-01101111	1000100111	1110111100	0111---
M. petkovskii	011101-00-	------0101	0-01101111	1000100111	1110111000	0001---
M. trispinosa	00*1010001	1010000101	0001101111	1000100111	1100111000	0000100
M. unispinosa	0011010001	1010000101	0001101111	1000100111	1100111000	0101100
N. insularis	0111010101	-11--0101	0011100111	1000100111	1101111001	1111---
P. boultoni	0111111001	1111111011	1111110111	1111110111	1101111001	0101---
P. longifurcata	0111111001	0011111011	1111110111	1111110111	11111110*1	*111---
R. borutzkyi	0011010001	------0001	0001100111	0000100111	*000111000	1111111
R. djirgalanica	0-1101-00-	------0001	-001100111	1000101111	1000111111	1111---
R. pseudotiansch.	001101-001	------0001	0-01110101	0000100111	1000111001	1111---
R. tianschanica	0011010001	------0001	0-01110101	0000100111	1000111000	1111---
S. dubia	0-1101-00-	------0001	1001110111	1000110111	1001011001	0000100
S. guadalfensis	0011010000	1000010101	1011110111	1000110111	1001011000	0111---
S. montana	011101-001	110---0001	0001110111	1000110111	1101011000	1111100
S. sequoyahi	0000110000	1000110001	0001110111	0001110111	0101011000	0001110

1978); *R. pseudotianschanica* (Sterba, 1973); *R. tianschanica* (Borutzky, 1972); *Stygonitocrella dubia* (Chappuis, 1937); *S. guadalfensis* Rouch, 1985; *S. montana* (Noodt, 1965); and *S. sequoyahi* Reid, Hunt & Stanley, 2003.

Phylogenetics

Our cladistic analysis with weighted characters resulted in only one tree (Fig. 3) with a length of 94 steps, a consistency index (Ci) of 0.43 and a retention index (Ri) of 0.64. The values obtained from the Ci and Ri indices indicate a relatively high proportion of convergencies (homoplastic changes). This is obviously a result of the character choice and taxon sampling, but it also reflects a general trait within the subterranean freshwater ameirids, which is an unusual proportion of convergencies within almost any of its groups. That is why at least some of the alleged 'symplesiomorphies' in this analysis must be interpreted in reality as constituting a series of homoplastic synapomorphies. Most importantly, our analysis suggests that the two new species are only remotely related, and even belong to different genera. In fact, *A. pardalotos* has no close relatives among recent species anywhere in the world, and we had to erect a new genus for this species. *Megastygonitocrella embe*, on the other hand, is nested deeply inside the *Megastygonitocrella* clade, and this genus already contains seven other species in Australia, although *M. embe* has no apparently very close relatives among currently known species. Autapomorphic features of the new taxa and their affinities are given in their respective 'Remarks' sections below.

With the inclusion of two new taxa in the cladistic analysis, and with the exclusion of *Stygonitocrella orghidani*, inevitably there are some changes in the tree topology between our current analysis (Fig. 3) and that published by Karanovic and Hancock (2009: fig. 18), although most major clades are still relatively well defined (especially the basal ones), the tree is even shorter (94 vs 107 steps) and the Ci and Ri indices are remarkably similar. The most obvious difference is that the two *Psammonitocrella* species are now nested within the *Stygonitocrella s. str.* clade, and the monophyly of the latter is not supported (the relationship between the South American *S. montana* and the rest of the species being unresolved). The monophyly of the *S. sequoyahi/P. boultoni/ P. longifurcata* clade is supported by at least two synapomophies, and interestingly all three taxa are North American, while the other clade (*S. dubia/S. guadalfensis*) is from Spain. If this topology is a reflection of true phylogenetic relationships, it would render the genus *Stygonitocrella* paraphyletic. In our view this is not problematic, as we philosophically accept paraphyletic taxa. The genus *Psammonitocrella* is well defined by several synapomorphies, and its position was also problematic in the analysis of Karanovic and Hancock (*op. cit.*). *Neonitocrella insularis* and *Inermipes humphreysi* are another two taxa that changed their position on the tree when compared with that of Karanovic and Hancock (*op. cit.*), now part of the terminal clade and with their relationship unresolved with the members of the genus *Megastygonitocrella*. This is not surprising for the Western Australian *I. humphreysi*, which shares the same armature formula of the second to fourth leg endopod with most Australian *Megastygonitocrella* species, and the close relationship between these two genera was already discussed by Karanovic and Hancock (*op. cit.*). As for the monospecific genus *Neonitocrella*, we expect its phylogenetic relationship to remain problematic as long as its males are unknown, as well as are many of its female characters due to an incomplete original description. Even though the phylogenetic position of the genera *Inermipes* and *Neonitocrella* is problematic, both are well defined by several autapomorphic features, which were not included in our analysis (as they are not phylogenetically informative). As both genera are still monospecific, their suggested close relationship may be a result of a long-branch attraction.

Analyses of unweighted characters predictably produced longer (152 steps) and more equally parsimonious trees (eight), which also lowers the Ci and Ri indices (0.37 and 0.58 respectively). However, the overall topology was quite similar to that in Fig. 3, except that *I. humphreysi* and *N. insularis* clustered together with *E. mexicana* and *Psammonitocrella*. The position of *A. pardalotos* was also unstable and *Reidnitocrella* appeared as monophyletic on four trees but paraphyletic on the rest. Most of these resulted from a large number of unknown characters in our matrix. Analyses with unweighted characters, but with blocked characters that were unknown or missing in many species, produced a very similar topology to that in Fig. 3, but understandably with more unresolved branches. As can be expected from an indicated large number of homoplasies and missing values in our dataset, bootstrap support was very low for most clades. Only three clades showed bootstrap values of more than 50%: *R. pseudotianschanica/tianschanica* (52%), *P. boultoni/longifurcata* (98%) and the ingroup (100%).

As briefly mentioned in the 'Introduction' *Megastygonitocrella embe* and *Antistygonitocrella pardalotos* do look quite similar at first glance, and it is easy to understand how para-taxonomists tasked with undertaking rapid biodiversity assessments (RBA) involving multiple undescribed taxa could misidentify them as belonging to the same species. In this particular case, many additional factors have contributed to this misidentification, including the same familial placement, proximity of their sampling sites, rarity of stygofauna in this region, intra-population variability of both taxa, and pressure from the mining company to deliver RBA. Superficial similarities between these species can be found not only in their extremely vermiform habitus, smooth somites (without any spinules, pits or cuticular windows), extremely short caudal rami, short swimming legs with the same segmentation and exopodal armature, and similar shape and armature of the fifth leg, but also in the number of armature elements on the endopodal segments of the swimming legs in most cases (these being variable in *A. pardalotos*). Both species have one short and one long element on the endopods of the second and third legs, and only one short element on the endopod of the fourth leg (compare Figs 6*B*, *C*, *D* and 9*C*, *D*, *E*, *H*, *I*, *J*). The two species differ in size (Figs 4*A*, *B* and 8*A*, *B* are drawn to the same scale) and even slightly in the habitus shape, the endopods of the second to fourth swimming legs are of different size and shape, and the short element on the endopods of the second and third legs are on the outer side in *M. embe* and on the inner side in *A. pardalotos*, in addition to somite ornamentation and proportions of armature elements on several appendages. Remarkable similarities in the armature of the exopods of the swimming legs and in the shape and armature of the fifth legs are real, but these could be suspected

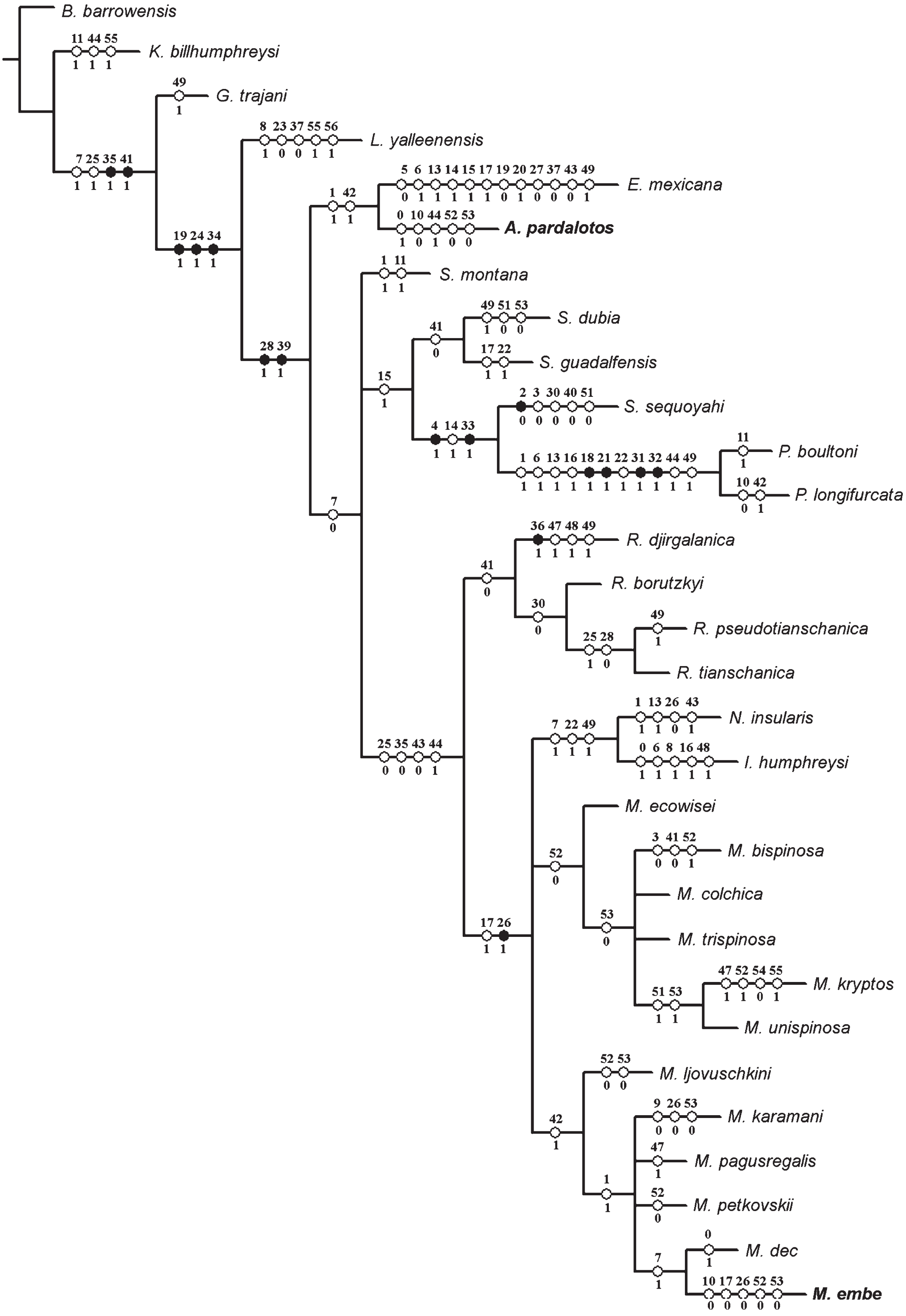

Fig. 3. The only cladogram resulting from analysis of 57 weighted morphological characters (Table 1) scored for 30 species of freshwater ameirids, 94 steps long, Ci = 0.43, Ri = 0.64. Full circles, apomorphies; empty circles, plesiomorphies or homplasies; numbers above branches, characters; numbers below branches, character states. See text for more details and non-abbreviated species names.

to be plesiomorphic characters in a larger group of species. All other superficial similarities could be explained as a result of convergent adaptation to the subterranean environment, i.e. they are apparent stygomorphies (Culver and Pipan 2009). Stygobitic animals usually exhibit a reduction or loss of eyes and pigments and have enhanced non-optic sense organs, and species that inhabit interstitial spaces are most often vermiform (Culver *et al.* 1995). Many convergent physiological adaptations also occur, especially lower metabolic rates, loss of circadian periodicity, and seasonal dynamics (Gibert *et al.* 1994; Langecker 2000). They lack resting stages, have fewer young, and live longer than their surface relatives (Coineau 2000). Case studies on population dynamics and seasonal variability of stygobitic animals indicated that subterranean ecosystems are very stable, slow to recover, and intrinsically vulnerable to anthropogenic effects (Lescher-Moutoué 1973; Pipan and Brancelj 2003, 2004; Culver and Pipan 2009).

Systematics and taxonomy

Subphylum **CRUSTACEA** Brünich, 1772

Class **MAXILLOPODA** Dahl, 1956

Subclass **COPEPODA** H. Milne Edwards, 1840

Order **HARPACTICOIDA** Dana, 1846

Family **AMEIRIDAE** Monard, 1927

Subfamily **AMEIRINAE** Monard, 1927

Genus ***Megastygonitocrella*** Karanovic & Hancock, 2009

Megastygonitocrella embe, sp. nov.

(Figs 4–7)

Type locality

Australia, Western Australia, near Forrestania (360 km E of Perth), Cosmic Boy mine, monitoring bore MB08, 32°35′03.31′S 119°45′27.30′E, elevation 430 m above sea level (ASL).

