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DIVERGENT MOLECULAR LINEAGES AND NOT-SO-CRYPTIC SPECIES: THE FIRST DESCRIPTIONS OF STYGOBITIC CHILTONIID AMPHIPODS (TALITROIDEA: CHILTONIIDAE) FROM WESTERN AUSTRALIA

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ABSTRACT

The Australian stygofauna comprises a unique and diverse assemblage of invertebrates, of which the amphipod crustaceans are a dominant but poorly described element. Recent exploration of the Western Australian stygofauna, in particular the Yilgarn region of central Western Australia, has shown evidence of great species diversity, with numerous individual calcrete aquifers found to contain unique assemblages of invertebrate species. A recent fine-scale biodiversity initiative, using *COI* barcoding, of a single calcrete aquifer (Sturt Meadows) in the Yilgarn region reported the presence of three divergent and morphologically cryptic stygobitic lineages of amphipods from Chiltoniidae, which represent undescribed taxa. This paper details the subsequent systematic analysis of these *COI* lineages and presents a broader phylogeny and detailed morphological analyses of the lineages. The report of cryptic species was not supported upon morphological examination and three new species from three new genera (*Scutachiltonia* n. gen., *Stygochiltonia* n. gen., and *Yilgarniella* n. gen.) are described from the Sturt Meadows calcrete aquifer. The three genera do not form a monophyletic group and are instead believed to have evolved from separate colonisation events from distinct ancestors rather than from speciation events within the aquifer. This work contributes to a broader research initiative, documenting the presence of a rich subterranean invertebrate fauna in the Yilgarn region.

KEY WORDS: Amphipoda, *COI*, cryptic species, groundwater, mtDNA, stygofauna

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INTRODUCTION

Within the last two decades, intensive exploration of Australian subterranean aquatic habitats and their associated fauna has revealed a unique and highly diverse assemblage of stygobitic invertebrates (stygofauna) (Humphreys, 2008; Humphreys et al., 2009; Guzik et al., 2011a). Once considered a depauperate fauna, in comparison to subterranean diversity hotspots in Europe and North America, many major faunal groups (Mollusca, Nematoda, Oligochaetea, Hexapoda, and Crustacea) have now been found in a wide variety of geological habitats across Australia that include karst, larval tubes, alluvial sediments, fractured rock aquifers and subterranean carbonate deposits (calcrete aquifers) (Humphreys, 2008; Väinölä et al., 2008; Guzik et al., 2011a).

Crustaceans are a dominant member of the Australian stygofauna, with Amphipoda particularly well represented, largely by Crangonyctoidea (Bradbury and Williams, 1999; Bradbury, 2000; Lowry and Stoddart, 2003; Väinölä et al., 2008). Whilst abundant, the Australian stygobitic amphipod fauna is poorly described; with estimates indicating that in the western half of Australia only about 20% of known and identified stygobitic amphipods have been formally described (28 species from four families – Bogidiellidae, Hadziidae, Melitidae, and Paramelitidae) and perhaps 20 times more remain to be discovered (Eberhard et al., 2005; Finston et al., 2008; Guzik et al., 2011a). The examination of molecular diversity in undescribed stygobitic amphipods has largely contributed to this estimate; with recent studies uncovering highly diverse mtDNA lineages equating to species endemic to individual aquifers (Cooper et al., 2007; Finston et al., 2007, 2008; Bradford et al., 2010; Guzik et al., 2011b). Descriptive taxonomic work to formally identify species has lagged behind molecular-based species discovery, in part due to a lack of specialised

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taxonomists, but also due to the detection of cryptic species (Finston et al., 2008). Adaptations to extreme conditions, such as in stygobitic habitats, have been shown to lead over large periods of time to phenotypic convergence with the potential to confound morphological study (Wiens et al., 2003; Lefébure et al., 2006; Trontelj et al., 2009; Zakšek et al., 2009); within Amphipoda adaptations to the stygobitic environment are typically the reduction or loss of pigment and eyes, reduction of coxae, and the elongation of antennae, body segments and posterior legs (Bradbury, 2000). The potential for phenotypic convergence in stygobitic habitats, combined with typically small sample sizes for stygofauna of a few individuals per collection, increases the difficulty in analysing morphological variation. Under these conditions it is likely that morphology-only identification approaches will lead to an underestimation of species diversity and a combination of techniques and data (molecular, morphological, biochemistry, and geographical) where available, are more appropriate to fully explore species boundaries and diversity of styogobitic amphipods (Lang et al., 2003; Tomikawa et al., 2007; Flot et al., 2010).

In Western Australia, the ancient Yilgarn craton (Fig. 1) is part of the Western shield, a region of stable continental crust thought to have been continually emergent from the sea since the Palaeozoic, more than 250 million years ago (Humphreys, 2001). This region was presumed to be deficient for stygobitic habitats until the 1998 discovery of stygofauna within numerous distinct subterranean calcrete aquifers (hereafter referred to as calcretes) (Fig. 1). These relatively thin (∼10 m deep) calcretes formed as carbonate deposited from groundwater flow along palaeo drainage channels during Pliocene aridification of inland Australia 5-10 million years ago (Humphreys, 2001; Cooper et al., 2007), and are thought to have become aquatic refugia for water dependent inland invertebrates as aridification altered the landscape from warm wet forests to arid and semi-arid desert (Leys et al., 2003; Byrne et al., 2008). Complex patterns of habitat connectivity and environmental gradients both within calcretes and broadly across the Yilgarn region, as well as large and small historical landscape changes (aridification, geological movements, flooding events) have combined to create highly structured subterranean systems and a richly diverse invertebrate fauna (Humphreys et al., 2009). The stygobitic fauna of the Yilgarn has only recently begun to be explored, yet the diversity of many associated aquatic invertebrates (water beetles, copepods, bathynellaceans, isopods, and crangonyctoid amphipods) within Yilgarn calcretes are high, with species restricted to single calcretes (Cooper et al., 2007, 2008; Leys and Watts, 2008; Guzik et al., 2009, 2011b).

Access to an extensive Yilgarn bore field on the Sturt Meadows pastoral property near Leonora in Western Australia has afforded opportunities to undertake comprehensive faunal surveys of the calcrete, and lead to the discovery of a diverse group of invertebrates, including amphipods belonging to Chiltoniidae (Bradford et al., 2010). The Australian chiltoniids are a small group of freshwater amphipods, historically represented by only two species in lowland surface water systems (creeks, dams, marshes) across south-eastern Australia and two species from groundwater-fed springs in South Australia (King, 2009a). Recent work, including both molecular and morphological analyses, has described a highly diverse group and provided new species and new genera as well as evidence of morphologically cryptic species (King, 2009a, b; Murphy et al., 2009; King and Leys, 2011). Whilst undescribed, stygobitic chiltoniid amphipods are known to exist in the Yilgarn region; populations from several calcretes were sequenced and mistakenly identified as belonging to Hyalidae, along with crangonyctoid amphipods by Cooper et al. (2007). Within the Sturt Meadows calcrete, three diverse sympatric chiltoniid amphipod lineages were detected using mitochondrial and allozyme molecular markers (Bradford et al., 2010). Each purported species occurred in differing abundances across the calcrete and each was reported to be more closely related to taxa in other regional calcretes than to each other. The lineages were also reported to be cryptic species with no morphological differences discerned (Bradford et al., 2010).

Evidence of high levels of diversity among morphologically cryptic species, coupled with the availability of mtDNA sequence data and extensive material collected from the Sturt Meadows calcrete presented an opportunity for subsequent systematic study, with the primary aim to examine and describe the new species from the Sturt Meadows calcrete. The availability of mtDNA COI sequence data from six Yilgarn calcrete chiltoniid populations including Sturt Meadows (Cooper et al., 2007; Bradford et al., 2010), as well as South Australian mound spring chiltoniid populations (Murphy et al., 2009) meant that a comparatively broad molecular framework was possible for phylogenetic examination and to inform taxonomic evaluation of the Sturt Meadows taxa. Taxonomic effort included the description of species using morphological and molecular evidence, detailed examination of phenotypic variation and an examination of the phenomenon of cryptic species within the stygobitic chiltoniid amphipods.

Materials and Methods

Morphological Methods

Material from the Bradford et al. (2010) study was examined for morphological differences, with 82 individuals identified to each of three purported species. With these individual samples frequently damaged through the mtDNA tissue extraction process, an additional 29 uncatalogued specimen lots from Sturt Meadows calcrete in the collections of the Western Australian Museum (WAM) (15 samples) and The University of Adelaide (12 samples) were also examined for the three species (Table 1). Additional undescribed material (20 samples) from 11 calcretes throughout the Yilgarn region from WAM were also examined for comparative purposes (Table 2).

Types were dissected along the left side and appendages illustrated with a drawing tube attachment to a Nikon Eclipse 80i microscope. All type material has been lodged with the Western Australian Museum. The family and higher systematic treatment follows that outlined by Serejo (2003).

All taxonomic descriptions in this work are attributed to R. A. King.

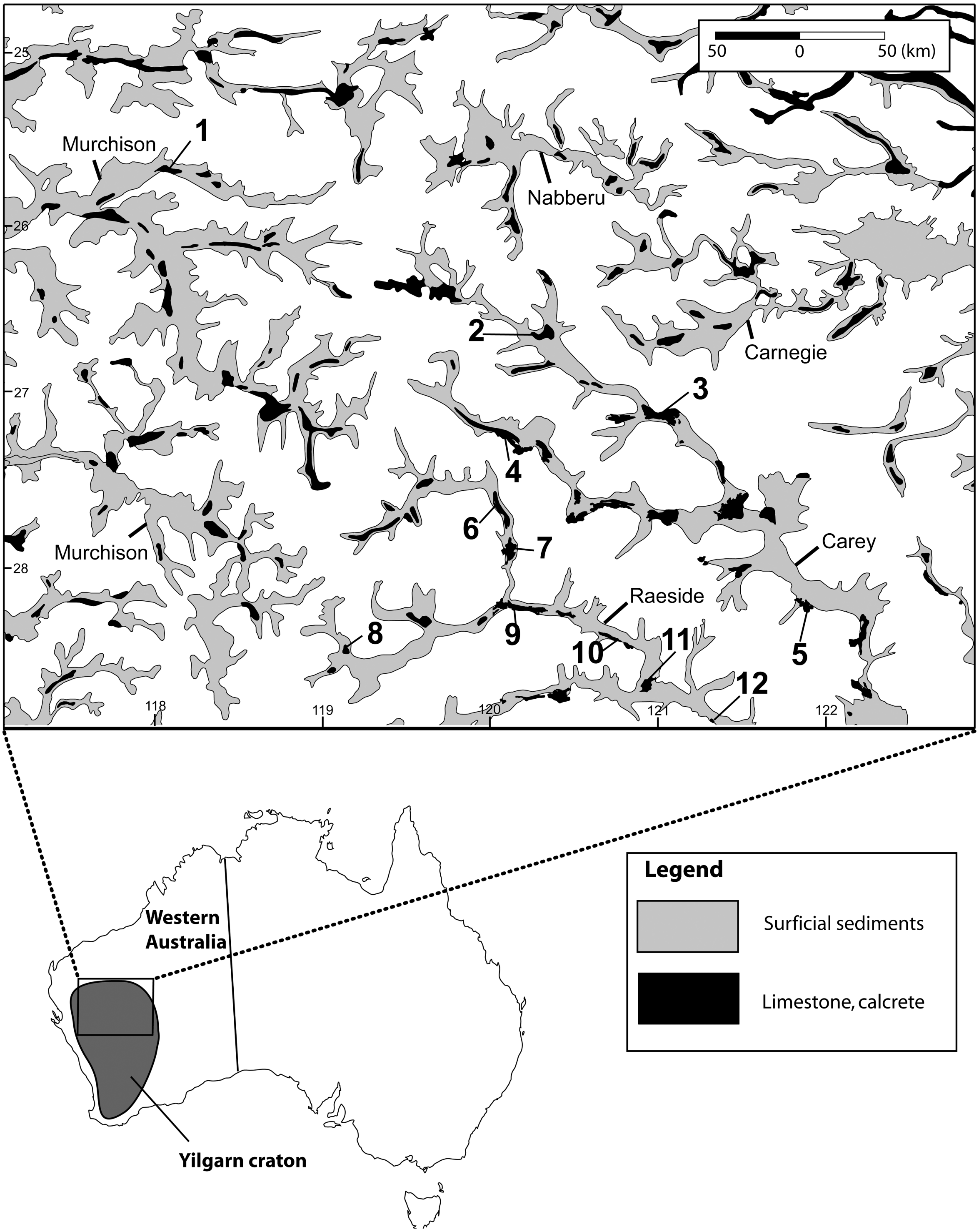

Fig. 1. The calcretes of the Yilgarn region in Western Australia. Each palaeodrainage is labelled. Calcretes are as follows: 1, Mt Padbury; 2, Lake Violet; 3, Barwidgee; 4, Lake Mason; 5, Nambi; 6, Kaluwiri; 7, Depot Springs; 8, Yuinmerry; 9, Pinnacles; 10, Perrinvale; 11, Sturt Meadows; 12, Sons of Gwalia.

Molecular Methods

Existing sequences from Sturt Meadows (from Bradford et al., 2010), five additional Yilgarn calcretes (Lake Mason, Lake Violet, Barwidgee, Mt. Padbury and Depot Springs) (from Cooper et al., 2007) and the four known species from the South Australian mound springs (*Arabunnachiltonia murphyi* King, 2009; *Austrochiltonia dalhousiensis* Zeidler, 1997; *Wangiannachiltonia guzikae* King, 2009; and *Phreatochiltonia anophthalma* Zeidler, 1991) (from Murphy et al., 2009) were included in the analysis. Examination and identification of specimens from all three Sturt Meadows lineages included further sequencing of 11 specimens to ensure

Table 1. Chiltoniid amphipod material examined from the Sturt Meadows calcrete from the collections of the Western Australian Museum (WAM) (BES field numbers, catalogue C numbers) and the University of Adelaide (SM field numbers). m = male; f = female; juv = juvenile.