Specimens examined

Types only: holotype ♀ dissected on one slide (WAM C47217), allotype ♂ dissected on one slide (WAM C47218), one paratype ♀ dissected on one slide (WAM C47219), one paratype ♂ dissected on one slide (WAM C47220), 46 paratypes (16 ♂ + 8 ♀ + 22 copepodids) together in 99% ethanol (WAM C47221); all collected from type locality, 7.v.2009, seLN5108, leg. Rockwater Pty Ltd. Additional one paratype ♂ and one paratype ♀ together on one slide in toto (WAM C47222), also collected from type locality, 6.iv.2009, seLN5106, leg. Rockwater Pty Ltd.

Description of female

Data from holotype and 10 paratypes. Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding appendages and caudal setae), from 595 to 644 µm (holotype 641 µm). Preserved specimens highly transparent, with white hue. Nauplius eye not visible. Prosome comprising cephalothorax with completely fused first pedigerous somite, and three free pedigerous somites; urosome comprising fifth pedigerous somite, genital somite, four abdominal somites, and caudal rami. Podoplean boundary inconspicuous (especially in dorsal view), with only a small sclerotised joint between prosome and urosome ventrally. Habitus (Fig. 4*A*, *B*) cylindrical, gently tapering towards posterior end, very slender; prosome : urosome ratio only 0.76 and greatest width in dorsal view very hard to establish (probably at second pedigerous somite). Body length : width ratio ~7.4; cephalothorax 1.05 times as wide as genital somite. Free pedigerous somites without lateral or dorsal expansions, pleural plates very short and not covering coxae of swimming legs in lateral view. Integument extremely weakly chitinised, soft, and without cuticular windows or pits anywhere. Surface ornamentation of somites consisting of 38 pairs of large sensilla (numbered with Arabic numerals consecutively from anterior to posterior end of body, and from dorsal to ventral side in Figs 4*A*, *B*, 5*A*), and two lateral pairs of cuticular pores on anal somite; several short rows of spinules present only on caudal rami. Rostrum small, membranous, linguiform, reaching just beyond half-length of first antennular segment, about twice as long as wide and not demarcated at base; ornamented with two dorsal sensilla (no. 1).

Cephalothorax (Fig. 4*A*, *B*) almost cylindrical in dorsal view and only gradually tapering towards anterior end in first quarter, ~1.4 times as long as wide; represents only 17% of total body length. Surface of cephalic shield ornamented with 15 pairs of long sensilla (nos 2–16); sensilla 12–16 belong to first pedigerous somite incorporated into cephalothorax. Second pedigerous somite (first free) ornamented with four pairs of long sensilla (nos 17–20); antero-dorsal pair of sensilla (no. 18) probably serially homologous to pair no. 12 on first pedigerous somite, while pair no. 17 probably serially homologous to pair no. 14. Third pedigerous somite ornamented with only three pairs of long sensilla (nos 21–23), antero-dorsal pair (no. 21) probably serially homologous to pairs nos 12 and 18 on first and second pedigerous somite respectively; dorsal pair of sensilla (that would be serially homologous to pairs nos 14 and 17 on first and second pedigerous somites) missing. Fourth pedigerous somite ornamented similarly to third one, with three pairs of sensilla (nos 24–26), all three also serially homologous to those on previous somite. Hyaline fringes of all prosomites extremely short and smooth, hardly visible, and mostly continuing gradually into arthroidal membrane. Fifth pedigerous (first urosomal) somite longer than any of the free prosomites, as well as than genital somite, ornamented with three pairs of large sensilla (nos 27–29), but their serial homologies are hard to establish; hyaline fringe smooth and very narrow.

Genital somite (Figs 4*A*, *B*, 5*A*) ~0.6 times as long as wide (ventral view), ornamented with only two pairs of large lateral pores (nos 30, 31); hyaline fringe smooth and hardly visible on ventral side. Female genital complex (Fig. 5*A*, *J*), occupying most of ventral surface, with single ellipsoid copulatory pore near posterior margin, weakly sclerotised and narrow copulatory duct and two large ovoid seminal receptacles. Single median genital aperture covered by fused and reduced sixth legs, represents 47%

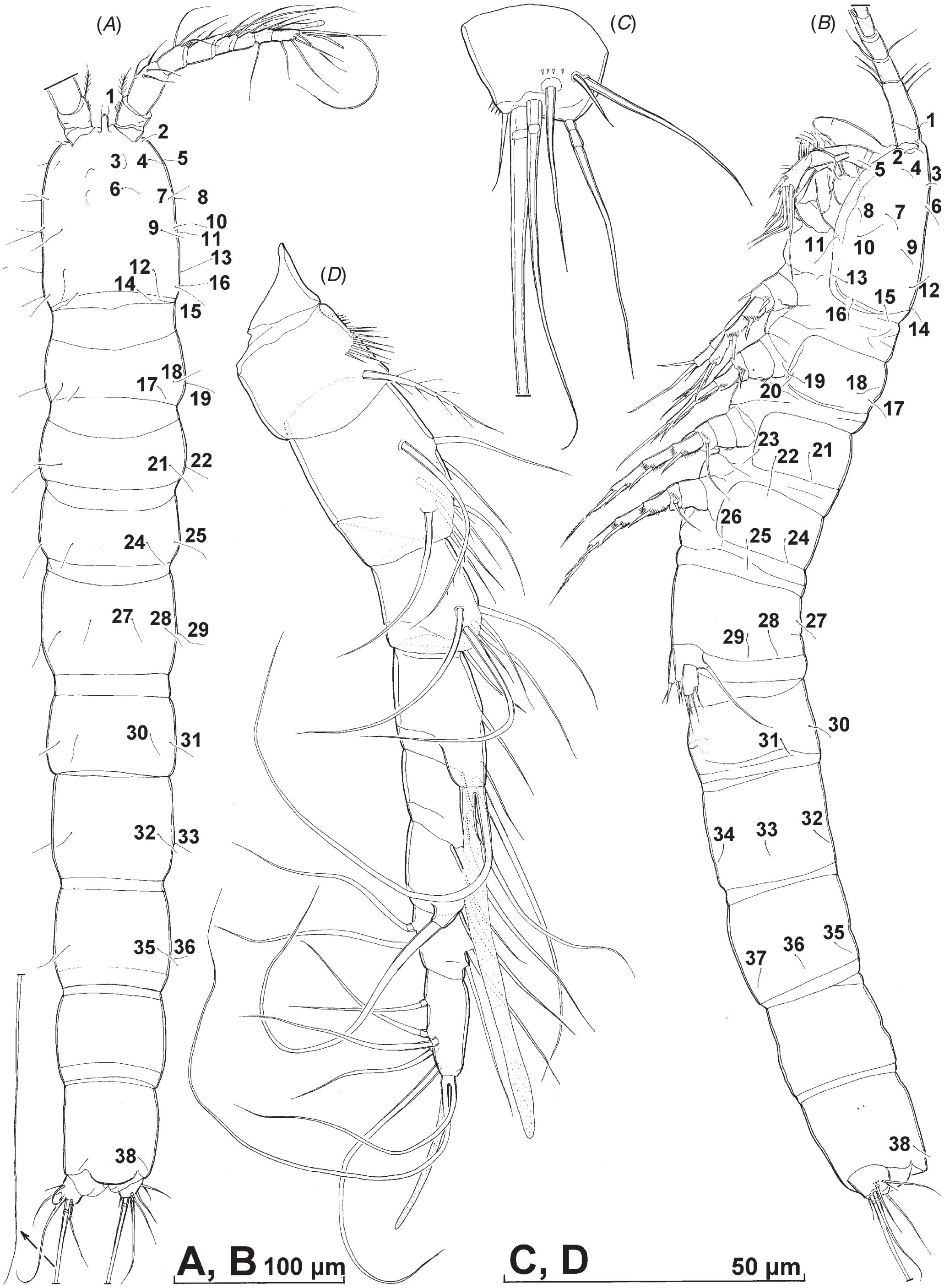

Fig. 4. *Megastygonitocrella embe*, sp. nov., holotype female: (*A*) habitus, dorsal view; (*B*) habitus, lateral view; (*C*) left caudal ramus, lateral view; (*D*) antennula, ventral view. Arabic numerals numbering sensilla consecutively from anterior to posterior end of body, and from dorsal to ventral side.

of somite width. Third urosomite (first abdominal somite) ornamented only with three pairs of posterior sensilla (nos 32–34); slightly narrower and nearly 1.4 times as long as genital somite; hyaline fringe smooth and narrow. Fourth urosomite about as long as third, and also ornamented only with three pairs of large posterior sensilla (nos 35–37), which are also clearly serially homologous (nos 23 and 35, 33 and 36, 34 and 37). Fifth urosomite (preanal) without ornamentation, slightly narrower and shorter than fourth urosomite. Anal somite (Figs 4*A*, *B*, 5*A*) cleft medially only at last quarter, 1.2 times as long as preanal somite and slightly narrower, ornamented with pair of large dorsal sensilla (no. 38) and two anterior lateral

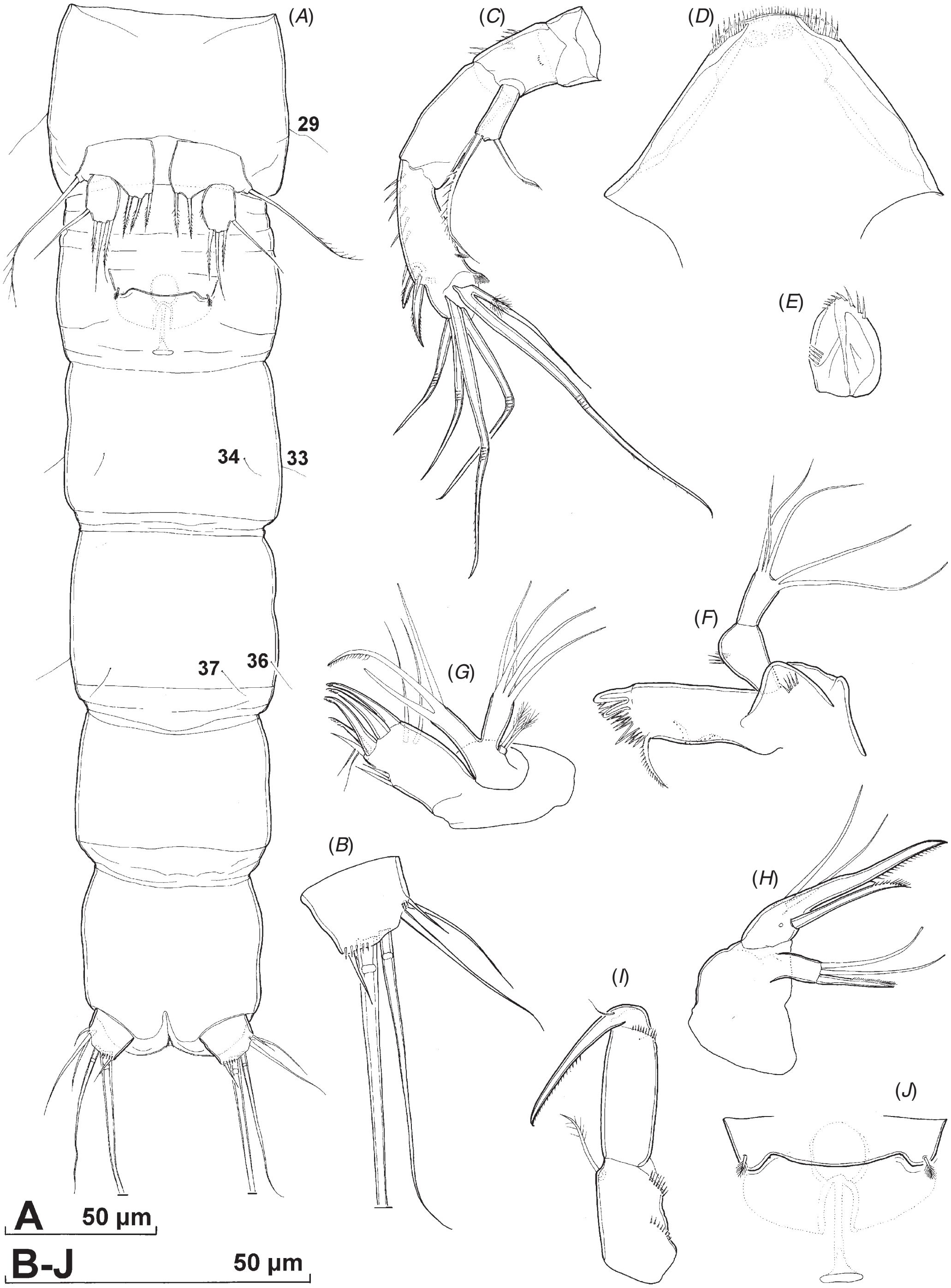

Fig. 5. *Megastygonitocrella embe*, sp. nov., holotype female: (*A*) urosome, ventral view; (*B*) left caudal ramus, ventral view; (*C*) antenna, anterior view; (*D*) labrum, anterior view; (*E*) right paragnath, anterior view; (*F*) mandibula, anterior view; (*G*) maxillula, anterior view; (*H*) maxilla, anterior view; (*I*) maxilliped, antero-lateral view; (*J*) genital field. Arabic numerals on urosome numbering sensilla consecutively from anterior to posterior end of body, and from dorsal to ventral side.

pairs of small cuticular pores; anal operculum convex, wide and long, almost reaching posterior margin of anal somite, represents 55% of somite's width, smooth; anal sinus unornamented, widely open, with weakly sclerotised walls, with inner medial corners slightly protruding posteriorly past anterior part of caudal rami.