No. of specimens	Collection no.	Latitude	Longitude	WAM accession no.
Yilgarniella sturtensis n. sp.				
>30 (m, f, juv)	BES 10241	28°42′0.9″S	120°50′27.42″E	C49178
1 juv	BES 10271	28°42′59.11″S	120°53′23.82″E	C49179
2 (1 f, 1 m)	BES 10280	28°41′54.13″S	120°53′36.24″E	C49180
9 (1 m, 3 f, 5 juv)	BES 10286	28°41′48.05″S	120°53′47.62″E	C49181
4 f	BES 12029	28°42′0.83″S	120°53′36.13″E	C49182
5 (1 m, 4 f)	BES 12045	28°43′2.35″S	120°53′27.6″E	C49183
1 f	BES 12057	28°41′51.32″S	120°54′2.2″E	C49184
9 (3 m, 2 f, 4 juv)	BES 12068	28°41′51.47″S	120°54′5.76″E	C49185
Stygochiltonia bradfordae n. sp.				
4 (2 f, 1 m, 1 juv)	BES 10240	28°42′0.9″S	120°50′27.42″E	C49186
4 (1 m, 3 f)	BES 10262	28°41′47.72″S	120°53′36.56″E	C49187
Scutachiltonia axfordi n. sp.				
1 f, 2 m	SM E13	28°41′56.57″S	120°54′18.18E	C49188
1 f	BES 11998	28°42′14.04″S	120°53′32.28″E	C49189
2 m	BES 12996	28°42′30.06″S	120°53′42.94″E	C49190
1 f	SM F13	28°41′59.81″S	120°54′18.03″E	C49191
1 m	SM N4	28°42′31.69″S	120°53′36.68″E	C49192
1 f	BES 12994	28°42′36.47″S	120°53′35.45″E	C49193
1 m	BES 11974	28°42′31.9″S	120°53′47.90″E	C49194
1 m	SM E5	28°41′56.04″S	120°53′48.67″E	C49195
2 m	SM B3	28°41′46.41″S	120°53′40.89″E	C49196
1 f	SM W3	28°42′57.67″S	120°53′36.43″E	C49197
1 f	SM E12	28°41′56.53″S	120°54′14.51″E	C49198
1 f	SM E11	28°41′56.49″S	120°54′10.63″E	C49199

that the identified morphospecies matched the molecular lineages identified by Bradford et al. (2010).

DNA was extracted from pereiopod tissue from 11 specimens using the Gentra (Puregene) method for fresh tissue and preserved in absolute ethanol. PCR amplification and sequencing were performed as described in Cooper et al. (2007). A ∼650 bp region of the mitochondrial Cytochrome oxidase subunit I (*COI*) gene was amplified using universal primers: M414 (forward, 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′, alias LCOI490, Folmer et al., 1994), M423 (reverse, 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′, alias LCO2198, Folmer et al., 1994).

MEGA (Tamura et al., 2007) was used to align sequences, for analyses of amino acids and to determine nucleotide pairwise distances. These were calculated using two models for comparison, the Kimura-2 and the Maximum Composite Likelihood with Gamma distribution, both widely used in crustacean studies (Lefébure et al., 2006; Pernet et al., 2010; Filipová et al., 2011). Baysian phylogenetic analyses of aligned sequences were carried out using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). As in Bradford et al. (2010), the data set was partitioned by codon using independent models of sequence evolution for the first codon position (TrN + I + G), second codon position (K81uf + I) and third codon position (HKY + G), which were with the optimal models specified using ModelTest 3.7 (Posada and Crandall, 1998), under the Alkaike Identification Criterion. Bayesian analyses were run using four chains for 10 million generations in two independent runs, sampling every 500 generations. The program Tracer 1.5 (Rambaut, 2003) was used to evaluate convergence of distribution: the likelihood values converged after ∼1 million generations. A burnin of 2000 was chosen and a 50% consensus tree was constructed from the remaining 18 001 trees.

Table 2. Chiltoniid amphipod material from additonal calcretes in the Yilgarn region examined from field collections of the Western Australian Museum (WAM).

WAM no.	Calcrete	Latitude	Longitude
BES 10377	Barwidgee	27°8′14.89″S	120°56′57.95″E
BES 6650	Depot Springs	28°3′36.25″S	120°4′2.78″E
BES 8382	Depot Springs	27°55′50.88″S	120°4′45.19″E
BES 8407	Depot Springs	28°2′59.57″S	120°2′21.08″E
BES 8408	Depot Springs	28°3′36.25″S	120°4′2.78″E
BES 8367	Kaluwiri	27°40′58.22″S	120°2′7.01″E
BES 8393	Kaluwiri	27°40′58.22″S	120°2′7.01″E
BES 8361	Lake Mason	27°32′24.03″S	119°37′27.41″E
BES 8363	Lake Mason	27°32′24.03″S	119°37′27.41″E
BES 6425	Lake Violet	26°40′29.53″S	120°13′55.2″E
BES 6434	Lake Violet	26°40′29.53″S	120°13′55.2″E
BES 9309	Mt. Padbury	25°41′41.93″S	118°4′46.49″E
BES 10317	Nambi	28°14′25.44″S	121°50′13.92″E
BES 10252	Perinvale	28°46′30.14″S	120°25′1.2″E
BES 10253	Perinvale	28°46′30.14″S	120°25′1.2″E
BES 6642	Pinnacles	28°15′26.78″S	120°7′36.84″E
BES 6643	Pinnacles	28°15′26.78″S	120°7′36.84″E
BES 8399	Pinnacles	28°12′43.74″S	120°2′36.53″E
BES 11830	Sons of Gwalia	28°56′3.3″S	121°18′4.39″E
BES 6657	Yuinmerry	28°32′55.03″S	119°5′28.07″E

RESULTS

Molecular Results

A 624 bp fragment of *COI* was obtained from 83 individuals. All *COI* sequences had an open reading frame, with no evidence of insertions/deletions or anomalies in the protein coding sequence, suggesting that they are all likely to be from functional mitochondrial *COI*. Phylogenetic analyses indicated that nine well supported and deeply divergent amphipod lineages exist within the sampled Yilgarn calcretes that include Sturt Meadows, Lake Mason, Lake Violet, Barwidgee, Mt. Padbury and Depot Springs (Fig. 2). These divergent molecular lineages were restricted in their distribution to single calcretes. Nucleotide divergence parameters, measured as pair wise distances (Tables 3, 4), were slight within the stygofaunal lineages (0.2-1%), while pair wise distances between stygofaunal lineages were between 9-39% (Table 3).

Strong support (100% Baysian Posterior Probability (BPP)) was found for three distinct lineages that do not form a reciprocally monophyletic clade within the Sturt Meadows calcrete (Fig. 2). The three lineages showed nucleotide divergence levels of 15-23%. Single lineages were observed in the majority of the Yilgarn calcretes (Lake Mason, Lake Violet, Mt. Padbury, Barwidgee), however two distinct lineages, equating possibly to separate species, were found within the Depot Springs calcrete (at 12-16% divergence) as well as the three from Sturt Meadows.

The South Australian chiltoniid taxa did not form a monophyletic group, instead four distinct lineages (93-100% BPP) were evident that included the southern mound spring taxa (*Ar. murphyi* and *W. guzikae*) and two northern mound spring groups (*Au. dalhousiensis* and *P. anophthalma*), which showed close affinities to Western Australian lineages: *Au. dalhousiensis* from Dalhousie springs, SA to the lineage from Lake Mason calcrete, WA and *P. anophthalma* from Dalhousie Springs, SA to a Sturt Meadows lineage (= *Scutachiltonia* n. gen.). Pairwise distances within lineages were higher in the South Australian mound spring taxa (8-13%) than in the stygofauna and the distances between lineages was comparable to that of the stygofauna (17-35%) (Tables 3, 4).

Morphological Results

Examination of the Sturt Meadow lineages did not uncover morphologically cryptic species, but instead supported the recognition of three distinct morphospecies equating to three new species recognised here as three new chiltoniid genera (*Scutachiltonia* n. gen., *Stygochiltonia* n. gen., and *Yilgarniella* n. gen). Compared to the known epigean chiltoniid

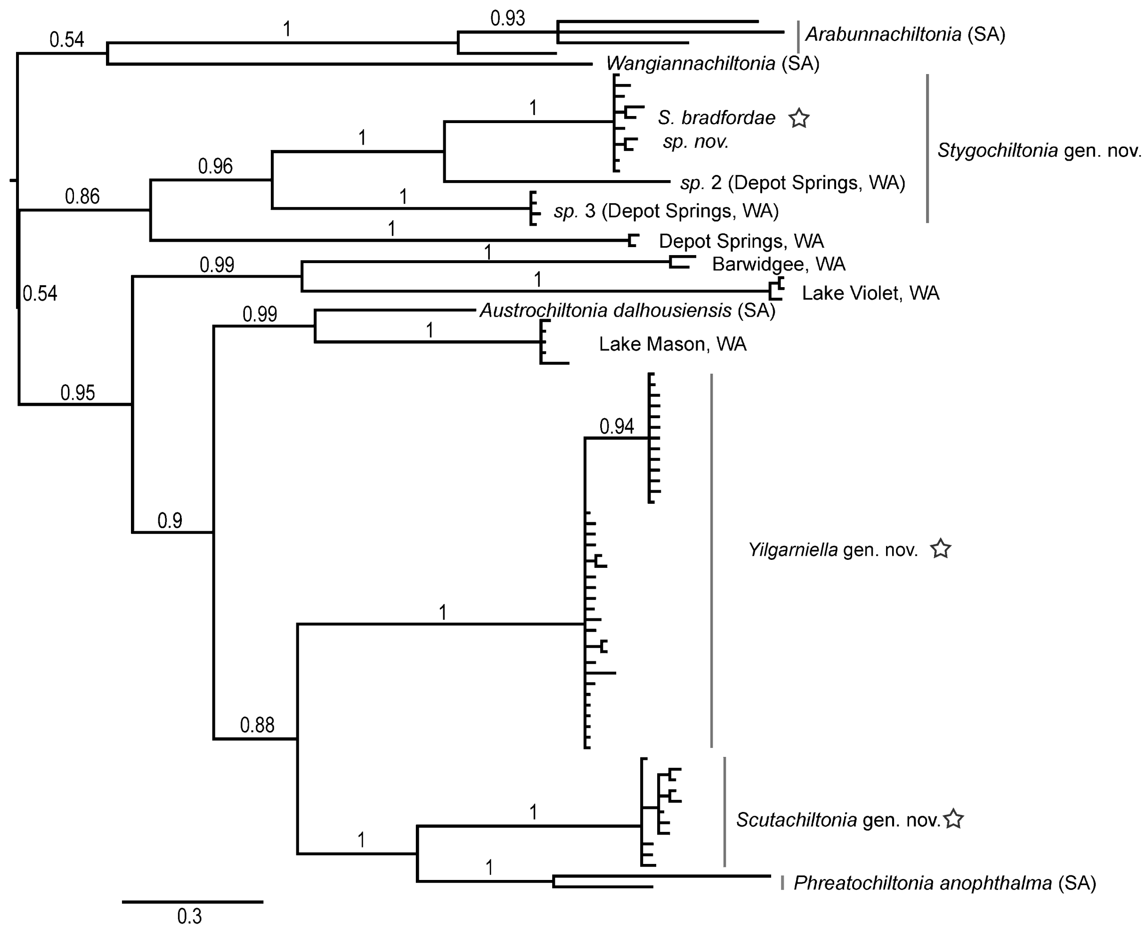

Fig. 2. Posterior probability (50%) Bayesian consensus tree of Yilgarn chiltoniid amphipod *COI* sequences, posterior probabilities are listed on corresponding branches. Star symbol indicates Sturt Meadows, WA groups. WA = Western Australia; SA = South Australia.

Table 3. mtDNA *COI* nucleotide pair wise distances between Yilgarn (WA) and South Australian stygobitic chiltoniid taxa. Values calculated via the Kimura-2 (bold, bottom left) and Maximum Composite Likelihood (Gamma) models (plain, top right). * includes Sturt meadows taxa only; + includes Sturt Meadows, and two putative Depot Springs species.

	South Australian taxa				Sturt Meadows (WA)				Additional Yilgarn calcretes (WA)					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Phreatochiltonia*		0.345	0.198	0.320	0.220	0.150	0.220	0.358	0.358	0.231	0.252	0.267	0.312	0.250
2. *Wangiannachiltonia*	**0.209**		0.314	0.355	0.370	0.317	0.342	0.395	0.395	0.350	0.285	0.293	0.390	0.364
3. *Au. dalhousiensis*	**0.139**	**0.202**		0.263	0.201	0.228	0.224	0.292	0.292	0.215	0.256	0.231	0.240	0.133
4. *Arabunnachiltonia*	**0.191**	**0.208**	**0.175**		0.366	0.318	0.309	0.325	0.325	0.293	0.297	0.316	0.372	0.294
5. *Yilgarniella*	**0.153**	**0.216**	**0.142**	**0.210**		0.203	0.223	0.302	0.302	0.237	0.270	0.262	0.330	0.219
6. *Scutachiltonia*	**0.115**	**0.190**	**0.156**	**0.189**	**0.148**		0.199	0.372	0.372	0.176	0.216	0.220	0.263	0.264
7. *Stygochiltonia* SM*	**0.147**	**0.207**	**0.151**	**0.189**	**0.151**	**0.138**		na	0.219	0.108	0.116	0.261	0.268	0.242
8. *Stygochiltonia* (all)+	**0.152**	**0.202**	**0.155**	**0.187**	**0.159**	**0.139**	**na**		0.235	na	na	0.261	0.271	0.251
9. Mt. Padbury	**0.202**	**0.234**	**0.187**	**0.202**	**0.187**	**0.207**	**0.150**	**0.167**		0.252	0.269	0.386	0.359	0.269
10. Depot Springs sp. 2	**0.152**	**0.211**	**0.149**	**0.182**	**0.165**	**0.126**	**0.093**	**na**	**0.172**		0.158	0.261	0.289	0.222
11. Depot Springs sp. 3	**0.163**	**0.185**	**0.167**	**0.185**	**0.175**	**0.144**	**0.095**	**na**	**0.180**	**0.122**		0.259	0.273	0.282
12. Barwidgee	**0.169**	**0.187**	**0.154**	**0.196**	**0.165**	**0.147**	**0.162**	**0.163**	**0.219**	**0.165**	**0.165**		0.259	0.266
13. Lake Violet	**0.194**	**0.243**	**0.167**	**0.214**	**0.202**	**0.177**	**0.178**	**0.180**	**0.216**	**0.181**	**0.182**	**0.175**		0.274
14. Lake Mason	**0.171**	**0.230**	**0.115**	**0.193**	**0.155**	**0.182**	**0.168**	**0.173**	**0.174**	**0.159**	**0.189**	**0.174**	**0.189**	

taxa, all three species from Sturt Meadows presented distinct evidence of stygobitic lifestyles: loss of eyes and pigment, elongation of antennae, elongation of pereiopods 6-7, elongation of the uropods.

Whilst all three species from Sturt Meadows have been sampled in sympatry (by Bradford et al., 2010) and *S. bradfordae* n. sp. and *Y. sturtensis* n. sp. found here in samples BES 10241, 10262 (see Table 1)), morphology with supporting distributional data suggests each may inhabit a somewhat distinct niche within the calcrete. *Yilgarniella*, represented by *Y. sturtensis*, is the most widespread of the three species within the calcrete and is the least specialised stygobite of the three, being closest in morphology to described epigean species from southeastern Australia (coxae 1-4 unmodified and similar length to pereion segments, pereiopods 6-7 not extremely elongate compared to pereiopod 5). Based on morphological examination, further species within *Yilgarniella* are suspected in other calcretes across the Yilgarn (including Nambi); sequence data from individuals from these calcretes was not available for comparison. *Scutachiltonia*, represented by the comparably large (5-6 mm) *S. axfordi* n. sp. and currently thought to be endemic to Sturt Meadows, is found in a somewhat patchy distribution across the calcrete and exhibits marked morphological characters (narrow elongate coxae 1-4, crenulated bases on pereiopods 5-7) making it a robust, presumably well protected species in larger spaces within the calcrete. *Styogochiltonia*, represented by *S. bradfordae* n. sp., is the rarest species of the three within the calcrete and presents the most characters of a stygobitic lifestyle (narrow elongate body segments, coxae 1-7 reduced, basis of pereiopods reduced, pereiopods 6-7 very long compared to pereiopod 5) and presumably is able to inhabit smaller interstitial spaces compared with the other two species. Examination of the two distinct Depot Springs molecular lineages indicates that they are congeneric with *Styochiltonia bradfordae*.

Table 4. Pairwise distances within Yilgarn and South Australian stygobitic chiltoniid amphipod groups (*A. dalhousiensis*, *Arabunnachiltonia*, Mt. Padbury, Depot Springs spp. 2 and 3 not included as too few sequences available for comparision). * includes Sturt meadows taxa only; + includes Sturt Meadows, and two putative Depot Springs species.

	Kimura-2	Maximum composite likelihood
Phreatochiltonia	0.0884	0.102031
Wangiannachiltonia	0.1109	0.130839
Yilgarniella	0.011	0.011244
Scutachiltonia	0.0046	0.004624
Stygochiltonia SM*	0.0039	0.003874
Stygochiltonia (all)+	0.051	0.062
Barwidgee	0.0029	0.002949
Lake Violet	0.002	0.001957
Lake Mason	0.0035	0.003538

SYSTEMATICS

Infraorder Talitrida Rafinesque, 1815
Talitroidea s.s. Rafinesque, 1815
Chiltoniidae Barnard, 1972
Scutachiltonia King, n. gen.

Type Species.—*Scutachiltonia axfordi* King, n. sp.

Diagnosis.—Eyes absent. Antenna 1 at least twice the size of antenna 2. Coxae 1-3 long (at least twice as long as broad). Coxa 4 longer than broad, with a defined proximal corner. Coxae 5-6 posterior lobe extending at least twice length of anterior lobe. Gnathopod 2 propodus in males elongate (at least two times as long as wide). Pereiopod 5-7 bases with postero-distal lobe; with distinct crenulation along posterior margins; anterior margins highly setose with up to ten robust setae. Pereiopods 6 and 7 about 1.5 times as long as pereiopod 5; carpus elongate and distinctly longer than merus; propodus elongate and around 1.3 times as long as carpus. Epimera 1-3 with postero-distal corners defined with a blunt spine.

Etymology.—Named for the elongate coxae and large bases of pereiopods 5-7, which together form long shields (latin:

"scuta") and give the species a robust shape, and "chiltonia" for its placement within Chiltoniidae.

Remarks.—*Scutachiltonia* includes "Clade 2" of Bradford et al. (2010) and comprises the largest of the three species present at Sturt Meadows. It is unique in its possession of elongate coxae 1-4, pereiopods 5-7 with anterior margin strongly setose, and epimera 1-3 with postero-distal corner spines. No other similar morphotype has been found in material examined from numerous regional calcrete aquifers. Extensive examination of the Sturt Meadows calcrete, together with molecular evidence confirming a divergent monotypic group indicate that *Scutachiltonia* will remain monotypic and endemic to the Sturt Meadows calcrete.

Our results indicate that *Scutachiltonia* is more closely related to the South Australian mound spring amphipod *P. anophthalma*, than it is to the other Sturt Meadows amphipods (Fig. 2). Whilst *P. anophthalma* is the only other described blind chiltoniid, the two species do not share any great morphological affinities and it is likely that as more calcrete populations are analysed, their relationship will become clearer.

Scutachiltonia axfordi King, n. sp.
Figs. 3-6

Morphological Diagnosis.—Antenna 1 greater than 3/4 body length, flagellum three times longer than peduncle; antenna 2 1/3 body length, flagellum at least 1.5 times longer than peduncle. Gnathopod 2 propodus (in males) two or more times as long as broad. Coxa 1-3 two times as long as broad; coxa 4 elongate and with defined proximal corner. Pereiopods 5-6 bases longer than broad, with postero-distal lobe, entire posterior margin crenulate with associated short setae, posterior margin with 7-10 robust setae concentrated distally; pereiopod 7 basis almost as long as broad, with poster-distal lobe, entire posterior margin crenulate with associated short setae, posterior margin with 7-20 robust setae concentrated distally. Uropod 1 outer ramus with 2-3 robust setae along length, with distal cluster of 2-3 small setae and single long robust seta (half-length of ramus); inner ramus with 1-3 robust setae in two rows along length, with distal cluster of 4 small setae and single long robust seta (half-length of ramus). Uropod 2 outer ramus with 1-3 robust setae along length, with distal cluster of 2-3 small setae and single long robust seta (half-length of ramus); inner ramus with 5-9 robust setae in two rows along length, with distal cluster of 4 small setae and single long robust seta (half-length of ramus). Uropod 3 bi-articulate with second article minute (less than 1/4 length of first article).

Material Examined.—Holotype, WAM C49169, male, 5.3 mm, Sturt Meadows calcrete, Western Australia, BES 12986, Bore N4, 28°42′36.396″S, 120°53′31.74″E, coll. S. Cooper, A. Allford, 4 Apr 2005. Allotype, WAM C49170, female, 5.6 mm, Sturt Meadows calcrete, Western Australia, BES 11836, Bore E13, 28°42′1.3314″S, 120°54′12.996″E, coll. W. F. Humphreys, S. Cooper, R. Leys, A. Allford, 31 Mar 2005. Paratypes (two males, three females), WAM C49171, Sturt Meadows calcrete, Western Australia, BES 12025, Bore E12, 28°42′1.224″S, 120°54′9.36″E, collected by W. F. Humphreys, S. Cooper, J. Bradbury, M. Guzik, 25 Sep 2004 (see Table 1 for additional material).

Distribution.—Western Australia: Sturt Meadows calcrete aquifer, situated on the Sturt Meadows Pastoral Property, near Leonora.

Description.—Holotype male (WAM C49169), length: 5.3 mm. Head about as long as deep (Fig. 3A).

Antenna 1 (Fig. 4B) 0.77 times body length, peduncular article 1 2.2 times as long as broad, inner lateral margin with two robust setae; peduncular article 2 slightly longer than article 1, almost four times as long as broad; peduncular article 3 shorter than article 2, four times as long as broad; flagellum three times longer than peduncle, of 22 articles, with ventral aesthetascs on the proximal margins of the eight distal articles. Antenna 2 (Fig. 4A) about 0.4 times length of antenna 1; peduncular article 3 as broad as long, inner-distal margin with 2-3 robust setae; peduncular article 4 two times as long as article 3, 3.5 times longer than broad, inner lateral margin with two robust setae, distal margin with one robust seta; peduncular article 5 longer than article 4, 4.75 times as long as broad; flagellum 1.5 times longer than peduncle, of 12 articles.

Mouthparts as for family (Fig. 4C-I) (see King, 2009b).

Gnathopod 1 (Fig. 5A) coxa two times as long as broad, distal margin with seven short simple setae; basis ventral margin with scattered simple setae; ischium, and merus distoventral corners with clusters of one or two simple setae; carpus with ventral-lateral lobe and row of 10 setulate setae becoming longer distally, distodorsal margin with long setae; propodus 2.2 times as long as broad, subchelate, palm acute, distoventral corner with one robust seta at corner of palm, medial palm margin with short robust and long simple setae, distodorsal margin with long simple setae, inner face with three robust plumose setae; dactylus with unguis, curved, fitting against palm, with proximal plumose seta. Gnathopod 2 (Fig. 5B) coxa two times as long as broad, distal margin with five short simple setae; basis dorsal and ventral margins without setae; ischium and merus with few setae on ventral margins; propodus two times as long as broad, subchelate, distoventral corner marked by dactylar socket, palm margin with numerous robust setae with subterminal spines. Pereiopod 3 (Fig. 5C) coxa 2.2 times as long as broad, distal margin with six short simple setae; basis dorsal and ventral margins with few simple setae, distoventral corner with cluster of setae; ischium distoventral corner with clusters of setae; merus with slight distodorsal lobe, ventral margin with scattered simple setae, distoventral corner with cluster of setae; carpus ventral margin with robust setae and scattered simple setae; propodus dorsal margin with few setae; ventral margin with seven clusters of robust and simple setae; dactylus dorsal margin with plumose seta, ventral margin with simple seta, unguis present. Pereiopod 4 (Fig. 5D) coxa 1.4 times as long as wide, with distinct proximal excavation, distal margin with 19 short simple setae; basis dorsal and ventral margins with scattered simple setae, distoventral corner with cluster of simple setae; ischium distoventral corner with cluster of setae; merus with slight distodorsal lobe, ventral margin with scattered simple setae, distoventral corner with cluster of setae; carpus ventral margin with scattered robust and simple setae; propodus ventral margin with seven clusters of robust and simple setae; dactylus dorsal margin with plumose seta,

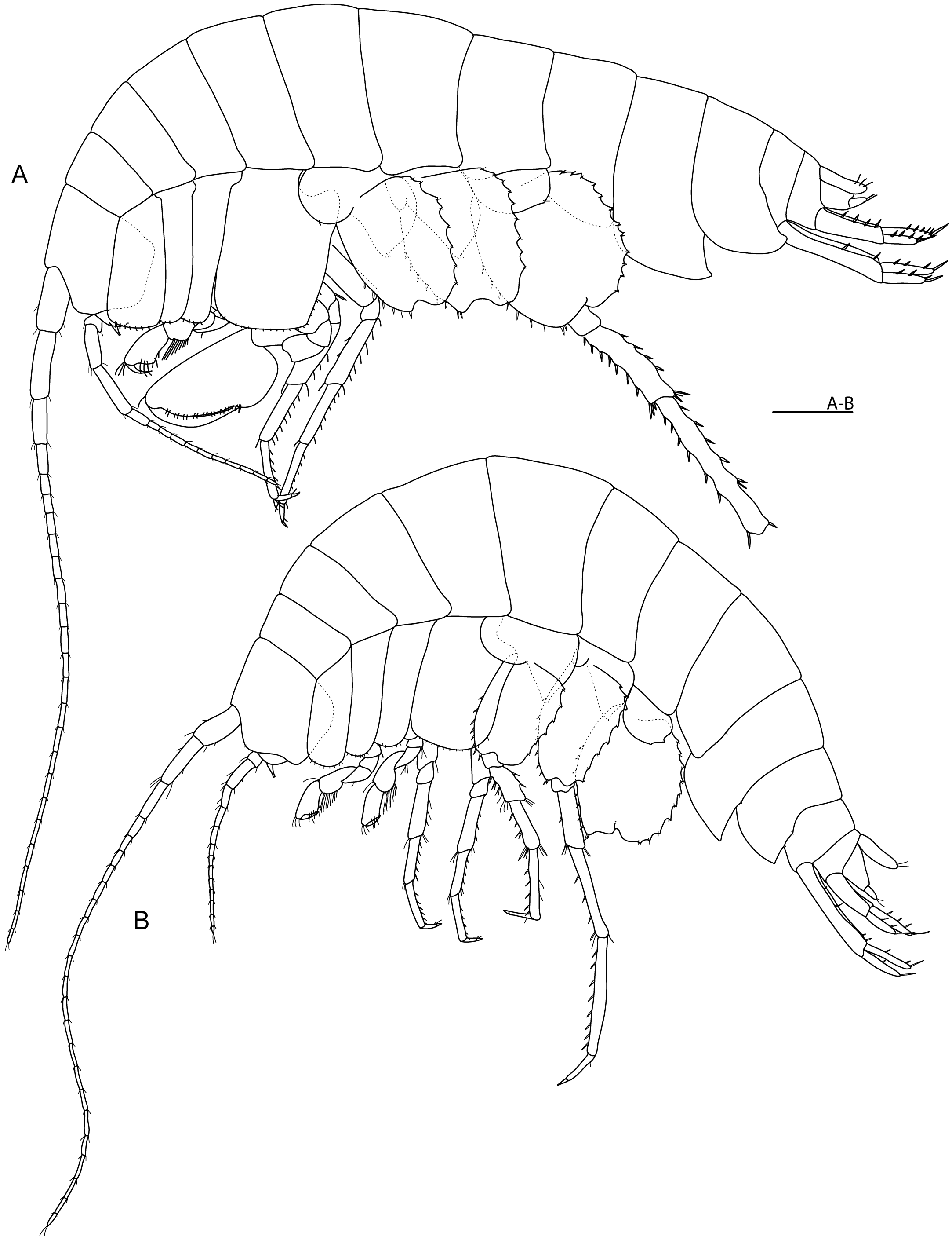

Fig. 3. *Scutachiltonia axfordi* King, n. sp. A, Holotype male lateral view, WAM C49169; B, Allotype female, lateral view, WAM C49170. Scale bar: 0.5 mm.