Caudal rami (Figs 4*A*, *C*, 5*A*, *B*) extremely short and small compared with anal somite, divergent at almost 90° angle, ~0.25 times as long as anal somite, 0.75 times as long as wide (ventral view), with space between them ~1.3 times one ramus width, and with dorsal anterior half covered by bulging thin

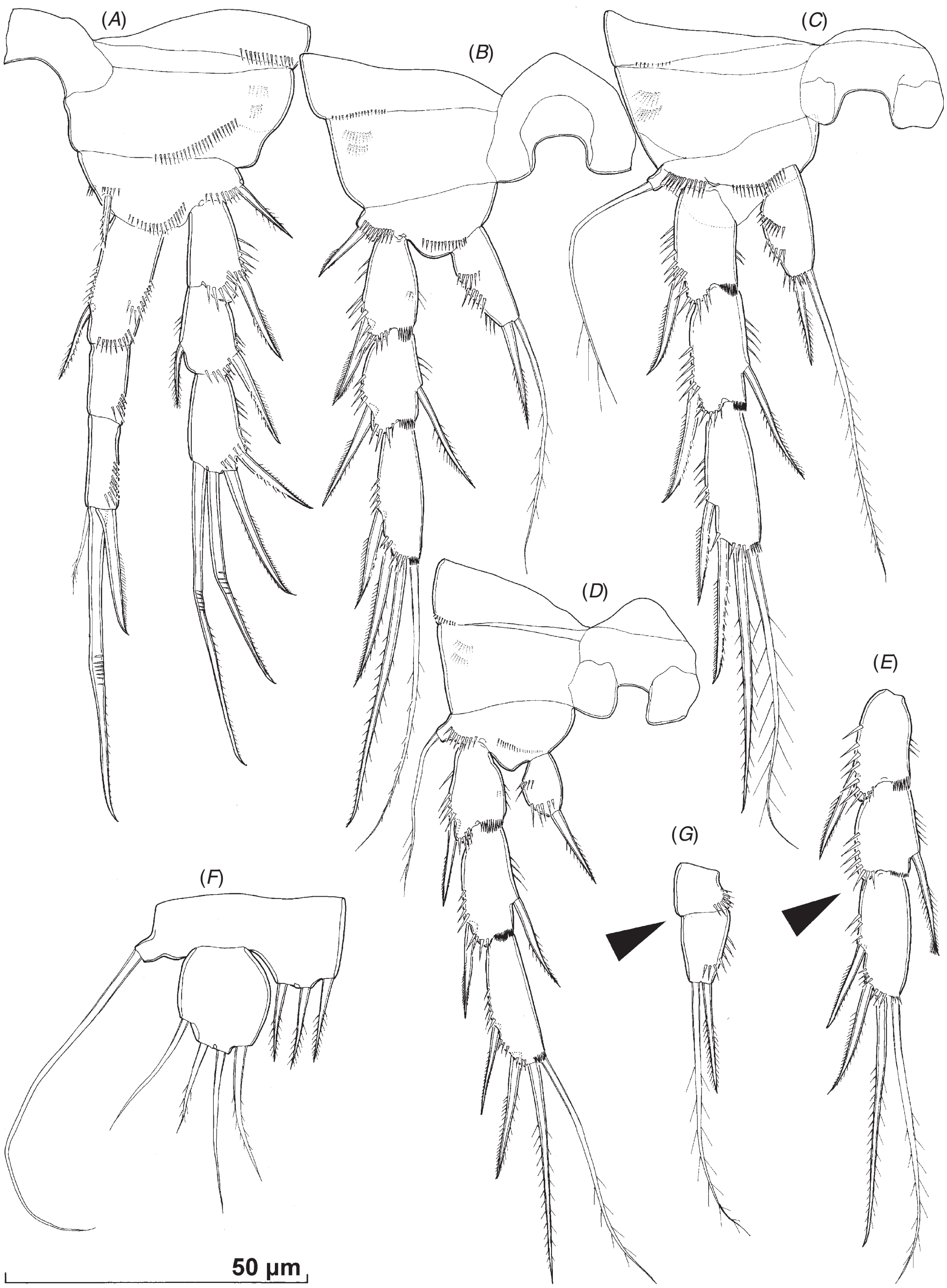

Fig. 6. *Megastygonitocrella embe*, sp. nov., (*A–D*) holotype female; (*E*, *F*) paratype female 1; (*G*) paratype female 2: (*A*) first swimming leg, anterior view; (*B*) second swimming leg, anterior view; (*C*) third swimming leg, anterior view; (*D*) fourth swimming leg, anterior view; (*E*) abnormal exopod of third swimming leg, anterior view; (*F*) fifth leg, anterior view; (*G*) endopod of second swimming leg, anterior view. Arrows pointing to variable features.

arthroidal membrane of anal somite; with seven armature elements (three lateral, one dorsal and three apical); ornamentation consists of four minute spinules at base of posterior lateral seta and five slightly larger spinules along posterior margin ventrally (at base of inner apical seta). Dorsal seta relatively long and slender, smooth, inserted close to postero-median corner, ~2.2 times as long as caudal ramus (lateral view), triarticulate (i.e. inserted on two pseudojoints). Lateral setae all smooth and slender; distalmost seta longest, inserted very close to posterior margin, more than 3.5 times as long as smaller

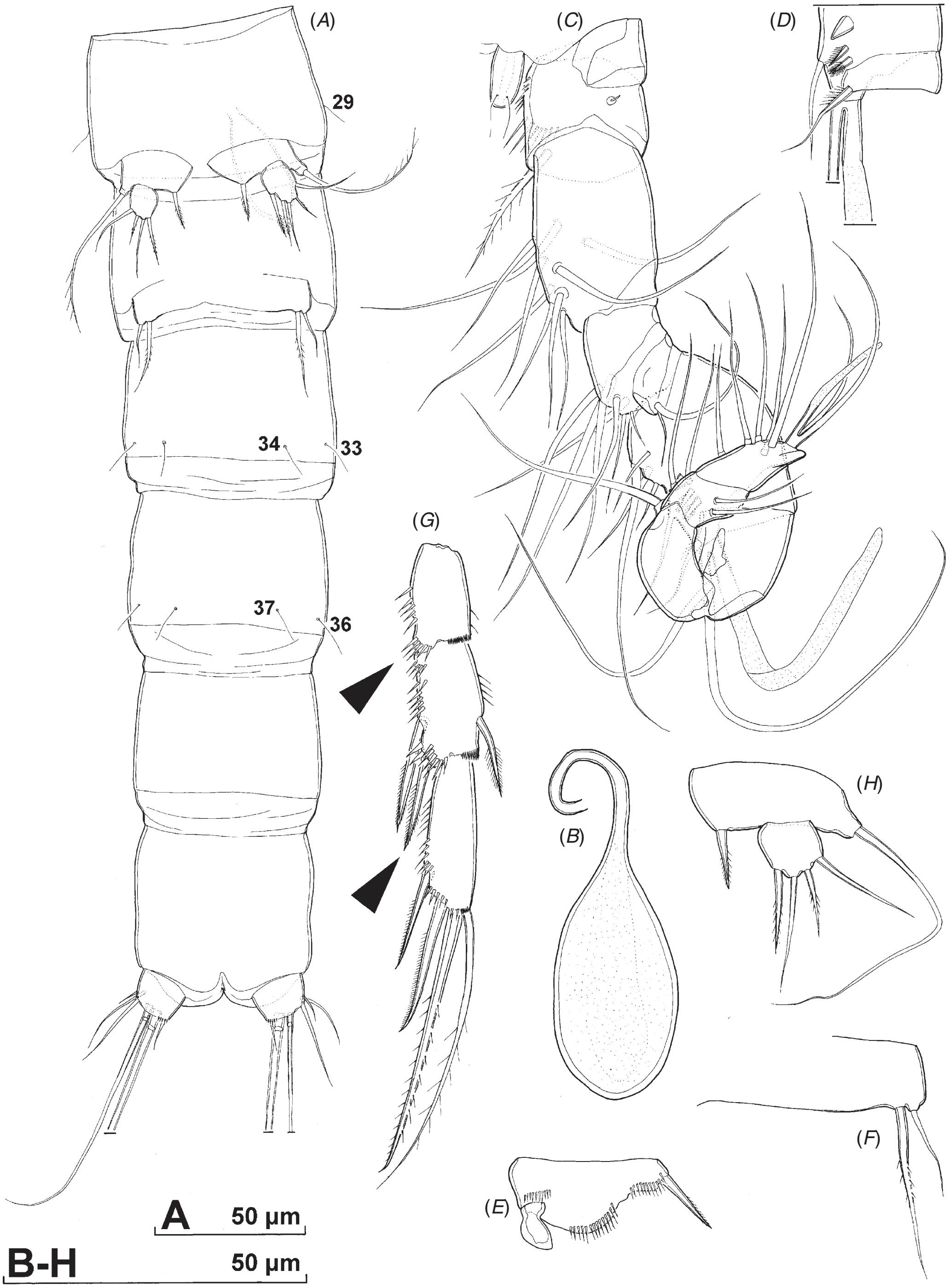

Fig. 7. *Megastygonitocrella embe*, sp. nov., (*A–F*) allotype male; (*G*, *H*) paratype male: (*A*) urosome, ventral view; (*B*) spermatophore; (*C*) rostrum and antennula, dorsal view; (*D*) antennula, detail of fifth and sixth segments, anterior view; (*E*) basis of first swimming leg, anterior view; (*F*) sixth leg, anterior view; (*G*) abnormal exopod of third swimming leg; (*H*) fifth leg, anterior view. Arrows pointing to abnormalities.

proximal seta, 1.3 times as long as larger proximal seta, and ~2.3 times as long as caudal ramus (ventral view); proximal lateral setae inserted very close to each other in cuticular depressions similar to those of typical sensilla, at ~3/5 of ramus length (lateral view) and close to dorsal side. Inner apical seta smooth and minute, only about as long as smallest lateral seta, i.e. ~0.7 times as long as ramus (ventral view). Middle apical seta strongest and longest, with breaking plane, only slightly shorter than four abdominal somites combined, and ~3.5 times as long as outer apical seta. Outer apical seta also with breaking plane and relatively strong, smooth, 3.8 times as long as caudal ramus (ventral view).

Antennula (Fig. 4*D*) eight-segmented, cylindrical, joined to cephalotholax with small triangular pseudosegment laterally, ~1.1 times as long as cephalothorax, ornamented with long spinules on first segment along anterior margin and tubular pore on dorsal surface. Long aesthetasc on fourth segment wide and robust, fused basally with adjacent large seta, and reaching beyond tip of appendage for less than length of last segment; slender apical aesthetasc on eighth segment fused basally with two apical setae, forming apical acrothek. Setal formula: 1.8.7.4.2.3.4.7. Only seta on first segment bipinnate and one seta on second segment unipinnate; all other setae smooth. Two lateral setae on seventh segment and four lateral setae on eighth segment biarticulate (i.e. inserted on small pseudojoint); all other setae uniarticulate and without breaking planes. Length ratio of antennular segments, from proximal end and along caudal margin 1 : 1.8 : 0.9 : 1 : 0.8 : 0.9 : 0.7 : 1.2.

Antenna (Fig. 5*C*) relatively short, curved, 0.6 times as long as cephalothorax, composed of coxa, basis, two-segmented endopod and one-segmented exopod. Coxa very short, unarmed and unornamented. Basis more than twice as long as coxa and ~1.3 times as long as wide, ornamented with several large spinules along inner margin and transverse short row of minute spinule on outer margin proximally, unarmed. First endopodal segment about twice as long as wide and nearly 1.5 times as long as basis, unornamented and unarmed. Second endopodal segment 1.5 times as long as first endopodal segment, with two surface frills subdistally, armed laterally with two spines flanking thin seta; apical armature consisting of five prehensile setae, longest one fused basally to additional smaller and slender seta; smallest seta bearing proximal tuft of fine setules; two longest prehensile setae unipinnate distally along concave margin, others smooth; ornamentation consisting two parallel rows of large spinules along ventral surface basally and several spinules at base of lateral spines. Exopod slightly shorter than basis, cylindrical, unornamented, armed with two unipinnate slender setae; apical seta twice as long as subapical seta and 2.2 times as long as exopod.