ventral margin with simple seta, unguis present. Pereiopod 5 (Fig. 5F) coxa posterior lobe with one short seta along distal margin; basis 1.4 times as long as broad, dorsal margin with seven robust setae along length, distodorsal margin with two robust setae, ventral margin distinctly crenulated and with nine short simple setae along length. Pereiopod 6 (Fig. 5G) coxa posterior lobe with one short seta along distal margin; basis 1.4 times as long as broad, dorsal margin with 10 robust setae along length, distal end of dorsal margin with one robust seta, ventral margin distinctly crenulated and with 11 short simple setae along length. Pereiopod 7 (Fig. 5E) coxa ventral margin with one short simple seta; basis 1.2 times as long as broad, dorsal margin with 10 robust setae along length, distal end of dorsal margin with two robust setae, ventral margin distinctly crenulated and with 10 short simple setae along length; ischium dorsal margin with distal

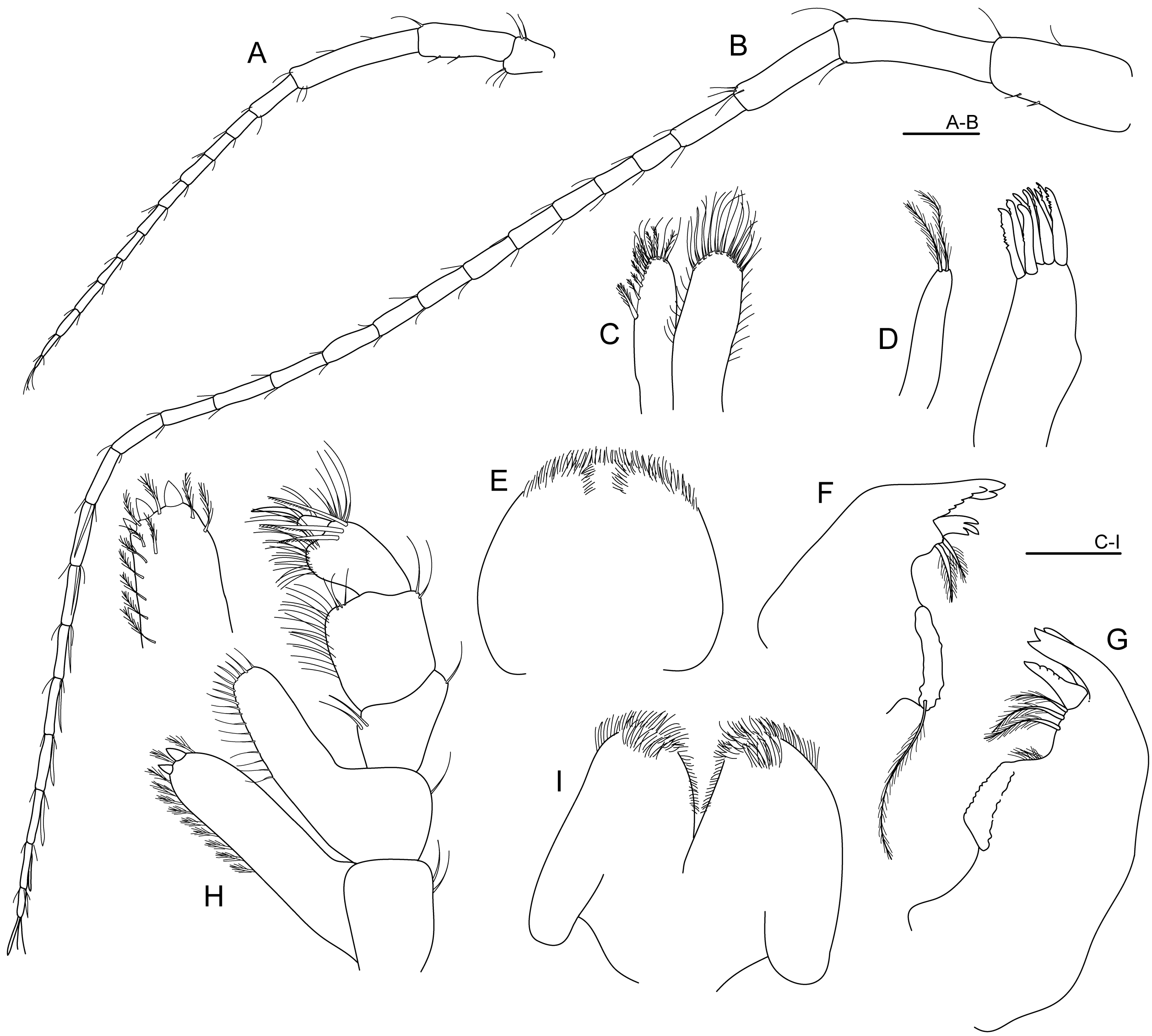

Fig. 4. *Scutachiltonia axfordi* King, n. sp. holotype male, 5.3 mm, WAM C49169. A, Antenna 2; B, Antenna 1; C, Maxilla 2; D, Maxilla 1; E, Upper lip; F, Mandible RHS; G, Mandible LHS; H, Maxilliped; I, Lower lip. Scale bars: (A-B) 0.2 mm, (C-I) 0.1 mm.

cluster of short robust setae; merus with slight postero-distal lobe, dorsal margin with robust setae in five clusters, ventral margin with robust setae in four clusters; carpus longer than merus, nine times as long as broad, dorsal margin with robust setae in six clusters, ventral margin with robust setae in six distal clusters.

Pleopod 1 (Fig. 5H) similar to pleopods 2-3, unmodified (compared to *Chiltonia* Stebbing, 1899), peduncle inner margins with three distal retinacula (coupling hooks), inner ramus of 10 articles, outer ramus of 13 articles. Uropod 1 (Fig. 5L) peduncle two times longer than rami, dorsal margin with two robust setae along the length of the outer margin and two along the inner margin; outer ramus with three apical robust setae and a row of two robust setae along length; inner ramus with five apical robust setae (distal-most seta as long as half-length of ramus) and two rows of robust setae along length, outer margin with one robust seta, inner margin with two robust setae. Uropod 2 (Fig. 5K) peduncle similar length to inner ramus, dorsal margin with two robust setae along the length of the outer margin and four along the inner margin; outer ramus smaller than inner ramus, with four apical robust setae (distal-most seta as long as half-length of ramus), with a row of two robust setae along length; inner ramus with four apical robust setae (distal-most seta as long as half-length of ramus), with two rows of robust setae along length, outer margin with five robust setae, inner margin with four robust setae. Uropod 3 (Fig. 5J) bi-articulate, article 2 less than 0.25 length of article 1, distal margin with one short robust seta and one long robust seta apically. Telson (Fig. 5I) as long as broad, apically blunt, with two apically divided robust setae and four simple setae distally.

Allotype Female (WAM C49170).—Length: 5.6 mm (Fig. 3B).

Similar morphology to male except for the following: Gnathopod 1 (Fig. 6A) propodus around two times as long

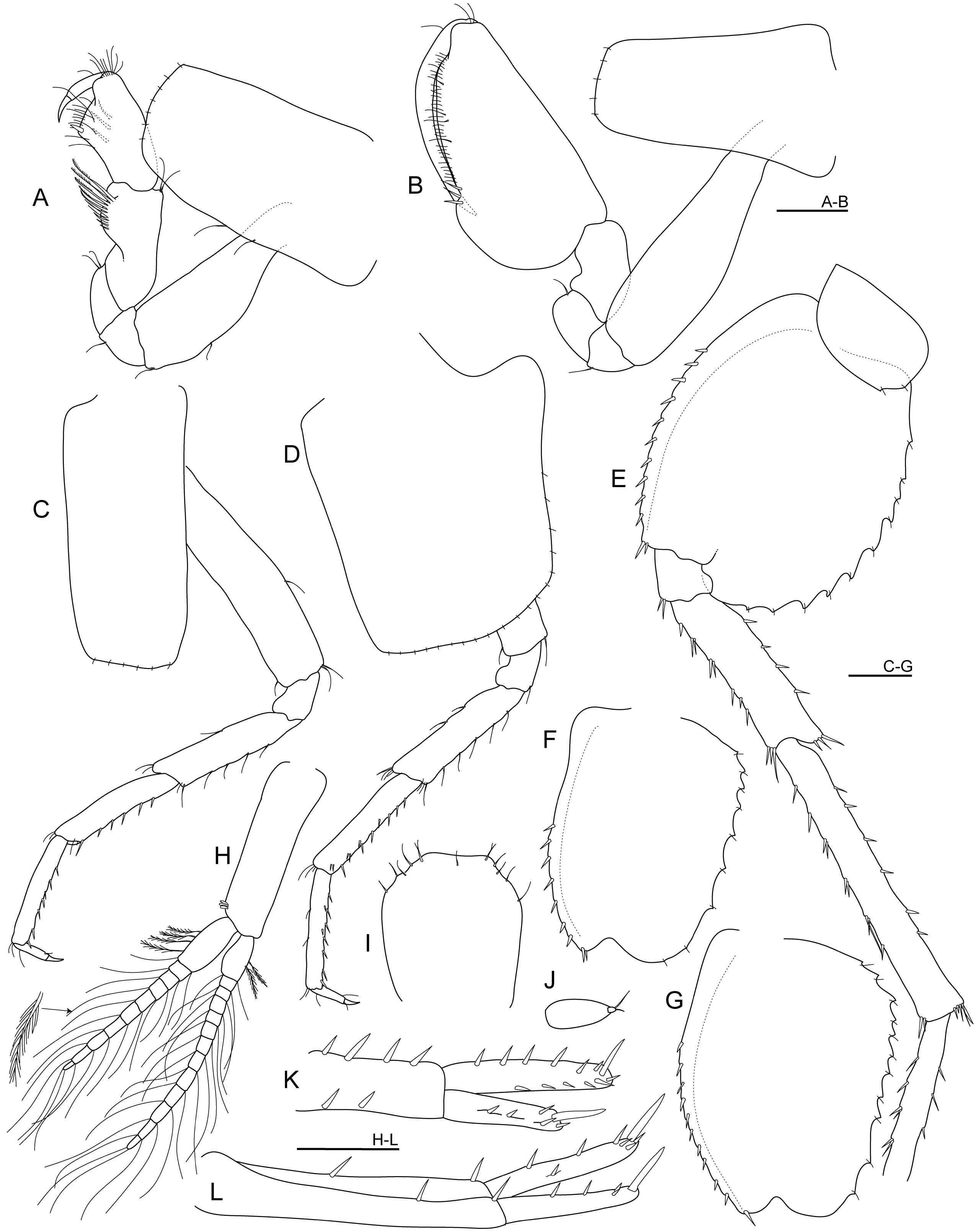

Fig. 5. *Scutachiltonia axfordi* King, n. sp. holotype male, 5.3 mm, WAM C49169. A, Gnathopod 1; B, Gnathopod 2; C, Pereiopod 3; D, Pereiopod 4; E, Pereiopod 7; F, Pereiopod 5 (basis); G, Pereiopod 6 (basis); H, Pleopod 1; I, Telson; J, Uropod 3; K, Uropod 2; L, Uropod 1. Scale bars: (A-B, H-L) 0.1 mm, (C-G) 0.2 mm.

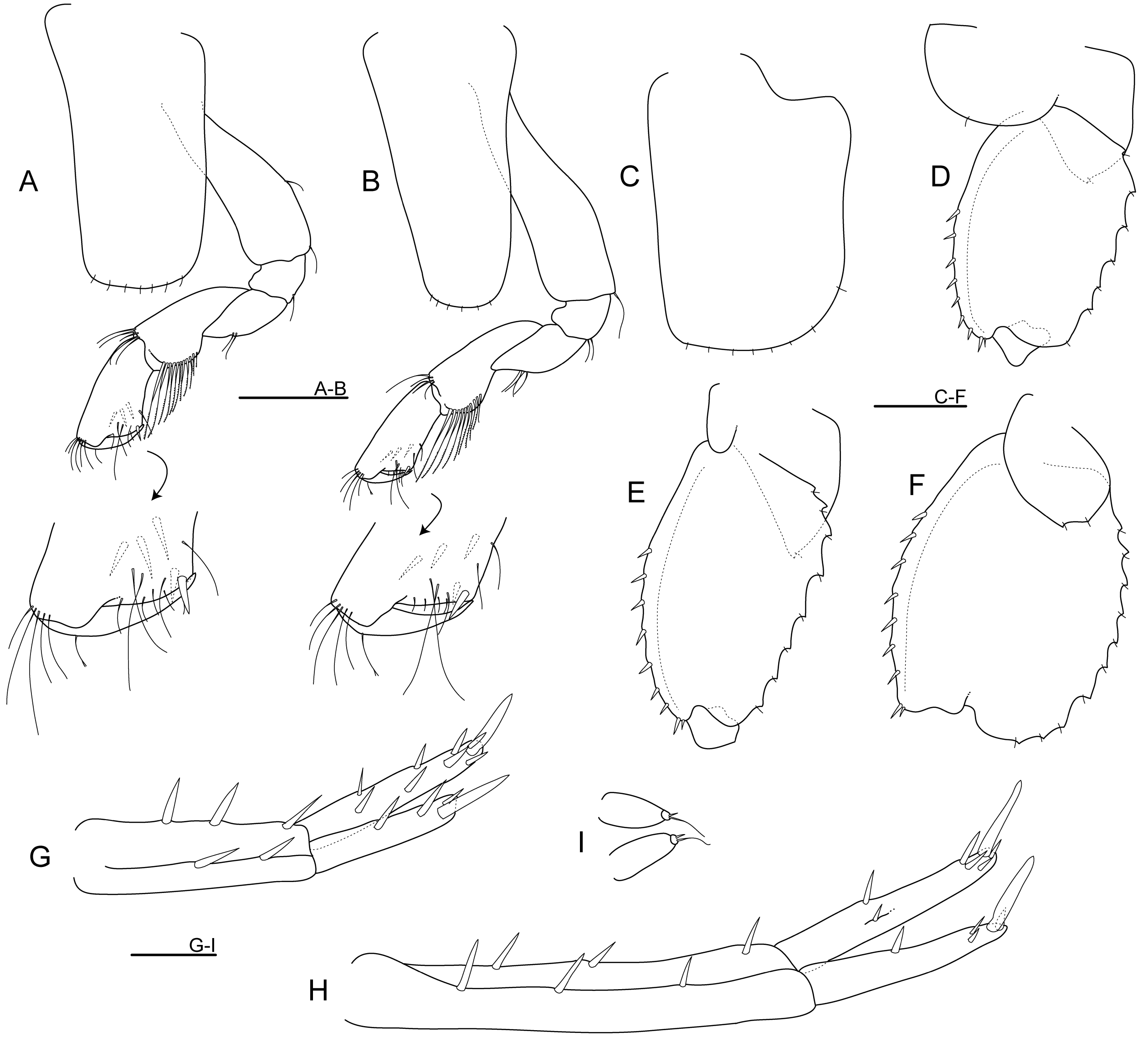

Fig. 6. *Scutachiltonia axfordi* King, n. sp. allotype female, 5.6 mm, WAM C49170. A, Gnathopod 1; B, Gnathopod 2; C, Pereiopod 4 (basis); D, Pereiopod 5 (basis); E, Pereiopod 6 (basis); F, Pereiopod 7 (basis); G, Uropod 2; H, Uropod 1; I, Uropod 3. Scale bars: (A-B, G-I) 0.1 mm, (C-F) 0.2 mm.

as broad, inner face with three robust setae. Gnathopod 2 (Fig. 6B) similar to gnathopod 1 except propodus 2.4 times as long as broad. Pereiopod 4 coxa (Fig. 6C) not as broad as in male. Pereiopods 5-7 bases (Fig. 6D-F) not as broad as in male.

Uropod 1 (Fig. 6H) peduncle dorsal margin with three robust setae along the length of the outer margin and three robust setae along the inner margin; outer ramus with four apical robust setae (distal-most seta as long as half-length of ramus) and one robust setae along length; inner ramus with five apical robust setae (distal-most seta as long as half-length of ramus) and two rows of robust setae along length, outer margin with one robust seta, inner margin with one robust seta. Uropod 2 (Fig. 6G) peduncle dorsal margin with two robust setae on outer margin and three robust setae on inner margin; outer ramus smaller than inner ramus, with three apical robust setae (distal-most seta as long as half-length of ramus), with one robust seta along length; inner ramus with five apical robust setae (distal-most seta as long as half-length of ramus), with two rows of robust setae along length, outer margin with two robust setae, inner margin with two robust setae.

Oöstegites present on coxae 2 to 5 to form the marsupium, margins with scattered curved hooks.

Etymology.—Named for the Axford family of the Sturt Meadows pastoral property.

Variation.—Mature males and females were a similar size in the samples studied. The number of robust setae on the anterior margin of the bases of pereiopods 5-7 differs by one or two setae dependent on the size of the animal (smaller animals had less setae). The number of robust setae on the peduncles and rami of uropods 1 and 2 also differs with size (larger individuals as illustrated, smaller individuals 2-3 less setae).

Remarks.—This species is distinctive primarily because of its elongate coxae and broad spinose bases of pereiopods 5-7. Individuals were fragile and many animals showed damage, specifically loss of pereiopods, presumably from collection and sample processing.

Stygochiltonia King, n. gen.

Type Species.—*Stygochiltonia bradfordae* n. sp.

Diagnosis.—Eyes absent. Antenna 1 at least twice the size of antenna 2. Coxae 1-3 short (about as long as broad). Coxa 4 broader than long, with poorly defined proximal corner. Coxae 5-6 posterior lobe reduced, not extending much further than anterior lobe. Gnathopod 2 in males short (1.5 or less times as long as wide). Pereiopod 5-6 bases with postero-distal lobe; pereiopod 7 without postero-distal lobe. Pereiopods 5-7 bases with subtle crenulation along posterior margin, concentrated proximally; anterior margin with up to five robust setae. Pereiopods 6 and 7 at least twice as long as pereiopod 5, with numerous elongate robust setae along anterior and posterior margins of all articles; carpus as long as merus; propodus elongate (1.7 to two times length of carpus). Epimera 1-3 with blunt squared postero-distal corners.

Etymology.—"Stygo" for the stygobitic environment of the species as well as the typical stygobitic morphology of elongate pereiopods and reduced coxae, and "chiltonia" for its placement within Chiltoniidae.

Remarks.—*Stygochiltonia* incorporates "clade 3" of Bradford et al. (2010) and represents the first chiltoniid amphipod with all the archetypal subterranean morphological features: loss of eyes; elongate antennae; narrow and elongate pereionites; pereiopods 6 and 7 elongate compared to pereiopod 5. It is unique in its possession of reduced coxae 1-6, short gnathopod 2 propodus in males, pereiopod 7 basis without postero-distal lobe and epimera 1-3 postero-distal corners blunt and squared. It is likely that individuals examined from other calcrete sites within Western Australia (Depot Springs, Pinnacles), some noted by Bradford et al. (2010) as potential sister species to *S. bradfordae* sp nov., belong within *Stygochiltonia*.

Stygochiltonia bradfordae King, n. sp.
Figs. 7-10

Morphological Diagnosis.—Antenna 1 greater than 3/4 body length, flagellum four times longer than peduncle; antenna 2 1/3 body length, flagellum two times longer than peduncle. Gnathopod 2 propodus (in males) 1.5 times as long as broad. Coxae 1-3 as long as broad; coxa 1 narrowing distally, coxae 2-3 squared; coxa 4 broad (1.2 times as broad as long) and with undefined proximal corner. Pereiopods 5-6 bases longer than broad, with postero-distal lobe, posterior margin with indistinct crenulations, with five to seven short setae along margin, posterior margin with three robust setae; pereiopod 7 basis longer than broad, without postero-distal lobe. Uropod 1 outer ramus with one to two robust setae along length, with distal cluster of three small setae and a single long robust seta (more than half-length of ramus); inner ramus with one or two setae along length, with distal cluster of four small setae and a single long robust seta (more than half-length of ramus). Uropod 2 outer ramus with one or two robust setae along length, with distal cluster of up to three small setae and a long robust setae (more than half-length of ramus); inner ramus with three to four setae in two rows along length, with distal cluster of four to five setae and one long robust seta (more than half-length of ramus). Uropod 3 bi-articulate with second article around 1/3 length of first article.

Material Examined.—Holotype, WAM C49172, male, 3.4 mm, Sturt Meadows calcrete, Western Australia, BES 10241, 28°42′0.9″S, 120°50′27.42″E, coll. W. F. Humphreys, S. Cooper, 16 Mar 2004. Allotype, WAM C49173, female, 4.5 mm, collected with holotype. Paratypes (two males, 1 female), WAM C49174, collected with holotype (see Table 1 for additional material).

Distribution.—Western Australia: Sturt Meadows calcrete aquifer, situated on the Sturt Meadows Pastoral Property, near Leonora.

Description.—Holotype male (based on WAM C49172), length: 3.4 mm. Head about as long as deep (Fig. 7A).

Antenna 1 (Fig. 8B) 0.8 times body length, peduncular article 1 three times as long as broad, inner lateral margin with two robust setae, distoventral margin with single robust seta; peduncular article 2 shorter than article 1 (0.7 times as long), 2.5 times as long as broad; peduncular article 3 shorter than article 2, three times as long as broad; flagellum longer than peduncle (3.4 times), of 20 articles, with ventral aesthetascs on the proximal margins of the eight distal articles. Antenna 2 (Fig. 8A) about 0.4 times length of antenna 1; peduncular article 3 broader than long, inner-distal margin with two robust setae; peduncular article 4 longer than article 3, three times longer than broad, inner lateral margin with two robust setae, distal margin with one robust seta; peduncular article 5 longer than article 4, four times as long as broad; flagellum longer than peduncle (1.6 times), of 9 articles.

Mouthparts as for family (Fig. 8C-I) (see King, 2009b).

Gnathopod 1 (Fig. 9A) coxa as long as broad, distal margin with three short simple setae; basis distoventral corner with cluster of simple setae; ischium, and merus distoventral corners with clusters of setae; carpus with ventral-lateral lobe and row of nine setulate setae becoming longer distally, distodorsal margin with long setae; propodus two times as long as broad, subchelate, palm acute, distoventral corner with one robust seta at corner of palm, medial palm margin with short robust and long simple setae, distodorsal margin with long simple setae, inner face with two robust plumose setae; dactylus curved, fitting against palm, with proximal plumose seta. Gnathopod 2 (Fig. 9B) coxa as long as broad, distal margin with six short simple setae; basis distoventral corner with scattered simple setae; ischium and merus with scattered setae on distoventral corners; propodus 1.5 times as long as broad, subchelate, distoventral corner marked by robust seta adjacent to dactylar socket, palm margin with numerous robust setae with subterminal spines. Pereiopod 3 (Fig. 9C) coxa distal margin with seven short simple setae; basis dorsal and ventral margins with scattered simple setae, distoventral corner with clusters of setae; ischium distoventral corner with clusters of setae; merus with slight

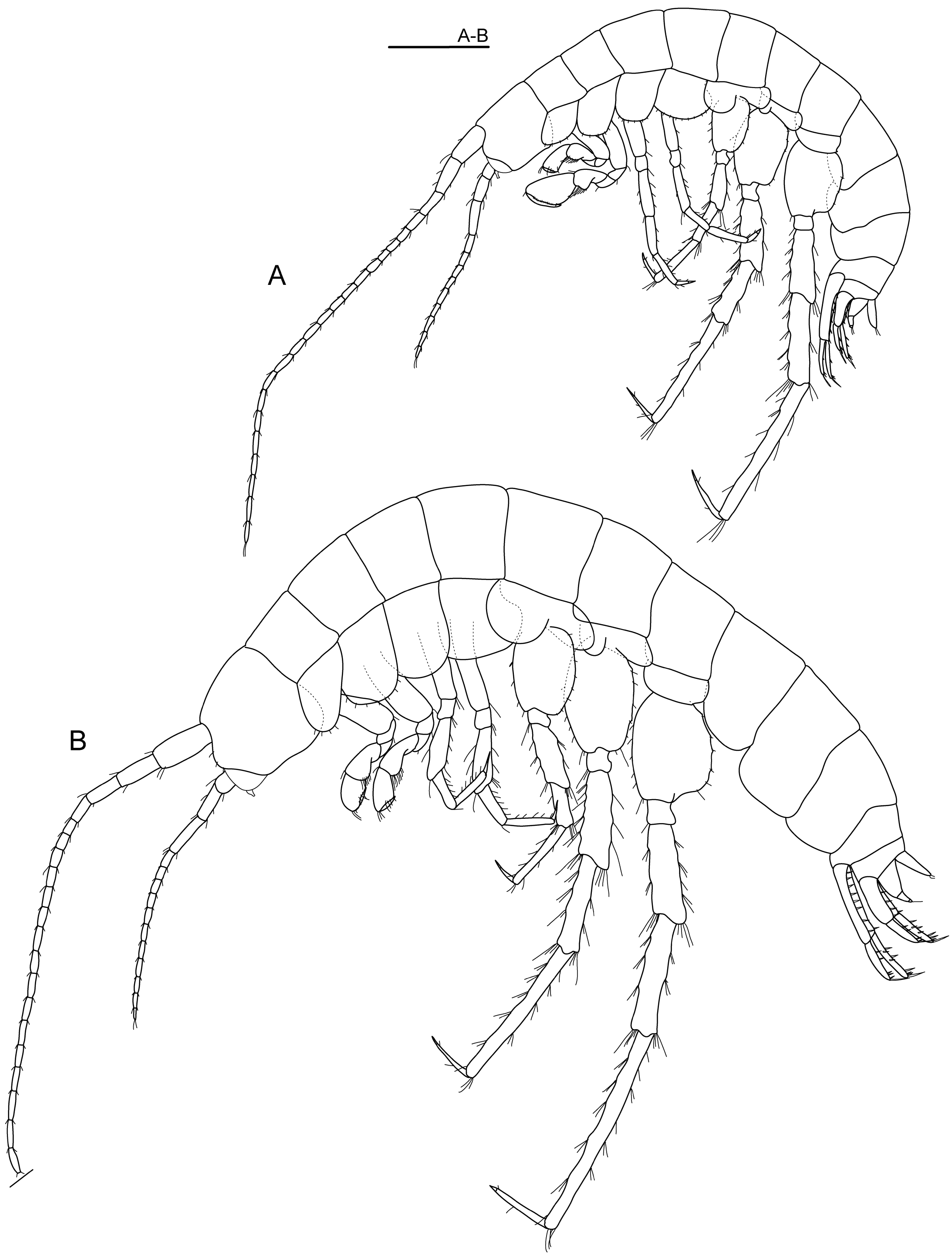

Fig. 7. *Stygochiltonia bradfordae* King, n. sp. A, Holotype male lateral view, WAM C49172; B, Allotype female, lateral view, WAM C49173. Scale bar: 0.5 mm.

distodorsal lobe, ventral margin with scattered simple setae, distoventral corner with cluster of setae; carpus ventral margin with robust setae and scattered simple setae; propodus dorsal margin with one cluster of simple setae; ventral margin with four clusters of robust and simple setae; dactylus dorsal margin with plumose seta, ventral margin with simple seta, unguis present. Pereiopod 4 (Fig. 9D) coxa as long as broad, with indistinct proximal excavation, distal margin with six short simple setae; basis dorsal and ventral margins with scattered simple setae, distoventral corner with cluster of simple setae; ischium distoventral corner with cluster of setae; merus with slight distodorsal lobe, ventral margin with scattered simple setae, distoventral corner with cluster of setae; carpus ventral margin with scattered robust and simple