Labrum (Fig. 5*D*) large compared with cephalothorax, clearly visible in lateral view, trapezoidal, rigidly sclerotised, with relatively short and somewhat concave cutting edge, ornamented subapically with two rows of eight strong spinules each and apically with numerous minute and slender spinules. Two ovoid fields of gustatory papillae on dorsal (posterior) surface, between rows of minute spinules and strongly chitinised medial horseshoe.

Paragnaths (Fig. 5*E*) ellipsoid, strongly sclerotised and each reinforced with central longitudinal ridge, ~1.4 times as long as wide, with row of large spinules of apically along outer margin, smaller spinule apically along inner margin, and diagonal row of four large spinules at midlength close to inner margin; lobes fused basally into medial linguiform plate, which is ornamented apically with row of hair-like spinules (not figured).

Mandibula (Fig. 5*F*) ornamented with five spinules on outer distal corner of coxa. Cutting edge not wider than rest of elongated coxa, armed with two tricuspidate strong teeth in ventral part, eight unicuspidate teeth and/or spinules in dorsal part, and single dorsal unipinnate seta; fourth unicuspidate tooth from dorsal side much longer and stronger than other unicuspidate teeth. Palp uniramous, comprising basis and one-segmented endopod. Basis with inflated distal part, ~1.6 times as long as wide, unarmed, ornamented with five spinules on inner margin. Endopod slender, unornamented, ~0.7 times as long as basis and 1.8 times as long as wide; armed with five slender and smooth setae apically.

Maxillula (Fig. 5*G*) with large praecoxa; arthrite rectangular, unornamented, armed with two smooth and slender anterior surface setae, three lateral and four apical elements (probably three spines and one seta); apical elements spiniform, with distal crown of spinules. Coxal endite much shorter than praecoxal arthrite, armed apically (on inner margin) with one curved and stout, unipinnate seta, and another two smooth and slender setae. Basis about as long as coxal endite, armed with five smooth setae apically and subapically. Endopod represented by minute but distinct segment, armed with two apical plumose slender setae of about same length, their tips reaching inner margin of basis.

Maxilla (Fig. 5*H*) strong, unornamented, without proximal endite of syncoxa. Distal endite of syncoxa cylindrical, well sclerotised and highly mobile, armed apically with one strong unipinnate seta, and two smooth and slender setae; smooth setae of subequal length, ~1.6 times as long as unipinnate seta, and 2.5 times as long as endite. Basis drawn out into long claw, with shorter spiniform and curved seta at base, ornamented with minute spinules along convex margin, and cuticular pore at base of seta. Endopod represented by minute segment, basally fused to basis, armed with two long and smooth apical setae of subequal length; endopodal setae ~1.1 times as long as basal seta, and all reaching 4/5 of basal claw in length.

Maxilliped (Fig. 5*I*) with short and stout syncoxa, 1.5 times as long as wide, ornamented with two diagonal rows of spinules along outer margin, armed with single bipinnate seta subapically near inner distal corner. Basis almost 2.7 times as long as wide and 1.3 times as long as syncoxa, unarmed, ornamented with transverse row of slender spinules close to distal margin. Endopod represented by long curved claw, about as long as basis, ornamented with row of spinules along concave side distally, accompanied at base by thin, smooth and very short seta.

All swimming legs (Fig. 6*A*, *B*, *C*, *D*, *E*, *G*) of similar size and short in comparison with body length, without armature, not longer than prosomites' width, composed of small triangular and unarmed praecoxa, large rectangular and unarmed coxa, shorter and nearly pentagonal basis, slender three-segmented exopod, also slender and one-segmented (first leg) or three-segmented (second to fourth legs) endopod; each leg joined to their pair on opposite side of body by simple smooth intercoxal sclerite.

First swimming leg (Fig. 6*A*) with short intercoxal sclerite, its distal margin concave, almost geometrically semicircular. Praecoxa ornamented with row of small spinules on anterior surface along distal margin. Coxa twice as wide as long, ornamented with long diagonal row of spinules on anterior surface, and two short transverse rows of spinules on posterior surface. Basis armed with one short and strong bipinnate spine on outer corner, and one even shorter unipinnate strong spine on inner distal corner; ornamented with row of spinules at base of each spine, as well as with distal row of spinules between exopod and endopod on anterior surface, and several spinules on inner margin. Exopod with all segments of about same length, each ~1.5 times as long as wide and ornamented with spinules along outer margin; first segment armed with single strong and finely bipinnate spine on outer distal corner, with smooth inner margin; second segment ornamented with three spinules along inner margin, armed with outer distal spine of about same length as that on first segment, and shorter inner seta, both bipinnate; third segment armed with two strong and finely bipinnate spines on outer margin and two prehensile apical setae, unipinnate distally along outer (concave) margin; inner apical seta 1.1 times as long as entire exopod, 1.4 times as long as outer apical seta, and 2.2 times as long as distal outer spine. Endopod slightly prehensile and slightly longer than exopod; first endopodal segment longest 2.3 times as long as wide, 1.8 times as long as second segment, and reaching slightly beyond midlength of second exopodal segment, ornamented with short and slender spinules along inner margin, outer and distal margins, armed with single bipinnate inner seta ~0.6 times as long as segment; second segment smallest, slender, ~1.9 times as long as wide, ornamented with five spinules on outer distal corner, unarmed; third segment also slender, 2.8 times as long as wide and more than 1.3 times as long as second segment, armed apically with three elements; innermost apical element probably spine, strong, nearly 1.4 times as long as segment, unipinnate along outer margin; middle element very strong and prehensile seta, nearly 2.5 times as long as outer element, and finely unipinnate along outer margin distally; innermost element slender bipinnate seta, slightly shorter than segment.

Second swimming leg (Fig. 6*B, G*) with intercoxal sclerite larger than in first leg, its distal margin deeply concave at middle. Praecoxa ornamented only with row of minute spinules on anterior surface along distal margin. Coxa nearly 1.8 times as wide as long, ornamented only with two transverse rows of spinules on posterior surface. Basis armed with single bipinnate, short and strong spine on outer corner; ornamented with spinules at base of spine, as well as with distal row of spinules between exopod and endopod on anterior surface. Exopod with all segments of about same width; first two segments of about same length, each 1.6 times as long as wide, ornamented with spinules along both inner and outer margins (those on outer margin somewhat stronger), and with inner distal frill; first segment armed with single strong and finely bipinnate spine on outer distal corner; second segment with similar outer distal spine, but additionally armed with strong and bipinnate inner seta, ~1.2 times as long as segment; third segment ~1.5 times as long as second segment, ornamented with strong spinules along outer margin and distal frill on inner distal corner (inner margin smooth), armed with two strong and finely bipinnate outer spines and two apical bipinnate setae; outer apical seta very strong (spiniform) with spinules along outer margin denser and somewhat shorter than those along inner margin, twice as long as segment, and 1.9 times as long as distal outer spine; inner apical seta slender, with sparse spinules on both sides, slightly longer than outer apical seta. Endopod one-segmented (normal condition; Fig. 6*B*) or incompletely two-segmented (abnormal and rare; arrowed in Fig. 6*G*), cylindrical but narrower at distal end than at proximal, slightly longer than first exopodal segment, 2.2 times as long as wide, ornamented with spinules along outer margin, armed apically with outer spine and inner seta; inner seta slender, sparsely bipinnate, ~2.4 times as long as outer spine, and 2.8 times as long as segment.

Third swimming leg (Fig. 6*C, E*) very similar to second swimming leg, except for slender outer seta on basis instead of spine and proportionately longer inner endopodal seta. One paratype specimen missing outer spine on second exopodal segment (abnormal condition; arrowed in Fig. 6*E*).

Fourth swimming leg (Fig. 6*D*) similar to third swimming leg, except for slightly shorter outer basal seta, smaller distal spinules on basis, and shape and armature of endopod. Intercoxal sclerite even more deeply concave than in third leg; coxa 1.6 times as long as wide. Endopodal segment small and ovoid, shorter than first exopodal segment, 1.5 times as long as wide, ornamented with two transverse rows of large spinules on anterior surface, armed with single finely bipinnate apical spine, 1.4 times as long as segment.

Fifth leg (Figs 5*A*, 6*G*) two-segmented, biramous, composed of wide baseoendopod (fused basis and endopod) and smaller ovoid exopod, without connecting plate and not fused medially. Baseoendopod unornamented, with outer basal seta long and smooth, arising from long setophore; endopodal lobe relatively wide and short, trapezoidal, not reaching first third of exopod in length, armed with two or three stout, bipinnate elements of similar length; tips of endopodal elements reaching distal margin of exopod in length. Exopod 1.1 times as long as maximum width, unornamented, armed with four slender setae; innermost and third seta from inner side bipinnate, other two setae smooth. Length ratio of five exopodal setae, from inner side 1 : 1.2 : 0.7 : 1.

Sixth legs (Fig. 5*J*) completely fused together, indistinct, forming simple operculum covering single gonopore, without any ornamentation, each armed with outer short pinnate seta and minute inner spine (hardly visible as small notch).

Description of male

Data from allotype and several paratypes. Body length, excluding caudal setae, from 565 to 612 µm (allotype 604 µm). Similar to female in habitus, segmentation, ornamentation of all somites and caudal rami (Fig. 7*A*), rostrum (Fig. 7*C*), colour, antenna, labrum, paragnaths, mandibula, maxillula, maxilla, maxilliped, and second, third and fourth swimming legs. Prosome : urosome ratio 0.9, greatest width at posterior end of cephalothorax, body length : width ratio ~7.5; cephalothorax 1.1 times as wide as genital somite.

Genital somite (Fig. 7*A*) 1.5 times as wide as long, with small and longitudinally positioned spermatophore (Fig. 7*B*) visible inside on left side, which is somewhat shorter than somite, nearly 1.9 times as long as wide, with very long neck, and positioned partly inside first urosomal somite. Third urosomite (first abdominal somite) slightly narrower and nearly 1.2 times as long as genital somite. Fourth urosomite about as long as third and 0.9 times as wide as third. Fifth urosomite (preanal) without ornamentation, slightly narrower and shorter than fourth urosomite. Anal somite slightly longer than preanal, very similar to female.

Caudal rami (Fig. 7*A*) perhaps slightly shorter than in female, but with similar armature and ornamentation; outer apical seta proportionately longer than in female, 1.4 times as long as anal somite.

Antennula (Fig. 7*C, D*) also eight-segmented as in female, but third and fourth segments with small pseudosegments bearing two and one setae respectively on dorsal surface (sutures not visible on ventral surface), prehensile and digeniculate, with first geniculation between third and fourth segments and second geniculation between fifth and sixth segments. Only ornamentation consisting of tubular pore and long spinules on first segment, as in female, but fifth and sixth segments with several chitinous surface sutures and internal ridges on anterior (geniculated) surface not present in female. Only seta on first segment bipinnate, all other setae smooth. Setae in apical acrothek proportionately shorter that in female, but general armature of first two and last two segments as in female. Setal formula: 1.9.9.10.1.1.4.7. Anterior distal corner of eighth segment produced somewhat as blunt and short process. Four dorsal setae on fourth segment (Fig. 7*D*) transformed, spiniform, proximal one smooth and very short, second short and unipinnate, third short and bipinnate, fourth strong and unipinnate in proximal half, slender and smooth in distal half.

First swimming leg (Fig. 7*E*) with smooth and distally inflated modified inner spine on basis, somewhat shorter than basis and very stout; other armature and all ornamentation as in female.

Exopodal segment of third swimming leg as in female, except in one specimen with abnormal armature, where outer spine from first segment absent and inserted instead on second segment (arrowed in Fig. 7*G*).

Fifth legs (Fig. 7*A, H*) slightly smaller than in female, but also not fused to each other and two-segmented. Endopodal lobe smaller than in female, triangular, short, not extending to first fifth of exopod, unornamented, armed with single short and bipinnate element. Exopod in most cases slightly shorter than in female, but with similar armature. In allotype and one paratype exopod of left leg more elongated and with additional inner seta (Fig. 7*A*), which is bipinnate and about as long as next seta.

Sixth legs (Fig. 7*A, F*) completely fused basally to each other and partly to somite, left more developed, forming simple flap with almost straight margin, each armed with two elements, unornamented; inner element stronger, bipinnate and ~1.9 times as long as slender and smooth outer element.

Variability

Endopod of the second swimming leg is normally one-segmented (Fig. 6*B*), but in one paratype female a remnant of the ancestral two-segmented state is present in the form of an arthroidal membrane visible only on the anterior surface (Fig. 6*G*). Second exopodal segment of all legs bears an outer distal spine (Fig. 6*A, B, C, D*), except in one paratype female on the right third leg, where this spine is absent (Fig. 6*E*). Another abnormal armature pattern was observed on the third leg exopod of one paratype male (Fig. 7*G*), where the outer exopodal spine is moved from first to second segment, and the latter consequently bears two outer spines. Fifth leg can be armed with two or three elements on the female endopodal lobe (Figs 5*A*, 6*F*), and with four or five setae on the male exopod (Fig. 7*A, H*).

Remarks

The new species differs from all seven Australian congeners by the armature of the second leg endopod, which bears one apical seta and one subapical outer spine (see Karanovic 2006; Karanovic and Hancock 2009). All other Australian species have this ramus armed only with the outer apical spine. The presence of an apical seta is a plesiomorphic character state in this genus, and only one other species shares it: *Megastygonitocrella karamani* (Petkovski, 1959) from Macedonia (Europe). *Megastygonitocrella karamani* differs from *M. embe*, sp. nov. by many characters, including the armed basis of mandibula, clearly two-segmented endopods of both second and third swimming legs, and longer caudal rami, which are all also plesiomorphic characters, and thus useless for reconstructing phylogenetic relationships. Interestingly, one paratype female of *M. embe* has a partly divided endopod of the second leg (i.e. two-segmented), which is probably an atavism and another indication that the armature of this ramus is more probably plesiomorphic than a reversal. Another unique character of *M. embe* among Australian congeners is the absence of any spinules on somites (they are only present on caudal rami and appendages), which is also an autapomorphic feature of this species in the genus *Megastygonitocrella* (but strangely enough shared with a completely unrelated new species and genus described below). Although some of the species descriptions in this group are lacking in detail (Karanovic and Hancock 2009), they were all illustrated and/or described with at least some spinules on urosomites. The new species differs from all other congeners by the presence of two setae on the maxillular endopod (another plesiomorphic character state), but the armature of this appendage is unknown in four species. All Australian congeners have only one seta on this segment, which may indicate that they share a common ancestor which was not an ancestor of *M. embe. Megastygonitocrella embe* has only two setae on the antennal exopod, which is a character it shares only with *M. dec* Karanvoic & Hancock, 2009 from the Pilbara region of Western Australia. The two came out as sister species in our cladistics analysis (Fig. 3), and their close relationship is indeed supported by the armature of the antennal exopod (character 7), but they additionally share a free female genital somite, and small and divergent caudal rami. They differ, however, in several morphological characters (habitus shape, armature of the second leg, armature of the fifth leg, ornamentation of urosome, etc.), and it is very unlikely they are indeed sister species.

Etymology

The species name comes from its type locality (bore MB spelled in English), but should be treated as comprising an arbitrary combination of letters that can be treated as a Latin word and may be conceived as a noun in apposition to the generic name.

Key to world species of *Megastygonitocrella* Karanovic & Hancock, 2009

1. Endopod of second leg with single apical element..................................3
   This ramus with two apical elements 2
2. Second exopodal segment of first leg without inner seta ....
   ....*M. karamani* (Petkovski, 1959)
   This segment with inner seta .... *M. embe*, sp. nov.
3. Endopods of second and third leg two-segmented 4
   These rami one-segmented 5
4. Third exopodal segment of fourth leg without inner seta.........................
   ....*M. petkovskii* (Pesce, 1985)
   This segment with inner seta ....
   .... *M. colchica* (Borutzky & Michailova-Neikova, 1970)

5. Fifth leg with endopodal armature completely reduced 6
This leg endopodal lobe armed...8
6. Fifth leg exopod free...7
This segment fused to somite..
....................................*M. pagusregalis* Karanovic & Hancock, 2009
7. Habitus constricted at genital somite ....
....*M. dec* Karanovic & Hancock, 2009
Habitus (male) unconstricted ....
....*M. kryptos* Karanovic & Hancock, 2009
8. Third exopodal segment of fourth leg with one or two inner setae.......9
This segment without inner setae ....*M. ljovuschkini* (Borutzky, 1967)
9. Third exopodal segment of fourth leg with one inner seta..................10
This segment with two inner setae........*M. bispinosa* (Karanovic, 2006)
10. Integument without cuticular windows...11
Cuticular windows present.................. *M. trispinosa* (Karanovic, 2006)
11. Fifth leg endopodal lobe with two armature elements............................
....*M. ecowisei* Karanovic & Hancock, 2009
This lobe with one element................*M. unispinosa* (Karanovic, 2006)

Genus ***Antistygonitocrella***, gen. nov.

Diagnosis

Small Ameiridae with cylindrical habitus and no distinct demarcation between prosome and urosome. Integument weakly chitinised, without cuticular windows or pits; hyaline fringe of all somites smooth. First pedigerous somite incorporated into cephalothorax. All somites ornamented only with large sensilla, except preanal somite which is unornamented; spinules present only on caudal rami and appendages. Genital somite free; genital complex occupying most of its ventral surface, with single small and ovoid copulatory pore near posterior margin, weakly sclerotised and narrow copulatory duct and two large ovoid seminal receptacles; single median genital aperture covered by fused and reduced sixth legs. Anal operculum wide and convex, not reaching to posterior end of anal somite, smooth. Caudal rami extremely small compared with anal somite, divergent, ~0.2 times as long as anal somite, about half as long as wide; armed with seven armature elements (three lateral, one dorsal and three apical); dorsal seta relatively long and slender, smooth, inserted close to postero-median corner, about twice as long as caudal ramus, triarticulate; lateral setae inserted in one plane and very close to each other; principal apical setae with breaking plane. Antennula short, eight-segmented, digeniculate in male, with bipinnate seta, large spinules, and tubular pore on first segment. Antenna composed of coxa, basis, two-segmented endopod and one-segmented exopod; exopod armed with two elements. Labrum with narrow and convex cutting edge. Mandibula with narrow cutting edge and two-segmented palp; basis unarmed, endopod with five apical setae. Maxillular endopod armed with two bipinnate apical setae. Maxilla with single endite on syncoxa; endopod minute, armed with two setae. Maxilliped three-segmented, armed with one seta on syncoxa and one on endopod. All swimming legs with three-segmented exopod. Endopod of first leg three-segmented; endopod of other legs one-segmented. First exopodal segment of all legs without inner seta but with outer spine, second with inner seta and outer spine, third with two outer spines and two apical setae. First endopodal segment of first leg reaching midlength of second exopodal segment, armed with short spiniform inner seta; endopods of second and third leg armed with apical slender seta and sometimes with inner spiniform seta; that of fourth armed with single spiniform seta, its inner margin straight, outer margin inflated. Inner spine on male basis of first leg transformed, smooth and inflated distally; no other sexual dimorphism in swimming legs. Female fifth leg with two or three elements on endopodal lobe and four elements on ovoid exopod; male fifth leg with single element on endopodal lobe and with additional inner seta on exopod. Sixth leg in male armed with two setae, in female with one minute seta and one even more minute spine.

Type and only species

Antistygonitocrella pardalotos, sp. nov.

Etymology

The genus name is composed of the Greek adjective ‘anti’ (opposed to, against) and the existing harpacticoid genus name *Stygonitocrella*. It refers to the superficial similarity of these two genera (and other recently erected genera that used to be part of *Stygonitocrella s.l.*), which is mostly a result of convergent evolution and not of a close phylogenetic relationship.

Antistygonitocrella pardalotos, sp. nov.

(Figs 8–10)

Type locality

Australia, Western Australia, near Forrestania (360 km E of Perth), Spotted Quoll mine (Mining Lease 77/583), bore WWMB4, 32°29′18.83′S 119°40′48.69′E.

Material examined

Types only: holotype ♀ dissected on one slide (WAM C47223), allotype ♂ dissected on one slide (WAM C47224), two paratype ♀ dissected on one slide each (WAM C47225 and C47226), one paratype ♂ dissected on one slide (WAM C47227), seven paratypes (two ♀ + two copepodids) together in 99% ethanol (WAM C47228); all collected from type locality, 6.iv.2009, seLN5101, leg. Rockwater Pty Ltd.

Description of female

Data from holotype and four paratypes. Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding appendages and caudal setae), from 519 to 531 µm (holotype 531 µm). Colour, nauplius eye and body segmentation as in previous species. Podoplean boundary inconspicuous (especially in dorsal view), with small sclerotised joint between prosome and urosome ventrally. Habitus (Fig. 8*A*, *B*) cylindrical, vermiform, constricted slightly at midlength between first and second urosomal somites (arrowed in Fig. 8*A*), very slender; prosome : urosome ratio only 0.78 and greatest width in dorsal view at posterior end of cephalothorax. Body length : width ratio ~7.6; cephalothorax 1.2 times as wide as genital somite. Free pedigerous somites without lateral or dorsal expansions, pleural plates very short and not covering coxae of swimming legs in lateral view. Integument of all somites extremely weakly chitinised, soft and without cuticular windows, pores or pits anywhere. Surface ornamentation of somites consisting of 33 pairs of large sensilla (those homologous to previous species indicated

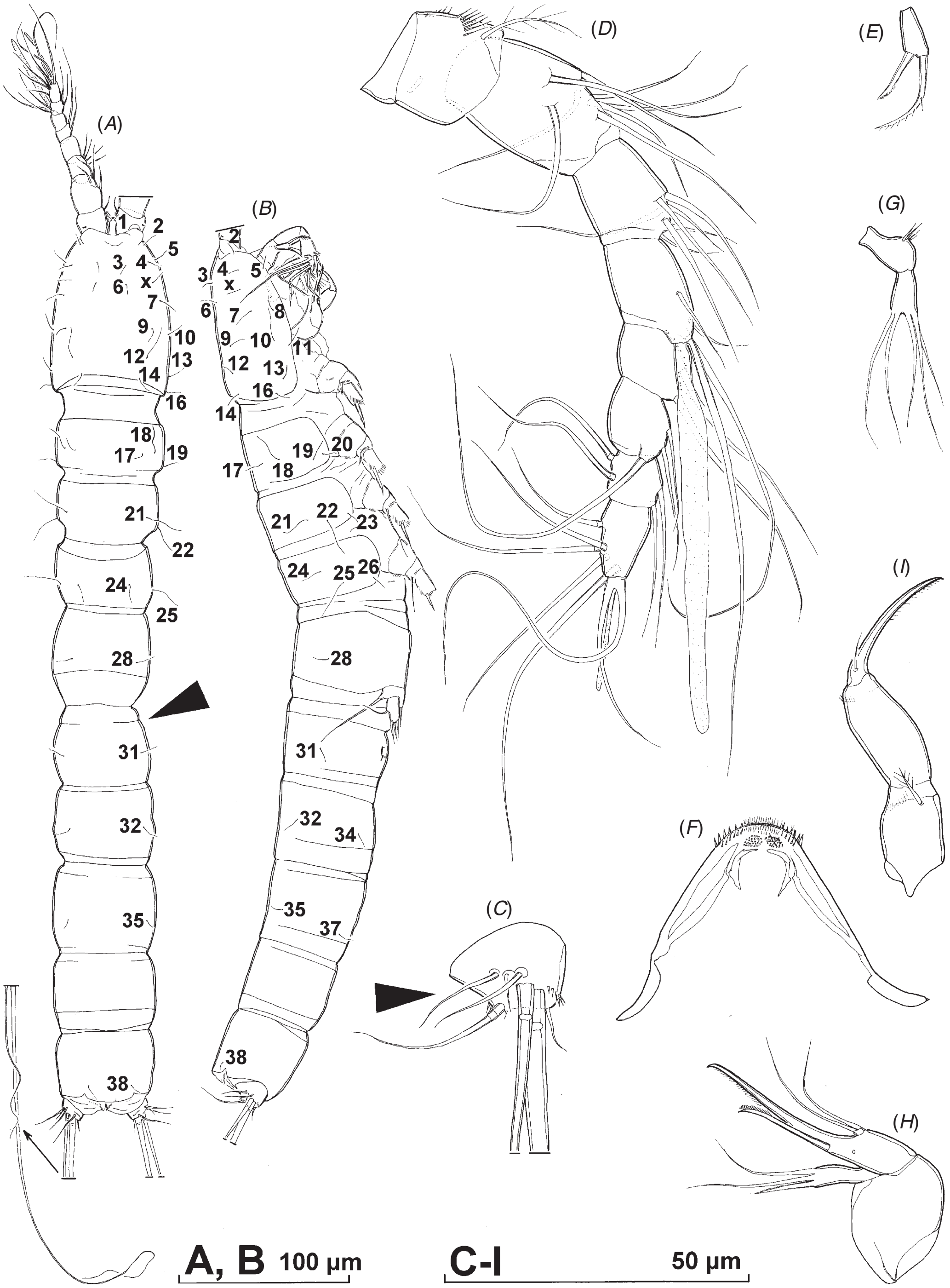

Fig. 8. *Antistygonitocrella pardalotos*, gen. et sp. nov., holotype female: (*A*) habitus, dorsal view; (*B*) habitus, lateral view; (*C*) right caudal ramus, lateral view; (*D*) antennula, ventral view; (*E*) exopod of antenna; (*F*) labrum, posterior view; (*G*) mandibular palp; (*H*) maxilla, anterior view. Arabic numerals indicating sensilla homologous to previous species; novel pair of sensilla on cephalothorax indicated with 'x'. Arrows pointing to characters different from previous species.

with same Arabic numerals in Fig. 8*A*, *B*; one novel pair of sensilla on cephalothorax indicated with 'x'; sensilla nos 15, 27, 29, 30, 33 and 36 missing); several short rows of spinules present only on caudal rami. Rostrum (Fig. 8*A*) small, membranous, linguiform, reaching just beyond half length of first antennular segment, about twice as long as wide and not demarcated at base; ornamented with two dorsal sensilla (no. 1).