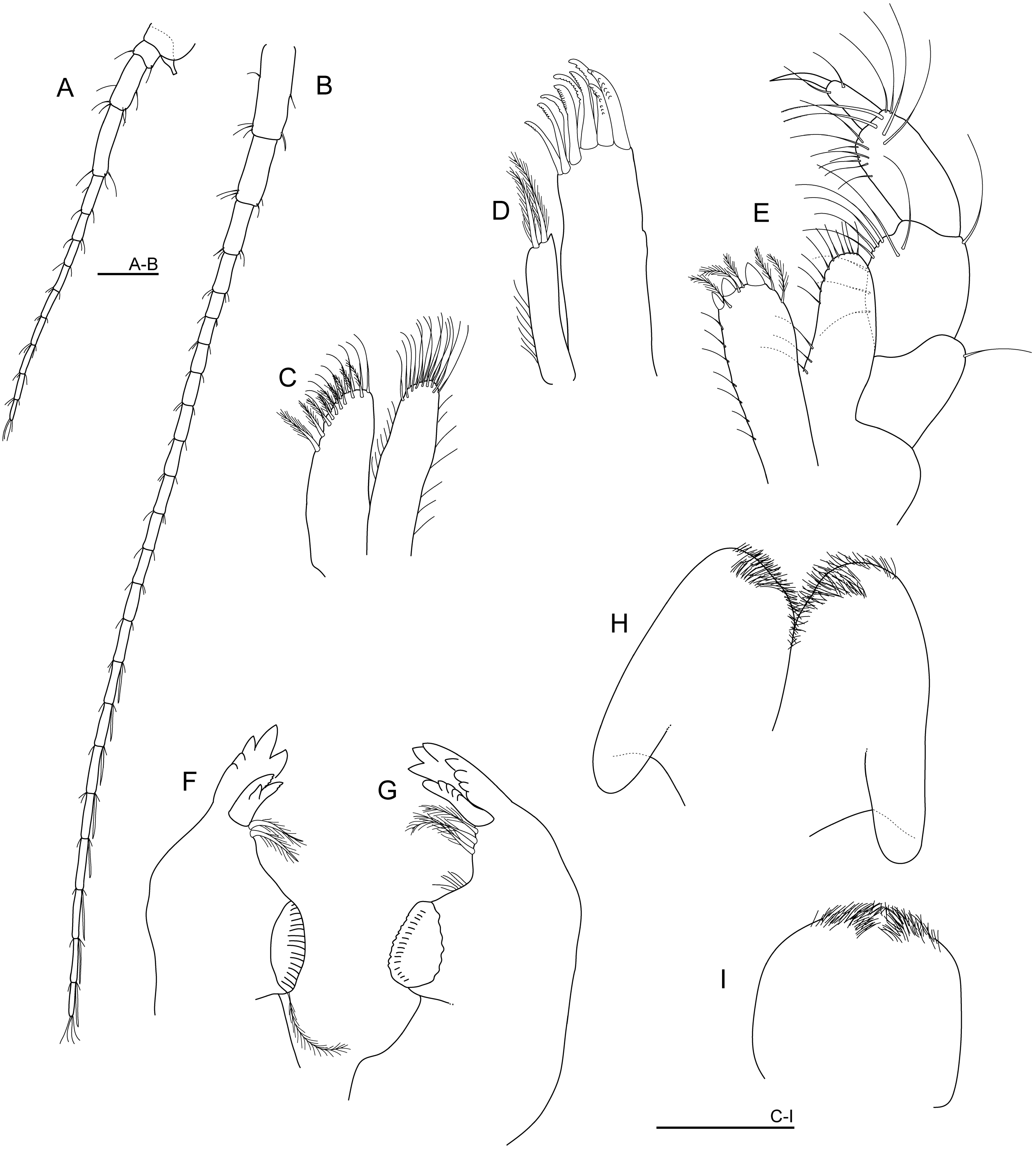

Fig. 8. *Stygochiltonia bradfordae* King, n. sp. holotype male, 3.4 mm, WAM C49172. A, Antenna 2; B, Antenna 1; C, Maxilla 2; D, Maxilla 1; E, Maxilliped; F, Mandible RHS; G, Mandible LHS; H, Lower lip; I, Upper lip. Scale bars: (A-B) 0.2 mm, (C-I) 0.1 mm.

setae; propodus ventral margin with four clusters of robust and simple setae; dactylus dorsal margin with plumose seta, ventral margin with simple seta, unguis present. Pereiopod 5 (Fig. 9E) coxa posterior lobe without setae along margin; basis 1.5 times as long as broad, dorsal margin with three robust setae along length, distodorsal margin with one robust seta, ventral margin indistinctly crenulated and with seven short simple setae along length; ischium distodorsal margin with distal robust setae; merus with strong postero-distal lobe, dorsal margin with robust setae in four clusters, ventral margin with robust setae in three clusters; carpus as long as merus, dorsal margin with robust setae in four clusters, ventral margin with robust setae in one cluster; propodus longer than merus, dorsal margin with five clusters of robust setae, ventral margin with two clusters of simple setae; dactylus with plumose seta on ventral margin, unguis present. Pereiopod 6 (Fig. 9F) coxa posterior lobe with one seta along margin; basis 1.6 times as long as broad, dorsal

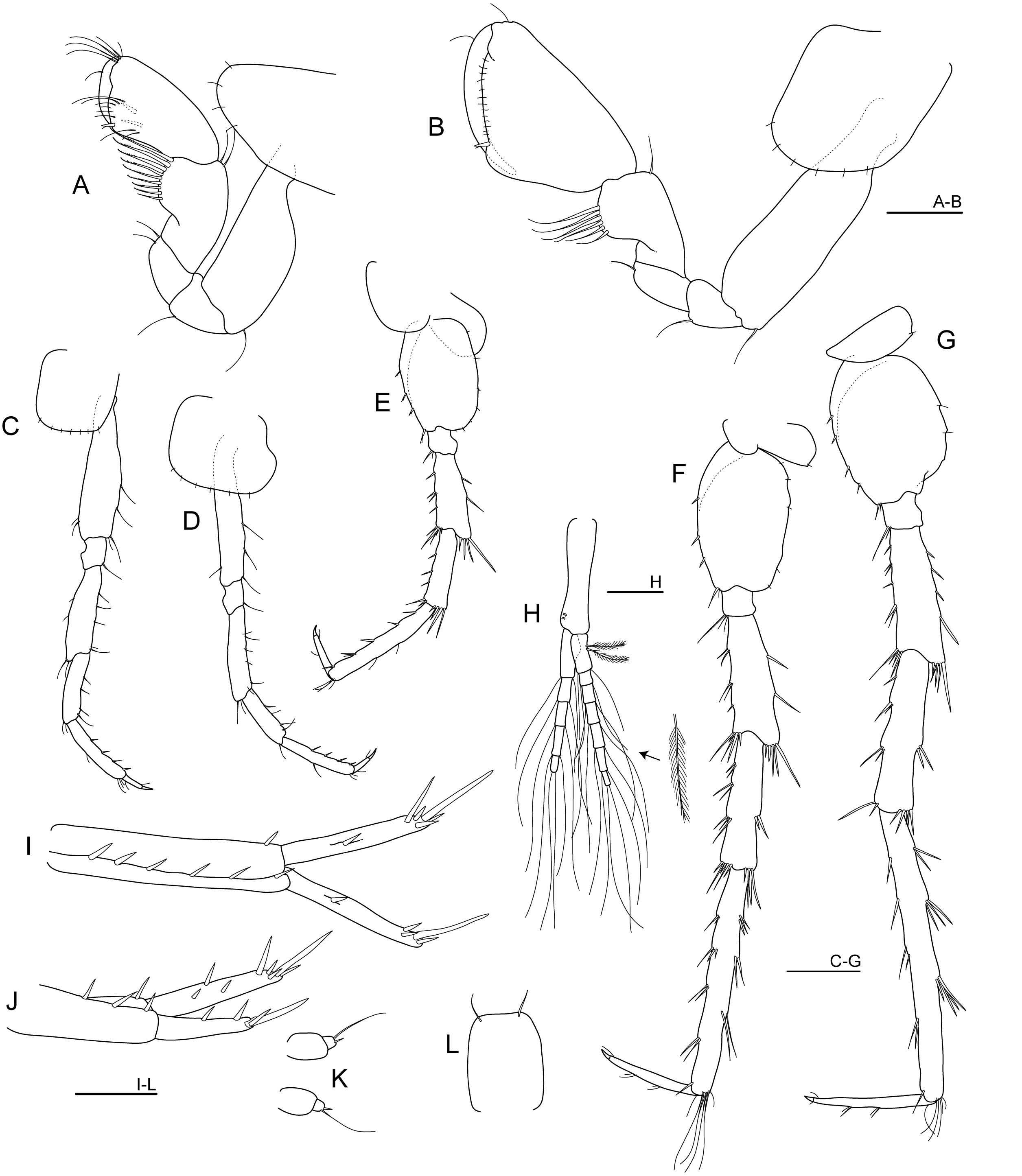

Fig. 9. *Stygochiltonia bradfordae* King, n. sp. holotype male, 3.4 mm, WAM C49172. A, Gnathopod 1; B, Gnathopod 2; C, Pereiopod 3; D, Pereiopod 4; E, Pereiopod 5; F, Pereiopod 6; G, Pereiopod 7; H, Pleopod 1; I, Uropod 1; J, Uropod 2; K, Uropod 3; L, Telson. Scale bars: (A-B, H, I-L) 0.1 mm, (C-G) 0.2 mm.

margin with three robust setae along length, distal end of dorsal margin with cluster of two robust setae, ventral margin indistinctly crenulated and with five short simple setae along length; ischium dorsal margin with distal robust setae; merus with strong postero-distal lobe, dorsal margin with robust setae in four clusters, ventral margin with robust setae in three clusters; carpus as long as merus, dorsal margin with robust setae in four clusters, ventral margin with robust setae in two clusters; propodus longer than merus, dorsal margin with five clusters of robust setae, ventral margin with three clusters of simple setae; dactylus with plumose seta on ventral margin, unguis present. Pereiopod 7 (Fig. 9G) coxa ventral margin with one short simple seta; basis 1.5 times as long as broad, dorsal margin with four robust setae along length, distal end of dorsal margin with two robust setae, ventral margin indistinctly crenulated and with two short simple setae along length; ischium dorsal margin with distal cluster of robust setae; merus with strong postero-distal lobe, dorsal margin with robust setae in five clusters, ventral margin with robust setae in five clusters; carpus as long as merus, dorsal margin with robust setae in four clusters, ventral margin with robust setae in two distal clusters; propodus longer than merus merus, dorsal margin with five clusters of robust setae, ventral margin with three clusters of simple setae; dactylus with plumose seta on ventral margin, unguis present.

Pleopod 1 (Fig. 9H) similar to pleopods 2-3, unmodified (compared to *Chiltonia*), peduncle inner margins with two distal retinacula (coupling hooks), inner ramus of five articles, outer ramus of seven articles. Uropod 1 (Fig. 9I) peduncle 1.5 times longer than rami, dorsal margin with six robust setae along the length of the outer margin and one seta along the inner margin; outer ramus with three apical small robust setae and one long robust seta (longer than half-length of ramus) and a robust seta along length; inner ramus with distal cluster of four robust setae (three small and one twice size of others) and one long robust seta (longer than half-length of ramus) and a seta along length. Uropod 2 (Fig. 9J) peduncle similar length to inner ramus, dorsal margin with three long robust setae along the outer margin and one robust seta along the inner margin; outer ramus slightly smaller than inner ramus, with apical cluster of two small robust setae and one long robust seta (longer than half-length of ramus), with one robust seta along length; inner ramus with distal cluster of four robust setae (three small and one twice size of others) and one long robust seta (longer than half-length of ramus), with two rows of robust setae along length, outer margin with one robust seta, inner margin with two robust setae. Uropod 3 (Fig. 9K) biarticulate, article 2 0.3 times length of article 1, distal margin with one short robust seta and one long robust seta apically. Telson (Fig. 9L) longer than broad, apically blunt, with two setae at each dorsal distal corner.

Allotype Female (WAM C49173).—Length: 4.5 mm (Fig. 7B).

Similar morphology to male except for the following: Gnathopod 1 (Fig. 10A) carpus with ventral-lateral lobe and row of 12 setulate setae becoming longer distally. Gnathopod 2 (Fig. 10B) similar to gnathopod 1 except propodus 2.6 times as long as broad. Pereiopods 5-7 bases (Figs. 10C-E) broader than in males.

Uropod 1 (Fig. 10F) peduncle dorsal margin with eight robust setae along length of the outer margin and one seta along the inner margin; outer ramus with two robust setae along length; inner ramus with two rows of robust setae along length, outer margin with two robust setae, inner margin with two robust setae. Uropod 2 (Fig. 10G) peduncle dorsal margin with four long robust setae along length of the outer margin and one robust seta along the inner margin; outer ramus with two robust setae along length; inner ramus with distal cluster of four robust setae (two small and two twice size of others) and one long robust seta (longer than half-length of ramus), with two rows of robust setae along length, outer margin with two robust setae, inner margin with two robust setae. Telson (Fig. 10I) as long as broad with three pairs of setae at each dorsal distal corner.

Etymology.—Named for Tessa Bradford who first detected the three Sturt Meadows lineages in her published study (Bradford et al., 2010).

Variation.—Mature females were distinctly larger than mature males. The holotype male is likely to be an early stage male, one of only two found in collections of this rare species. Whilst the penes were fully developed in both individuals, setation of the carpus of gnathopod 2 indicates only partial development from a juvenile morphotype. Although rare, this morphotype (mature male with setulate setae on the carpus of gnathopod 2) has been observed in populations of other chiltoniid species (personal observation, RAK). Examination of male specimens from the Depot Springs calcrete, a potentially con-generic species, showed fully developed males without this carpus setation. It is proposed that if later stage males of *S. bradfordae* are found in future collections, the propodus will be short (1.5 times length) and carpus free of the setulate setae, as seen in the Depot Springs males and adult males of other chiltoniid species.

Yilgarniella King, n. gen.

Type Species.—*Yilgarniella sturtensis* sp. nov.

Diagnosis.—Eyes absent. Antenna 1 at least twice the size of antenna 2. Coxae 1-3 about 1.5 times as long as broad. Coxa 4 about as broad as long, with defined proximal corner. Coxae 5-6 posterior lobe extending at least twice length of anterior lobe. Gnathopod 2 propodus in males elongate (two times as long as wide). Pereiopod 5-7 bases with postero-distal lobe. Pereiopods 5-6 bases with subtle crenulation along posterior margin, concentrated proximally; anterior margin with up to six robust setae. Pereiopod 7 basis posterior margin entirely crenulate, anterior margin with up to six robust setae. Epimera 1-3 with blunt postero-distal corners defined with a blunt spine.