Cephalothorax (Fig. 8*A*, *B*) almost ovoid in dorsal view and only gradually tapering towards anterior end in first half, ~1.4 times as long as wide; represents only 18% of total body length.

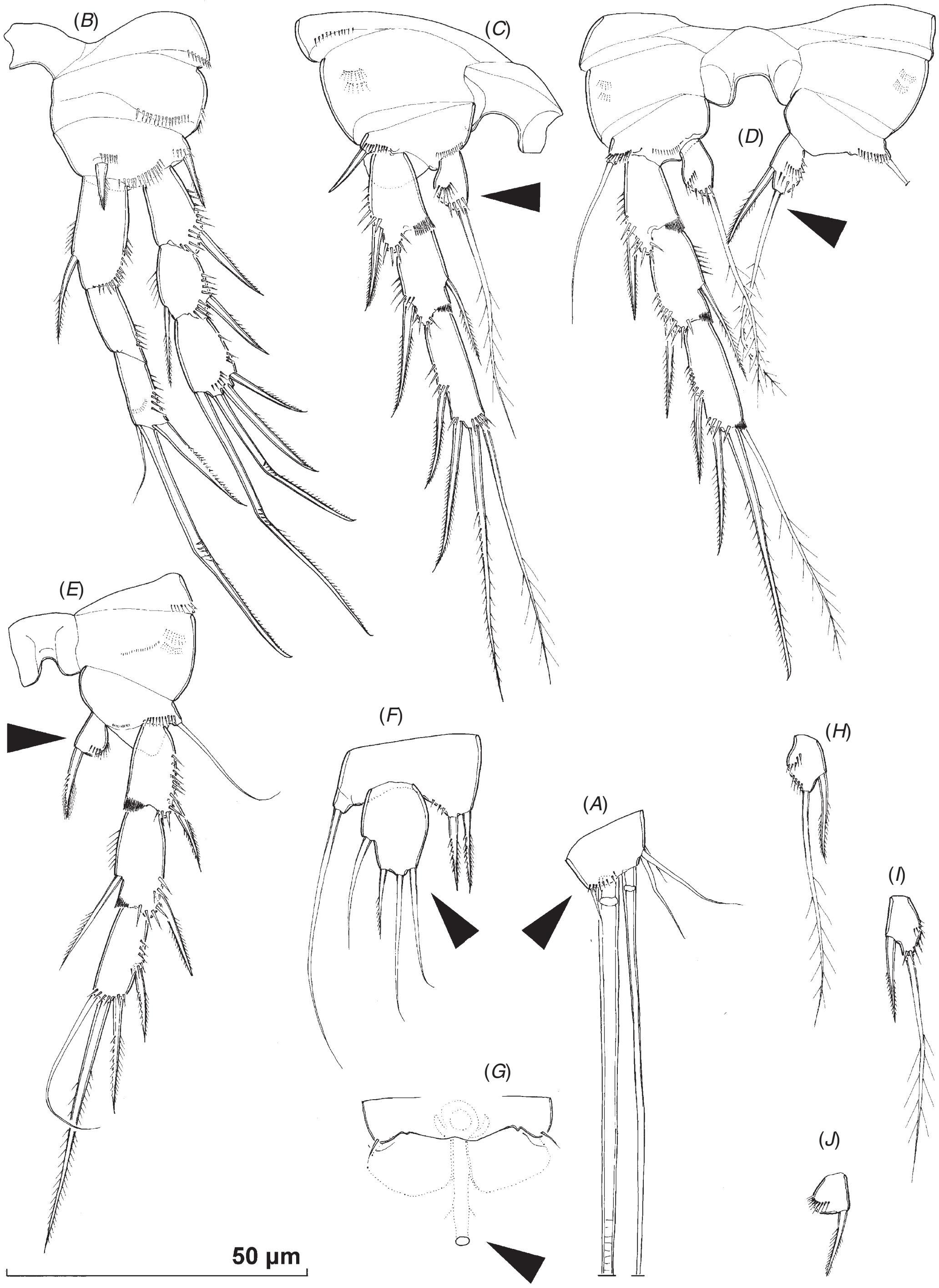

Fig. 9. *Antistygonitocrella pardalotos*, gen. et sp. nov., (*A*–*G*) holotype female; (*H*–*J*) paratype female: (*A*) left caudal ramus, ventral view; (*B*) first swimming leg, anterior view; (*C*) second swimming leg, anterior view; (*D*) right third swimming leg and coxa, basis, and endopod of left leg, anterior view; (*E*) fourth swimming leg, anterior view; (*F*) fifth leg, anterior view; (*G*) genital field; (*H*) endopod of second swimming leg, anterior view; (*I*) endopod of third swimming leg, anterior view; (*J*) endopod of fourth swimming leg, anterior view. Arrow in *D* pointing to a variable feature; other arrows pointing to characters different from previous species.

Surface of cephalic shield ornamented with 15 pairs of long sensilla (nos 2–14, 16, x); sensilla 12, 13, 14 and 16 belong to first pedigerous somite incorporated into cephalothorax. All three free pedigerous somites (Fig. 8*A*, *B*) ornamented as in previous species. Hyaline fringes of all prosomites extremely short and smooth, hardly visible, and mostly continuing gradually into

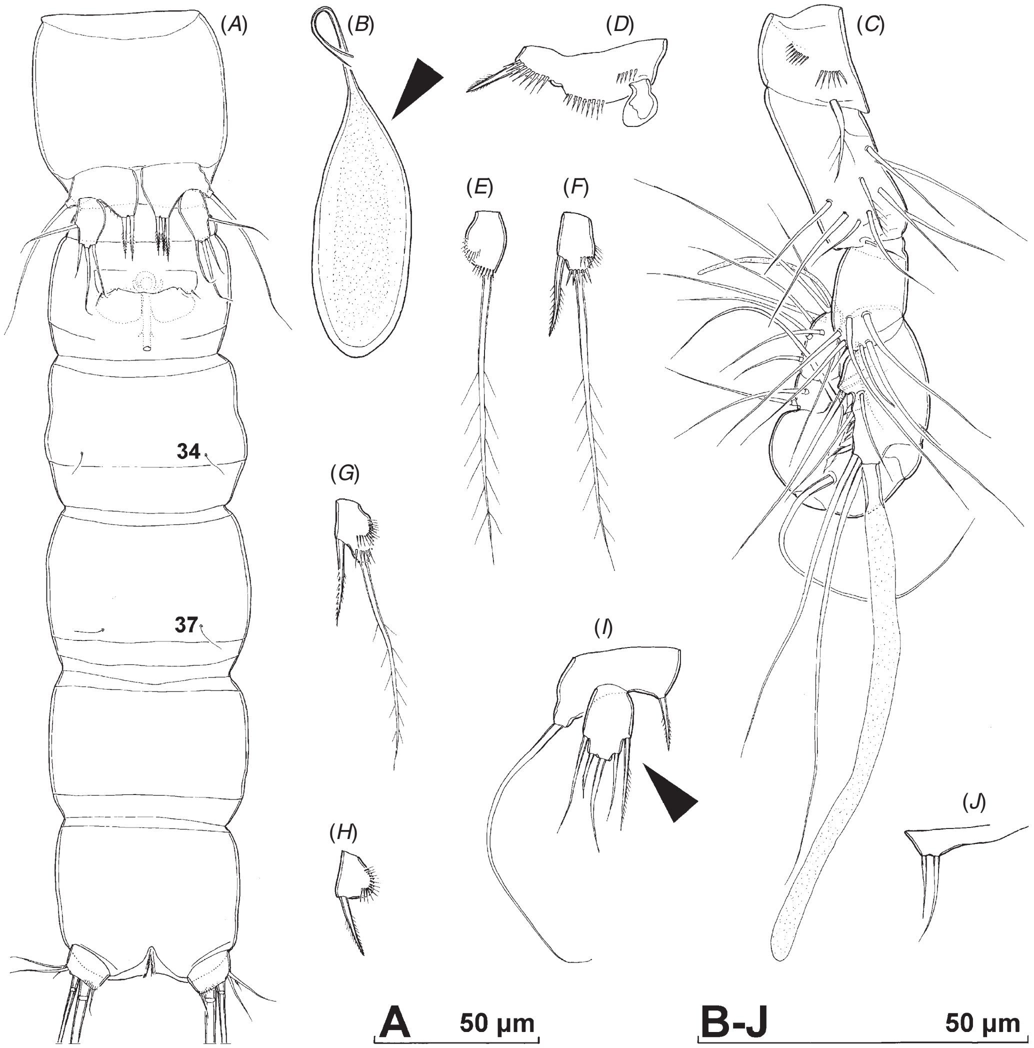

Fig. 10. *Antistygonitocrella pardalotos*, gen. et sp. nov., (*A*) holotype female; (*B–J*) allotype male: (*A*) urosome, ventral view; (*B*) spermatophore; (*C*) antennula, ventral view; (*D*) basis of first swimming leg; (*E*) right endopod of second swimming leg, anterior view; (*F*) left endopod of second swimming leg, anterior view; (*G*) endopod of third swimming leg; (*H*) endopod of fourth swimming leg, anterior view; (*I*) fifth leg, anterior view; (*J*) sixth leg, anterior view. Arabic numerals on urosome indicating sensilla homologous to previous species. Arrows pointing to characters different from previous species.

arthroidal membrane. Fifth pedigerous (first urosomal) somite longer than any free prosomites, as well as than genital somite, ornamented with single pairs of large sensilla (no. 28), but their serial homologies are hard to establish; hyaline fringe smooth and very narrow.

Genital somite (Figs 8*A*, *B*, 9*G*, 10*A*) ~0.64 times as long as wide (ventral view), ornamented with single pair of large lateral sensilla (no. 31); hyaline fringe smooth and hardly visible on ventral side. Female genital field (Figs 9*G*, 10*A*) occupying most of ventral surface, with single small and ovoid copulatory pore near posterior margin, weakly sclerotised and narrow copulatory duct and two large ovoid seminal receptacles. Single median genital aperture covered by fused and reduced sixth legs, represents 56% of somite's width. Third urosomite (first abdominal somite) ornamented only with two pairs of posterior sensilla (nos 32 and 34); in dorsal view slightly wider and ~1.1 times as long as genital somite; in lateral view slightly narrower than genital somite; hyaline fringe smooth and narrow. Fourth urosomite about as long as third and in dorsal view slightly wider, also ornamented only with two pairs of large posterior sensilla (nos 35 and 37), clearly serially homologous to those on third urosomite (nos 32 and 34). Fifth urosomite (preanal) without ornamentation, slightly narrower and shorter than fourth urosomite. Anal somite (Figs 8*A*, *B*, 10*A*) cleft medially only at last sixth, proportionately shorter than in previous species, only 1.1 times as long as preanal somite and only slightly narrower, ornamented only with pair of large dorsal sensilla (no. 38); anal operculum convex, wide and long, almost reaching posterior margin of anal somite, represents 51% of somite's width, smooth; anal sinus ornamented with two diagonal and parallel rows of minute spinules on each side, widely open, with weakly sclerotised walls, with inner medial corners slightly protruding posteriorly past anterior part of caudal rami.

Caudal rami (Figs 8*A, C*, 9*A*, 10*A*) even shorter than in previous species, and with shorter lateral setae inserted closer to each other (arrowed in Fig. 8*C*), extremely small compared with anal somite, divergent at almost 90° angle, ~0.2 times as long as anal somite, only 0.6 times as long as wide in ventral view (arrowed in Fig. 9*A*), with space between them ~1.8 times one ramus width, and with dorsal anterior half covered by bulging thin arthroidal membrane of anal somite; armature as in previous species; ornamentation consists of posterior row of small spinules on ventral surface (at base of inner apical seta), and row of even smaller spinules at base of dorsal seta; no spinules at base of lateral setae. Dorsal seta relatively long and slender, smooth, inserted close to postero-median corner, about twice as long as caudal ramus (lateral view), also triarticulate as in previous species. Lateral setae all smooth and slender, inserted in one plane and very close to each other; two larger ones of about same length, 2.6 times as long as middle minute seta, and 1.2 times as long as caudal ramus. Inner apical seta smooth and minute, only about1.2 times as long as smallest lateral seta, i.e. ~0.7 times as long as ramus (ventral view). Middle apical seta strongest and longest, with breaking plane, about as long as urosome and ~2.4 times as long as outer apical seta, with very slender and curled distal tip. Outer apical seta also with breaking plane and relatively strong, smooth, nearly eight times as long as caudal ramus (lateral view).

Antennula (Fig. 8*D*) segmentation, ornamentation, armature formula and general shape as in previous species, approximately as long as cephalothorax. Long aesthetasc on fourth segment wide and robust, reaching beyond tip of appendage for length of last two segments. Only seta on first segment bipinnate, all other setae smooth; same setae biarticulate as in previous species. Length ratio of antennular segments, from proximal end and along caudal margin, 1 : 2 : 1 : 1.1 : 0.7 : 1 : 0.7 : 1.1.

Antenna (Fig. 8*E*), labrum (Fig. 8*F*), paragnaths, mandibula (Fig. 8*G*), maxillula and maxilla (Fig. 8*H*) similar to previous species, except antennular exopod narrower at base and with shorter apical seta, basis of mandibula proportionately smaller, and basal spine of maxilla more slender.

Maxilliped (Fig. 8*I*) also similar to previous species, but without proximal row of spinules on syncoxa, and with proportionately shorter basis.

All swimming legs (Fig. 9*B, C, D, E, G*) of similar size and short in comparison with body length, not longer than prosomites' width, with segmentation, ornamentation, shape and armature of paecoxa, coxa, intercoxal sclerite, basis and endopod as in previous species. Endopods of second to fourth leg also one-segmented, but proportionately smaller and differently shaped, some also with different armature.

First swimming leg (Fig. 9*B*) with slightly shorter first endopodal segment than in previous species, with proportionately narrower coxa, and with additional rows on spinules on posterior margin of third endopodal segment; inner seta on second exopodal segment proportionately longer than in previous species (almost reaching distal tip of third exopodal segment vs reaching midlength of that segment).

Second swimming leg endopod (Fig. 9*C, H*) much smaller than first exopodal segment, conical in shape (arrowed in Fig. 9*C*), ornamented with two rows of large spinules on anterior surface, and usually armed with single apical, slender and bipinnate seta; one paratype female with additional distal inner spiniform seta ~1.6 times as long as segment; endopodal apical seta about 4.0 times as long as segment and 2.7 times as long as distal inner seta (when present).

Third swimming leg endopod (Fig. 9*D, I*) very similar to endopod of second swimming leg, also conical in shape and armed with apical slender seta (arrowed in Fig. 9*D*), with or without distal inner spiniform.

Fourth swimming leg endopod (Fig. 9*E, J*) very small, only half as long as first exopodal segment, always with straight and smooth inner margin (arrowed in Fig. 9*E*) and with very convex outer margin ornamented with long row of spinules, armed with single apical spiniform element (probably distal inner spiniform seta) nearly twice as long as segment. Inner apical seta on third exopodal segment much shorter than in previous species.

Fifth leg (Figs 9*F, 9A*) similar to previous species, also with two or three spinulose bipinnate elements on endopodal lobe, but with several small spinules on outer margin of endopodal lobe. Exopod 1.4 times as long as maximum width (i.e. more elongated than in previous species), unornamented, armed with four slender setae as in previous species, but innermost seta smooth (arrowed in Fig. 9*F*). Length ratio of five exopodal setae, from inner side 1 : 1.2 : 0.6 : 0.9.

Sixth legs (Fig. 9*J*) completely fused together, indistinct, forming simple operculum covering single gonopore, without any ornamentation, each armed with outer minute smooth seta and even more minute inner spine (hardly visible as small notch).

Description of male

Data from allotype and one paratype. Body length, excluding caudal setae, from 492 to 503 µm (allotype 503 µm). Similar to female in habitus, colour, segmentation, ornamentation of all somites and caudal rami, rostrum, antenna, labrum, paragnaths, mandibula, maxillula, maxilla, maxilliped, second swimming leg (Fig. 10*E, F*), third swimming leg (Fig. 10*G*) and fourth swimming leg (Fig. 10*H*). Prosome : urosome ratio 0.8, greatest width at posterior end of cephalothorax, body length : width ratio ~7.8; cephalothorax 1.3 times as wide as genital somite.

Genital somite ~1.5 times as wide as long, with small and longitudinally positioned spermatophore (Fig. 10*B*) visible inside on left side, which is somewhat shorter than somite, nearly 2.7 times as long as wide (i.e. much longer than in previous species; arrowed in Fig. 10*B*), with long and slender neck. Other urosomites and caudal rami as in female.

Antennula (Fig. 10*C*) segmentation, geniculation and ornamentation as in previous species. Armature also generally similar to previous species, but one additional seta present on second segment, one less seta present on fourth segment, one seta on second segment unipinnate (vs smooth in previous species), and aesthetasc on fourth segment proportionately much longer.

First swimming leg (Fig. 10*D*) with smooth and distally inflated modified inner spine on basis, somewhat shorter than basis and very stout, distally more inflated than in previous species; other armature and all ornamentation as in female.

Fifth leg (Fig. 10*I*) slightly smaller than in female, but also two-segmented. Endopodal lobe smaller than in female, triangular, short, not extending to first fifth of exopod, unornamented, armed with single short and bipinnate element as in previous species. Exopod similar to female, but with additional inner unipinnate element (arrowed in Fig. 10*I*), always slightly longer than next smooth element.

Sixth legs (Fig. 10*J*) completely fused basally to each other and partly to somite, left more developed, forming simple flap with almost straight margin, each armed with two smooth elements, unornamented; inner element ~1.4 times as long as outer element.

Variability

Endopod of the second and third swimming legs can be with or without the distal inner spiniform seta in both female and male (Figs 9*C, D, H, I,* 10*E, F, G*), and sometimes it is even an asymmetrical feature (Figs 9*D*, 10*E, F*). Endopodal lobe of the female fifth leg can be armed with two or three elements (Figs 9*F*, 10*A*). The shape of the fourth leg endopod in both sexes, however, is not variable (Figs 9*E, J,* 10*H*), always with straight inner margin and very convex outer margin.

Remarks

We had to erect a new genus to accommodate *Antistygonitocrella pardalotos*, gen. et. sp. nov., because this species could not be assigned to any known ameirid genus without expanding its diagnosis significantly. Our cladistic analysis suggests that this species has no close relatives among Australian ameirids (Fig. 3), nor is it closely related to any other species in the family. Its one-segmented endopods of the fourth swimming leg put it in the *Stygonitocrella*-group of genera (see Karanovic and Hancock 2009), but its unique swimming legs armature formula testifies its long independent evolutionary history. It is the only member of this group with the ultimate endopodal segment of the fourth leg armed only with distal inner seta (all other armature absent; arrowed in Fig. 9*E*), which is evident both from the shape of the segment (outer margin significantly inflated) and serial homologies of the armature elements (when present) on the second and third legs. The constricted habitus of female *A. pardalotos* is also an unusual feature, shared only with *Inermipes humphreysi* Lee & Huys, 2002 and *Megastygonitocrella dec* Karanovic & Hancock, 2009, although this constriction in the anterior part of the genital somite is much more pronounced in the latter two species. This character state is obviously a homoplastic feature, as all three species differ significantly in morphology. For example, *I. humphreysi* has a large genital double-somite, minute endopods of second to fourth swimming legs, and antennal exopod armed with a single apical seta. *Megastygonitocrella dec* has the copulatory pore situated in the middle on the genital somite, widely separated lateral setae on the caudal rami, and urosome ornamented with several rows of spinules and more sensilla than in *A. pardalotos*. All three species have a different shape and armature of the fifth leg. *Inermipes humphreysi* and *M. dec* have the same armature formula of the second to fourth swimming leg endopods and exopods, and are probably more closely related to each other than any of them is to *A. pardalotos.*

Another unusual feature of the new genus is the presence of two setae on the maxillular endopod, shared only with above described *Megastygonitocrella embe*, sp. nov. and the American *Psammonitocrella longifurcata* Rouch, 1992, but this is a plesiomorphic character state, and also unknown for 11 species of freshwater ameirids with one-segmented endopods of the fourth swimming leg (Table 1; Rouch 1992; Karanovic and Hancock 2009).

Our cladistic analysis (Fig. 3) positions the node that contains *A. pardalotos* quite basally and suggests a weak relationship with the Mexican *Eduardonitocrella mexicana* (Suárez-Morales & Iliffe, 2005), mostly based on the free genital somite (character 1) and absence of inner distal seta on the third exopodal segment of the fourth swimming leg (character 42), in addition to many plesiomorphic features in the armature of their swimming legs. It is obvious, however, that the first two characters are homoplasies in this group of freshwater ameirids that originated independently several times (Table 1). The two species differ in many morphological characters, and must be distantly related. For example, *E. mexicana* has a robust habitus, urosome ornamented with numerous spinules, a long labrum, unarmed maxilliped, different (more plesiomorphic) armature of the second to fourth swimming leg endopods, and the female fifth leg reduced to a small trilobate knob armed with three setae. Both species have the distal inner seta on the fourth leg endopod present (character 46), which is a rare feature in this group, only additionally shared with *Lucionitocrella yalleenensis* Karanovic & Hancock, 2009, described from the Pilbara region in Western Australia (Karanovic and Hancock 2009). The latter species also shares with *A. pardalotos* some features in the armature of other swimming legs (their endopods of the second leg are remarkably similar), but it differs in so many characters that a close phylogenetic relationship is very unlikely. *Lucionitocrella yaleenensis* has a more robust habitus, urosomites ornamented with spinules, genital double-somite, longer caudal rami, only one armature element on the antennal exopod, more armature elements on the exopods of second and fourth leg (three outer spines on the third exopodal segment of the second leg), more armature elements on the endopods of third and fourth leg, a very reduced fifth leg, and even a completely unarmed male sixth leg. Although most of the features in the habitus shape and the swimming legs armature are plesiomorphic in this species when compared with *A. pardalotos*, those in the shape and armature of the antenna, fifth and sixth legs are apomorphic, and this combination suggests a long independent evolutionary path from a common ancestor. Thus, we had to erect a new genus to accommodate *A. pardalotos*.

Etymology

The species name *pardalotos* (Greek, spotted like a leopard), refers to the name of the mine site (Spotted Quoll mine) type locality. Spotted quolls, also known as tiger quolls, are carnivorous marsupials native to Australia which are listed by the International Union for Conservation of Nature (IUCN) on the Red List of Threatened Species. One of the biggest threats to all quolls is habitat destruction (Jones *et al*. 2001).

Key to ameirid genera of the *Stygonitocrella*-group

1. Endopod of fourth leg one-segmented	2
This ramus two- or three-segmented	other Ameiridae
2. Ultimate exopodal segment of first leg with five elements	3

This segment with four elements 5

3. Endopod of fourth leg as small knob, unarmed .... *Kimberleynitocrella* Karanovic & Hancock, 2009

This segment well developed, armed 4

4. Third exopodal segments of second and third legs with inner setae .... *Gordanitocrella* Karanovic & Hancock, 2009

This segment without inner setae .... *Eduardonitocrella* Karanovic & Hancock, 2009

5. Third exopodal segment of second leg with two outer spines 6

This segment with three outer spines .... *Lucionitocrella* Karanovic & Hancock, 2009

6. Endopod of fourth leg as small knob, unarmed 7

This segment well developed, armed with one element 8

7. Second exopodal segment of first leg without outer spine .... *Psammonitocrella* Rouch, 1992

This segment with outer spine .... *Neonitocrella* Lee & Huys, 2002

8. Endopod of fourth leg ovoid, armed with outer distal spine 9

This segment with straight inner margin, armed with inner distal seta .... *Antistygonitocrella*, gen. nov.

9. Endopod of second leg with single element .... *Stygonitocrella* Reid, Hunt & Stanley, 2003

This ramus with two armature elements 10

10. Lateral seta on endopod of second leg present .... *Reidnitocrella* Karanovic & Hancock, 2009

This seta absent 11

11. Swimming legs without outer seta on basis .... *Inermipes* Lee & Huys, 2002

This seta present .... *Megastygonitocrella* Karanovic & Hancock, 2009

Discussion

Diversity and distribution patterns

This paper provides the first taxonomic description of stygofauna from groundwater in the Yilgarn craton south of the Menzies Line. This discovery significantly extends the biogeographic range and hydrogeologic habitats (fractured-rock aquifers developed in Archaean greenstone) known to be occupied by stygofauna in Western Australia. Already, stygofauna are known to be highly diverse and abundant in the semi-arid Pilbara and northern Yilgarn regions (Fig. 1). In both regions, progressive Quaternary climatic aridity is considered the major driver for subterranean colonisation (Karanovic and Tang 2009; Humphreys 2012), and particularly in case of the northern Yilgarn's 'archipelago' of hydrogeologically isolated groundwater calcrete 'islands', which have facilitated multiple independent synchronic radiations (Cooper *et al*. 2002; Leys *et al*. 2003; Cooper *et al*. 2007, 2008). As noted in the 'Introduction', the southern Yilgarn stygo-region contrasts with the northern stygo-regions in possessing a more temperate (palaeo-) climate and waning of groundwater calcretes; however, these factors have not precluded colonisation of groundwater in the south.

At continental scale, the group of species comprising *Stygonitocrella s.l.* appears to exhibit a widely disjunct distribution pattern across Australia, with isolated occurrences in the southern Yilgarn, Pilbara, Kimberley and Queensland (Fig. 1). This pattern is undoubtedly a sampling artefact to a certain extent because many parts of Australia have not been sampled for stygofauna; however, the absence of any members of the group in the northern Yilgarn is certainly real because a great deal of sampling has been undertaken there and the copepod fauna is well known and described (Karanovic 2004; Karanovic and Cooper 2011). This major stygo-geographic boundary between the Pilbara and northern Yilgarn cratons is geologically well demarcated and mirrored at (sub-) generic levels across multiple stygobitic taxa including Ostracoda, Amphipoda, Bathynellacea, Isopoda and Dytsicidae (see Humphreys 2012). An explanation for this great biogeographic disjunction remains wanting.