Etymology.—Named for the Yilgarn region of Western Australia where the specimens were sampled.

Remarks.—*Yilgarniella* includes "clade 1" of Bradford et al. (2010) and is a mostly unspecialised chiltoniid species in terms of stygobitic morphology, looking much more like epigean chiltoniids; pereiopods 6 and 7 are not particularly long, nor are their articles elongate compared to pereiopod 5, and the coxae are not particularly reduced or enlarged. It is easily recognised by these and a combination of other characters: lack of eyes, elongate antennae, elongate gnathopod 2 propodus in males, bases of pereiopods 5-7 dorsal margin with few robust setae and ventral margin with subtle to distinct crenulation, and pereiopod 7 basis with postero-distal lobe.

Individuals from the Nambi calcrete in Western Australia have been examined and determined to nominally be an additional species of *Yilgarniella*. Further descriptive work is ongoing.

Yilgarniella sturtensis King, n. sp.
Figs. 11-14

Morphological Diagnosis.—Antenna 1 greater than 3/4 body length, flagellum more than three times longer than

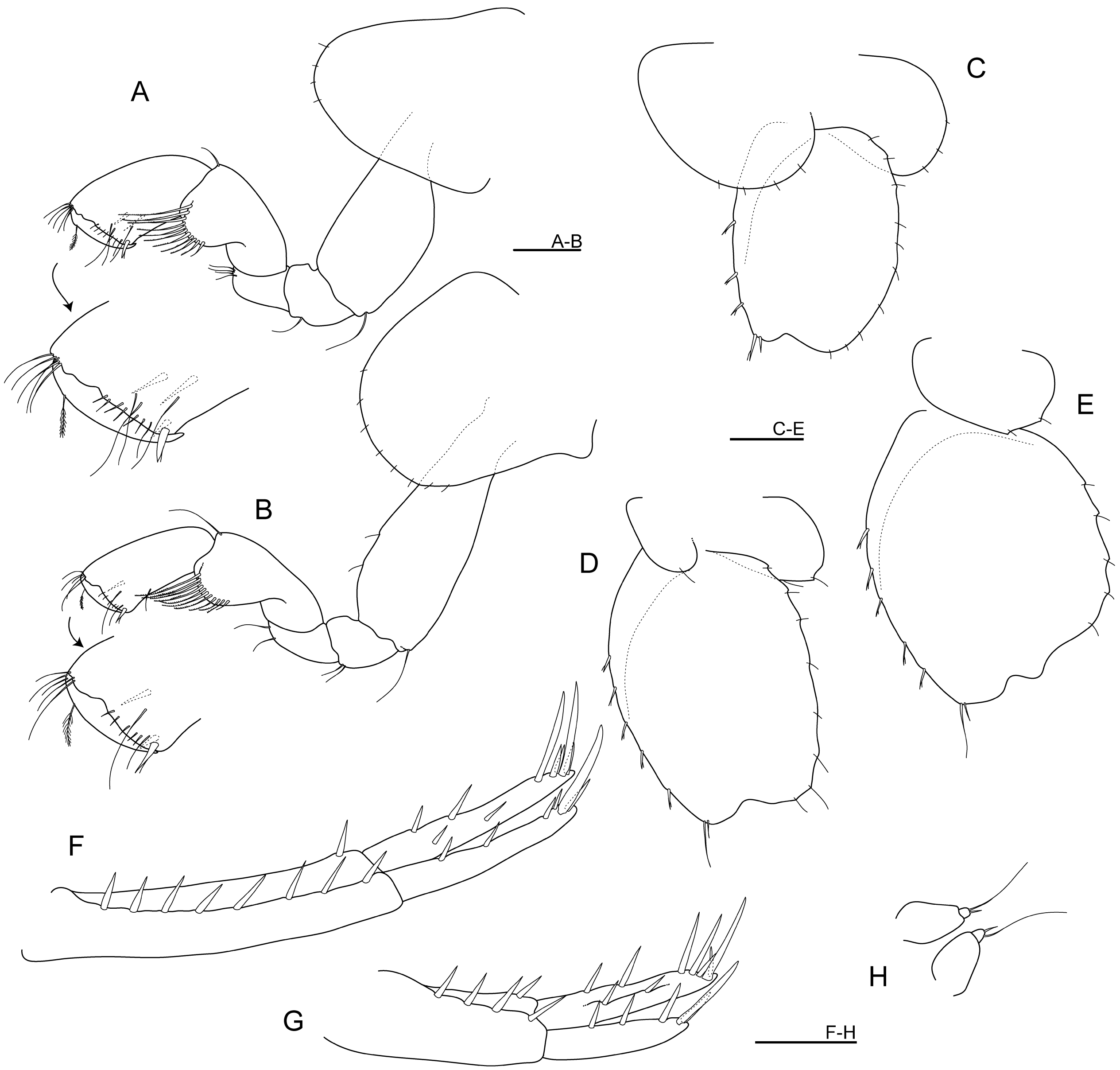

Fig. 10. *Stygochiltonia bradfordae* King, n. sp. allotype female, 4.5 mm, WAM C49173. A, Gnathopod 1; B, Gnathopod 2; C, Pereiopod 5 (basis); D, Pereiopod 6 (basis); E, Pereiopod 7 (basis); F, Uropod 1; G, Uropod 2; H, Uropod 3. Scale bars: (A-B, F-H) 0.1 mm, (C-E) 0.2 mm.

peduncle; antenna 2 1/3 body length, flagellum at least 1.3 times longer than peduncle. Gnathopod 2 propodus (in males) two times as long as broad. Coxae 1-3 1.5 times as long as broad; coxa 4 slightly broader than long, with defined posterior corner. Pereiopods 5-6 bases longer than broad, with postero-distal lobe, ventral margin subtly crenulate (concentrated proximally) with associated short setae, posterior margin with 2-5 robust setae concentrated distally. Pereiopod 7 basis longer than broad, with postero-distal lobe entire margin subtly crenulate with associated setae, posterior margin with five robust setae concentrated distally. Uropod 1 outer ramus with 4-7 robust setae along length, with distal cluster of 3 small setae and single long robust seta (about half-length of ramus); inner ramus with 5 robust setae in two rows along length, with distal cluster of 3 small setae and single long robust seta (about half-length of ramus). Uropod 2 outer ramus with 4-5 robust setae along length, with distal cluster of 3-4 small setae and single long robust seta (about half-length of ramus); inner ramus with 5-7 robust setae in two rows along length, with distal cluster of 3-5 small setae and single long robust seta (half-length of ramus). Uropod 3 bi-articulate, with second article around 1/3 length of first article.

Material Examined.—Holotype, WAM C49175, male, 3.7 mm, Sturt Meadows calcrete, Western Australia, BES 10262, 28°41′47.724″S, 120°53′36.5634″E, coll. W. Humphreys, S. Cooper, 18 Mar 2004. Allotype, WAM C49176, female, 4.7 mm, collected with holotype. Paratypes (two males, six females, five juveniles), WAM C49177, collected with holotype (see Table 1 for additional material).

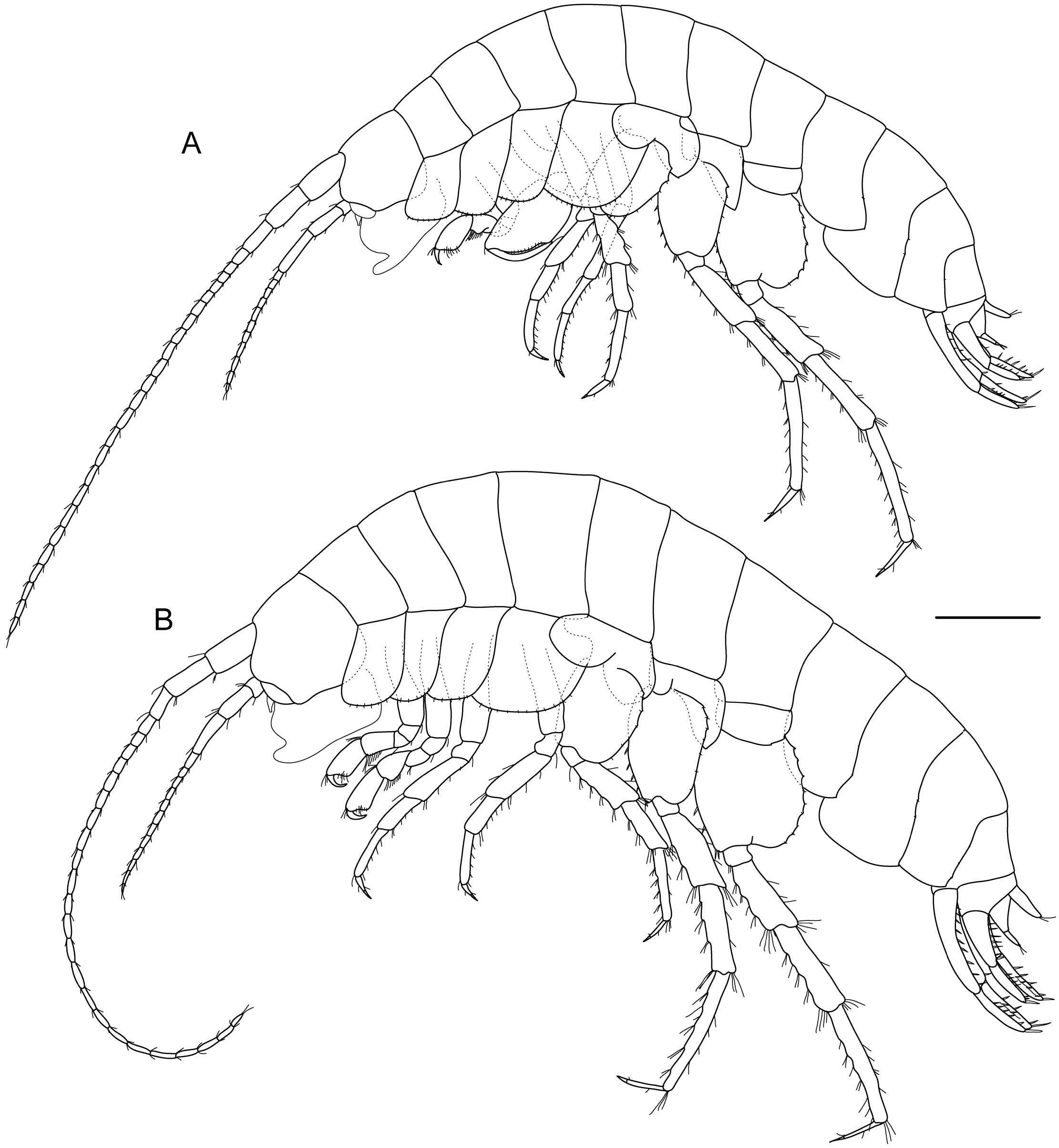

Fig. 11. *Yilgarniella sturtensis* King, n. sp. A, Holotype male lateral view, WAM C49175; B, Allotype female, lateral view, WAM C49176. Scale bar: 0.5 mm.

Distribution.—Western Australia: Yilgarn region (currently only known from Sturt Meadows calcrete aquifer, situated on the Sturt Meadows pastoral property, near Leonora).

Description.—Holotype male (WAM C49175), length: 3.7 mm. Head about as long as deep (Fig. 11A).

Antenna 1 (Fig. 12A) 0.8 times body length, peduncular article 1 2.4 times as long as broad, inner lateral margin with two robust setae, distoventral margin with single robust seta; peduncular article 2 shorter than article 1 (0.7 times as long), 2.8 times as long as broad; peduncular article 3 shorter than article 2, three times as long as broad; flagellum three times longer than peduncle, of 24 articles, with ventral aesthetascs on the proximal margins of the nine distal articles. Antenna 2 (Fig. 12B) about 0.4 times length of antenna 1; peduncular article 3 as broad as long, inner-distal margin with robust setae; peduncular article 4 longer than article 3, 2.2 times longer than broad, inner lateral margin with two robust setae, distal margin with one robust seta; peduncular article 5 longer than article 4, 4.5 times as long as broad; flagellum longer than peduncle (1.3 times), of 11 articles.

Mouthparts as for family (Fig. 12C-I) (see King, 2009b).