Like the northern Yilgarn, the Pilbara region has also been well sampled and the *Stygonitocrella s.l.* group has radiated extensively there with seven stygobitic species in three genera described (Karanovic 2006; Karanovic and Hancock 2009). Six of these Pilbara species appear to be short-range endemics known only from their type localities within discrete river catchments, while one species, *Megastygonitocrella trispinosa*, is more widespread with a distribution spanning several adjacent river catchments. There is even a case of sympatry in one bore where *M. trispinosa* was found together with the relatively closely related *M. ecowisei* (Karanovic and Hancock 2009). In Queensland, two species, *M. kryptos* and *M. pagusregalis*, are described from the Pioneer Valley alluvial aquifer (Karanovic and Hancock 2009), while the two species described in this paper occur in bores only 12.5 km apart in adjoining palaeodrainage systems. The observed patterns in localised speciation, endemism and sympatry within the *Stygonitocrella s.l.*-group suggest that further sampling of ameirids in the southern Yilgarn, Kimberley, Queensland and other parts of Australia, in tandem with close taxonomic scrutiny, will reveal addition examples of small-scale radiations and short-range endemism in this group.

Local groundwater environment

The measured range in groundwater physico-chemistry in the two bores containing the ameirids, and 45 other sampled bores, is summarised in Table 2. The ameirids inhabited groundwater characterised by slightly acid conditions (pH 5.98 to 6.70), salinity approximating seawater (26 000 to 34 000 mg L^{-1} TDS) and dissolved oxygen (DO) concentration ranging from suboxic to dysoxic (0.16 to 0.68 mg L^{-1}). The high salinity tolerance of stygofauna in Western Australia (up to 69 000 mg L^{-1} TDS) has previously been noted by Humphreys (2008), while the measured DO concentrations indicate the ameirids are very tolerant of low DO, as is expected for stygobitic crustaceans (Malard and Hervant 1999). Forty-five other bores sampled were similarly characterised by acid to circum-neutral pH (median 5.9), TDS typically highly saline (mean 34 110 mg L^{-1}) and DO ranging from suboxic (<0.3 mg L^{-1}) to oxic (>3.0 mg L^{-1}). Nine bores sampled at one locality (Jackson Rock) situated 41.5 km south of Spotted Quoll were notably fresh (<200 mg L^{-1}) and two of these bores recorded stygofauna including canthocamptid copepods, and parabathynellids; however, no ameirids were detected (Rockwater 2009*b*). The failure to detect ameirids in the other 45 bores sampled therefore cannot be accounted for on the basis of groundwater conditions in pH, salinity or DO exceeding the general tolerance limits known for stygofauna.

The drill log and construction details for monitoring bore WWMB4 provided additional information on the aquifer type and local groundwater environment inhabited by *A. pardalotos*. This bore, developed entirely in basalt, was drilled to a depth of 49 m below the ground surface and cased with PVC pipe with a

Table 2. Range in groundwater physico-chemistry in the two bores containing the ameirids (WWMB4 and MB08), and 45 other sampled bores in the study area

Summarised from Rockwater (2009*b*)

Bore identification	Temperature °C	Salinity mg L^{-1} TDS	Dissolved oxygen mg L^{-1}	pH
WWMB4 *A. pardalotos*	19.03–21.08	26 040–26 560	0.16	6.13–6.50
MB08 *M. embe*	20.86	33 000–34 000	0.68	5.98–6.70
Other bores (*n* = 45)	16.43–24.76	15 800–63 960*	0.09–6.82	3.09–7.45
Mean ± s.d.	20.21 ± 1.88	34 110 ± 9 620*	2.3 ± 1.96	

*Excluding Jackson Rock bores (*n* = 9) with salinity 1000–2000 mg L^{-1} TDS.

screened interval (between 22 and 49 m depth) spanning a zone of higher water flow in the aquifer coinciding with a likely fracture in the basalt (Rockwater 2009*a*). Fifteen other bores developed at Spotted Quoll were drilled to depths ranging from 49 to 67 m (106 m maximum) with slotted intervals spanning most of their saturated profile and intersecting various geologic strata including basalt, granite, meta-sediments, saprolite and clay. These bores were also sampled for stygofauna but none were detected.

Monitoring bore MB08 was drilled to 40 m below the ground surface with the water table intersected at 34 m depth. The slotted interval and specific lithology at the slotted depth in bore MB08 was not available for this study; however, the gross surface geology at Cosmic Boy deposit is mapped as greenstone metasediments (Fig. 2). Eight other bores at Cosmic Boy were also sampled for stygofauna but none were detected.

Conservation status

Antistygonitocrella pardalotos is known only from its type locality (bore WWMB4) at the Spotted Quoll mine. *Megastygonitocrella embe* is similarly known only from its type locality (bore MB08) at Cosmic Boy mine. The sites are 12.5 km apart.

Species with naturally small distribution ranges are termed short-range endemic species (Harvey 2002). Harvey (2002) suggested a nominal distribution range of less than 10 000 km^2 as a working definition for short-range endemic species, although Eberhard *et al*. (2009) recommended a smaller area of less than 1000 km^2 for stygofauna in the Pilbara region. Some species of stygofauna in Western Australia, most notably in the small and hydrogeologically isolated calcrete aquifers of the northern Yilgarn, have ranges one to two orders of magnitude lower than this (range 0.89–2205 km^2) (Harvey *et al*. 2011). Similarly in cave systems in south-west Western Australia there occur stygofauna species with ranges of less than 2 km^2 (Eberhard *et al*. 2005). Because of their restricted distribution short-range endemics are vulnerable to extinction from environmental change and human impact (Harvey *et al*. 2011).

Despite the substantial sampling effort applied in 60 groundwater bores, including bores located in the same aquifer(s) and in close proximity (500 to 3000 m) to the type locality bores, in addition to bores located in other aquifers with similar groundwater physico-chemistry distributed across the Forrestania study area, the two species described herein were only detected from one bore each. While it may seem unlikely that the distribution range of either species is restricted to the immediate vicinity of the bores in which they were collected, their real range remains uncertain. Nonetheless, survey evidence to date suggests that *Megastygonitocrella embe* and *Antistygonitocrella pardalotos* are short-range endemic species, and based on their known distribution and locality-specific threatening processes, both species are considered vulnerable, especially the population of *A. pardalotos* at Spotted Quoll.

The Spotted Quoll mine pit will be 150 m deep, 550 m long and 350 m wide at the surface (EPA 2009). The depth to groundwater is ~20 to 40 m so dry-floor mining will require dewatering of up to 4.7 gigalitres per year. The model-calculated dewatering drawdown will impact an area of ~10 km^2 and extend laterally up to 4 km from the pit (Rockwater 2009*b*). The population of *A. pardalotos* will be impacted by dewatering activities at the Spotted Quoll mine where the groundwater level will be lowered by ~50 m at bore WWMB4 (EPA 2009), which is situated ~900 m from the Spotted Quoll mine pit. The natural pre-mining groundwater level at this site was around 22 m below ground level (Rockwater 2009*a*) and the dewatering will completely dehydrate bore WWMB4 (drilled depth 49 m below ground level) so the survival of this population is under threat and the conservation status of the species a matter of concern considering this is the only known site of occurrence.

The population of *M. embe* at the currently inoperative Cosmic Boy mine is also potentially threatened by the mounding and contamination of groundwater due to the operation of the Cosmic Boy nickel concentrator and associated tailings dam (EPA 2009). The mining company proposes to protect the Cosmic Boy MB08 population through annual monitoring of stygofauna, and groundwater levels and quality, in addition to developing a stygofauna management plan which will outline contingency measures in the event of detrimental changes to water quality (EPA 2009). While this surveillance monitoring may help to protect *M. embe*, no monitoring conditions were applied to *A. pardalotos* owing to its misidentification as belonging to the same species, although the EPA did recommend that: 'In order to reduce the impact to the population found at WWMB4 [Spotted Quoll] the EPA recommends that the proponent takes all practicable measures to minimise dewatering'.

Broader implications for stygofauna surveys and EIAs

Despite the advanced level of environmental protection policy in Western Australia (EPA 2003, 2007, 2012) our taxonomic study has highlighted limitations in current EIA practices relating to subterranean fauna. These limitations are discussed further below in consideration of the EPA's (2012) proposed move towards a strategic risk-based approach to EIA. We suggest some modifications in survey and assessment practices that have global relevance in regions where short-range endemic subterranean species coincide with mineral resources exploitation. Since most environmental law in Australia is state-based, the challenge for better subterranean EIA may need to be faced multiple times because policy in other states is further behind that in Western Australia.

Identification

The observed patterns in localised speciation, endemism and sympatry within the *Stygonitocrella s.l.* group are likely to pose continuing challenges for para-taxonomists attempting to identify members of this group, which is characterised by a convergent and conservative morphology, but that is true for many other subterranean groups (see, for example, Page *et al.* 2008). As shown in this study, misidentification of species can have significant implications in EIA outcomes, as well as for the conservation of short-range endemic species. We advocate that para-taxonomists seek independent confirmation of identifications from a recognised taxonomic authority where possible and/or validate morpho-species identifications with DNA sequencing, especially in cases where opinions differ, or a taxonomist or taxonomic framework is unavailable.

Survey effort

In stygo-regions and/or aquifer types characterised by low diversity and abundance we advocate greater survey effort than currently recommended by the EPA (2007) guidance statement. Of the 60 groundwater bores sampled in the Forrestania study area (southern Yilgarn stygo-region), stygofauna were detected in only four bores. This 6% ‘strike rate’ of sampling success (the proportion of bores yielding stygofauna divided by the number of bores sampled) is low compared with other geographic regions and geology and aquifer types in Western Australia, where strike rates upwards of 60% can be expected in calcrete aquifers of the northern Yilgarn, and porous, fractured-rock or (pseudo-) karstic aquifers in the Pilbara (Subterranean Ecology, unpubl. data). Despite the considerable survey effort in the Forrestania study, the two species described herein were only detected from their individual type locality bores. This suggests that in geographic regions and geology and aquifer types where stygofauna are not diverse or abundant, repeated sampling of many bores is required to confirm the presence of stygofauna. Even in stygo-regions characterised by high diversity and abundance, such as the Pilbara, sampling frequency fails to detect species present at a site (Eberhard *et al.* 2009). The survey effort recommended by the EPA guidance statement (EPA 2007), especially for pilot surveys, is 6 to 10 samples, which is unlikely to adequately sample stygofauna in non-calcrete aquifers of the southern Yilgarn where regional diversity and local abundance are depauperate compared with hotspots such as the Pilbara, and calcrete aquifers in the northern Yilgarn. We recommend that design of survey effort take into consideration not only regional biogeography patterns but also local influences on habitat suitability including aquifer type. Survey adequacy is more appropriately assessed within a local context by species accumulation curves (Eberhard *et al.* 2009) rather than relying on a pre-determined number of samples, and we support implementation of this approach, which may be taken into account by the EPA (2007).

Habitat mapping and strategic risk-based assessment

The EPA (2012) is proposing to move towards a strategic risk-based approach that could use regions or geology to predict the likelihood of finding subterranean fauna. The concept of biogeographic regions for subterranean fauna is valid, with recognisable ‘stygo-regions’ for the Kimberley, Pilbara, northern Yilgarn and Nullarbor, for example (Subterranean Ecology 2007). For other regions, including the southern Yilgarn, the fauna is less sampled and characterised. While regional-scale patterns in biogeography, geology, groundwater condition (including for example salinity, pH, dissolved oxygen) and/or aquifer type can usefully inform preliminary desktop assessment of the likelihood of stygofauna occurrence, our study has demonstrated that regional groundwater conditions in the southern Yilgarn, characterised by waning of calcretes south of the Menzies Line and combined with mostly acid groundwater and extensive anoxic conditions (Morgan 1993), do not preclude stygofauna. Where there is uncertainty about regional patterns or boundaries, we advocate a precautionary approach backed up by field survey in EIA of mine projects.

The EPA (2012) is also proposing that habitat mapping be used as a surrogate to demonstrate landscape connectivity, or as an alternative to additional sampling for species found in low numbers. This approach requires acceptance of the view that it is scientifically defensible to use habitat mapping and analysis of gene flow in widespread species as a surrogate to demonstrate landscape connectivity, and as an alternative to additional sampling for species found in low numbers (EPA 2012). We do not accept this view as scientifically defensible. In granting approval for the mine, the EPA (2009) accepted a technical opinion that the aquifers at Spotted Quoll and Cosmic Boy formed part of a regionally interconnected aquifer system giving opportunity for regional distribution of stygofauna. The sampling evidence from this study provides no support for assumptions that hydro-geologic connectivity equates to: (1) wider habitat connectivity, and; (2) likelihood of wider fauna or species occurrence. While these assumptions are valid in some cases they are not universal and contrary patterns are regularly observed (S. Eberhard, pers. obs.).

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