Gnathopod 1 (Fig. 13A) coxa 1.5 times as long as broad, distal margin with nine short simple setae; basis distoventral corner with cluster of simple setae; ischium, and merus distoventral corners with clusters of setae; carpus with ventral-lateral lobe and row of nine setulate setae becoming longer distally, distodorsal margin with long setae; propodus two times as long as broad, subchelate, palm acute, distoventral corner with one robust seta at corner of palm, medial palm margin with short robust and long simple setae, distodorsal margin with long simple setae,

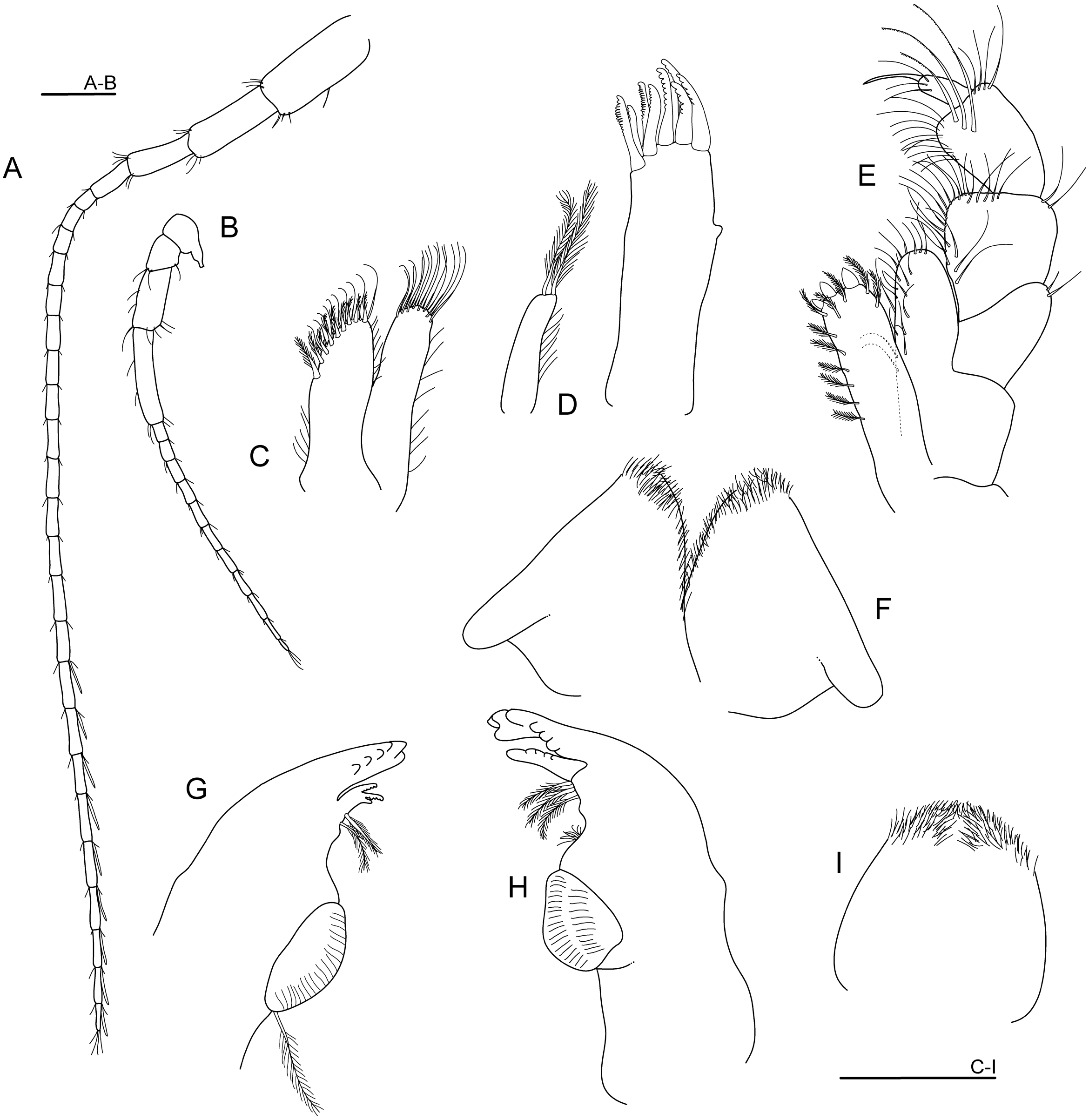

Fig. 12. *Yilgarniella sturtensis* King, n. sp. holotype male, 3.7 mm, WAM C49175. A, Antenna 1; B, Antenna 2; C, Maxilla 2; D, Maxilla 1; E, Maxilliped; F, Lower lip; G, Mandible RHS; H, Mandible LHS; I, Upper lip. Scale bars: (A-B) 0.2 mm, (C-I) 0.1 mm.

inner face with five robust plumose setae; dactylus with unguis, curved, fitting against palm, with proximal plumose seta. Gnathopod 2 (Fig. 13B) coxa 1.6 times as long as broad, distal margin with seven short simple setae; basis distoventral corner with scattered simple setae; ischium and merus distoventral corner with scattered setae; propodus two times as long as broad, subchelate, distoventral corner marked by robust seta adjacent to dactylar socket, palm margin with numerous robust setae with subterminal spines. Pereiopod 3 (Fig. 13C) coxa 1.5 times as long as broad, distal margin with eight short simple setae; basis dorsal and ventral margins with scattered simple setae, distoventral corner with clusters of setae; ischium distoventral corner with clusters of setae; merus with slight distodorsal lobe, ventral margin with scattered simple setae, distoventral corner with cluster of setae; carpus ventral margin with robust setae and scattered simple setae; propodus ventral margin with five clusters of robust and simple setae; dactylus dorsal margin with plumose seta, ventral margin with simple seta, unguis present. Pereiopod 4 (Fig. 13D) coxa 4 slightly broader than long, with distinct proximal excavation, distal margin with 12 short simple setae; basis ventral margin with scattered simple setae, distoventral corner with cluster of simple setae; ischium distoventral corner with cluster of setae; merus with slight distodorsal lobe, ventral margin with scattered simple setae, distoventral corner with cluster of setae; carpus ventral margin with scattered robust and simple setae; propodus ventral margin with five clusters of robust and simple setae; dactylus dorsal margin with plumose seta, ventral margin with simple seta, unguis present. Pereiopod

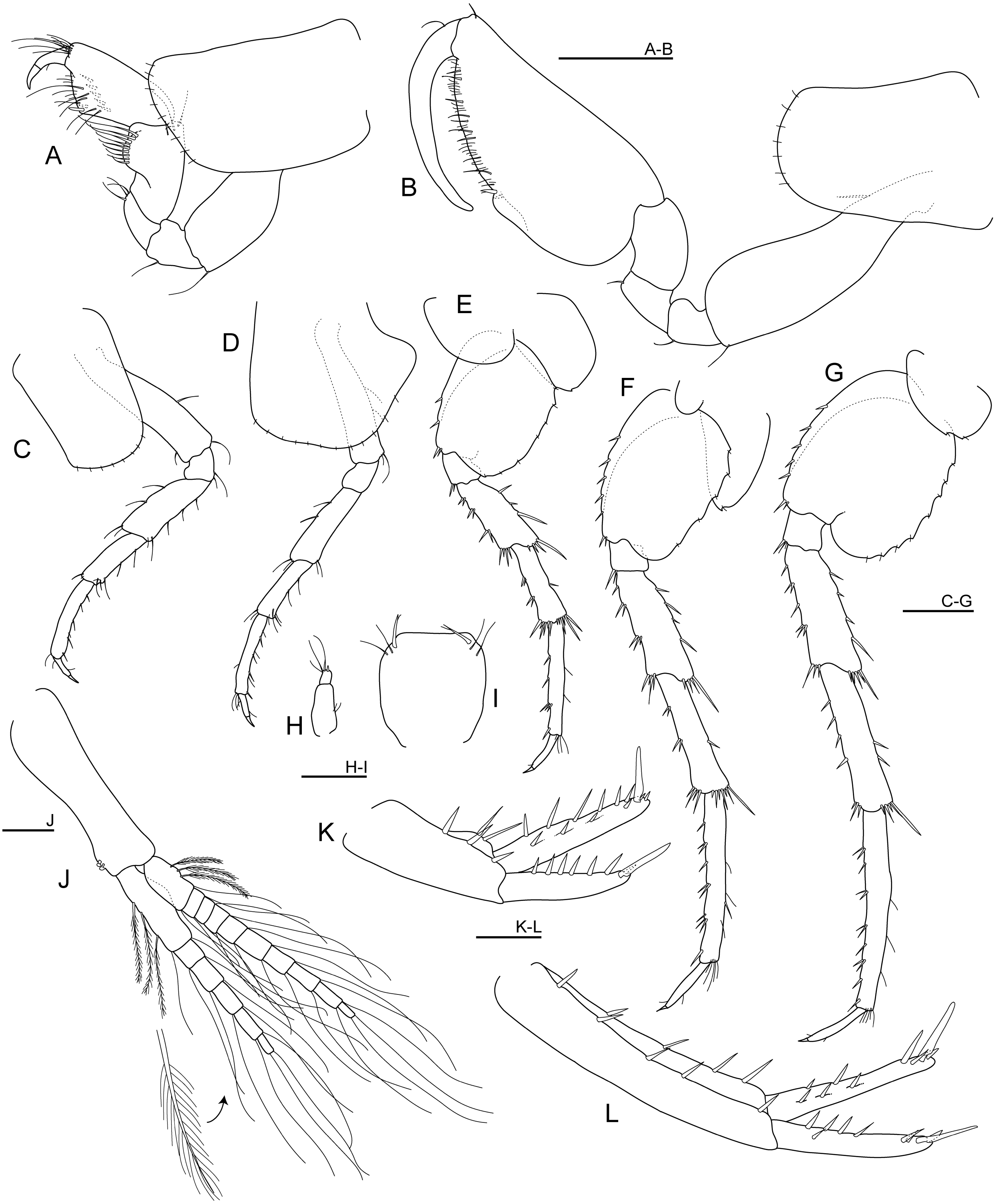

Fig. 13. *Yilgarniella sturtensis* King, n. sp. holotype male, 3.7 mm, WAM C49175. A, Gnathopod 1; B, Gnathopod 2; C, Pereiopod 3; D, Pereiopod 4; E, Pereiopod 5; F, Pereiopod 6; G, Pereiopod 7; H, Uropod 3; I, Telson; J, Pleopod 1; K, Uropod 2; L, Uropod 1. Scale bars: (A-B, C-G) 0.2 mm, (H-I, J, K-L) 0.1 mm.

5 (Fig. 13E) coxa posterior lobe with one short seta along margin; basis 1.4 times as long as broad, dorsal margin with two robust setae along length, distodorsal margin with two robust setae, ventral margin subtly crenulated and with seven short simple setae along length; ischium distodorsal margin with distal robust setae; merus with strong postero-distal lobe, dorsal margin with robust setae in four clusters, ventral margin with robust setae in four clusters; carpus as long as merus, dorsal margin with robust setae in three clusters, ventral margin with robust setae in two clusters;

propodus longer than merus, dorsal margin with four clusters of robust setae, ventral margin with two clusters of simple setae; dactylus with plumose seta on ventral margin, unguis present. Pereiopod 6 (Fig. 13F) coxa posterior lobe with one seta along margin; basis 1.4 times as long as broad, dorsal margin with five robust setae along length, distal end of dorsal margin with cluster of robust setae, ventral margin subtly crenulated and with five short simple setae along length; ischium dorsal margin with distal robust setae; merus with strong postero-distal lobe, dorsal margin with robust setae in four clusters, ventral margin with robust setae in five clusters; carpus as long as merus, dorsal margin with robust setae in three clusters, ventral margin with robust setae in two clusters; propodus longer than merus, dorsal margin with six clusters of robust setae, ventral margin with four clusters of simple setae; dactylus with plumose seta on ventral margin, unguis present. Pereiopod 7 (Fig. 13G) coxa ventral margin with two short simple setae; basis 1.3 times as long as broad, dorsal margin with five robust setae along length, distal end of dorsal margin with two robust setae, ventral margin subtly crenulated and with eight short simple setae along length; ischium dorsal margin with distal cluster of robust setae; merus with strong postero-distal lobe, dorsal margin with robust setae in five clusters, ventral margin with robust setae in five clusters; carpus as long as merus, dorsal margin with robust setae in five clusters, ventral margin with robust setae in three distal clusters; propodus longer than merus, dorsal margin with eight clusters of robust setae, ventral margin with three clusters of simple setae; dactylus with plumose seta on ventral margin, unguis present.

Pleopod 1 (Fig. 13J) similar to pleopods 2-3, unmodified (compared to *Chiltonia*), peduncle inner margins with two distal retinacula (coupling hooks), inner ramus of six articles, outer ramus of 10 articles. Uropod 1 (Fig. 13L) peduncle distinctly longer than rami, dorsal margin with five robust setae along the length of the outer margin and four along the inner margin; outer ramus with apical cluster of three small robust setae and one long robust seta (about half-length of ramus), a row of four robust setae along length and one inner robust seta; inner ramus with apical cluster of three small robust setae and one long robust seta (about half-length of ramus) and two rows of robust setae along length, outer margin with three robust setae, inner margin with two robust setae. Uropod 2 (Fig. 13K) peduncle shorter than inner ramus, dorsal margin with three robust setae along the length of the outer margin and two along the inner margin; outer ramus slightly smaller than inner ramus, with apical cluster of two small robust setae, with a row of five robust setae along length; inner ramus with apical cluster of five robust setae and one long robust seta (about half-length of ramus), with two rows of robust setae along length, outer margin with three robust setae, inner margin with five robust setae. Uropod 3 (Fig. 13H) bi-articulate, article 2 about 0.25 times length of article 1, distal margin with one short robust seta and three long robust setae. Telson (Fig. 13I) as long as broad, apically slightly blunt, with three pairs of setae around each dorsal distal corner.

Allotype Female (WAM C49176).—Length: 4.7 mm (Fig. 11B).

Similar morphology to male except for the following: Gnathopod 1 (Fig. 14A) carpus ventral-lateral lobe with row of 12 setulate setae; inner face with three robust setae. Gnathopod 2 (Fig. 14B) similar to gnathopod 1 except propodus 2.4 times as long as broad. Pereiopod 4 (Fig. 14C) coxa broader than in male. Pereiopods 5-7 (Fig. 14D-F) slightly broader than in male.

Uropod 1 (Fig. 14H) peduncle dorsal margin with 10 robust setae along the length of the outer margin and one along the inner margin; outer ramus with a row of seven robust setae along length. Uropod 2 (Fig. 14G) peduncle as long as inner ramus, dorsal margin with seven robust setae along the length of the outer margin and one along the inner margin; outer ramus with a row of four robust setae along length; inner ramus with apical cluster of three robust setae and one long robust seta (about half-length of ramus), with two rows of robust setae along length, outer margin with two robust setae, inner margin with three robust setae. Uropod 3 (Fig. 14I) article 2 with one short robust seta, and two long robust seta apically. Telson (Fig. 14J) slightly broader than in male.

Oöstegites present on coxae 2 to 5, forming the marsupium, margins with scattered curved hooks.

Etymology.—Named for the Sturt Meadows calcrete, where the species is found.

Variation.—Mature females were generally larger in size than mature males. Slight differences were noted in the number of flagellar articles in antennae 1 and 2: larger animals as illustrated, smaller individuals with 1-3 less articles. Setation of the pereiopods also differed slightly with size of the individuals with smaller animals having slightly fewer setae.

Discussion

Diversity of stygobitic amphipods from Western Australia has recently been estimated to be high (Guzik et al., 2011a). The results presented here support this assertion, formally adding a new stygobitic amphipod family (Chiltoniidae) for Western Australia and three new genera from the exploration of a single calcrete. Each of the three Sturt Meadows species is endemic to that calcrete, corroborating the results of other studies within the Yilgarn region of central Western Australia that strongly suggest that calcretes exist as 'closed island habitats' with sometimes highly structured populations of locally endemic aquatic invertebrate species (Cooper et al., 2002, 2007, 2008; Leys et al., 2003; Guzik et al., 2008; Leys and Watts, 2008; Bradford et al., 2010).

True cryptic stygobitic amphipod species have been reported in European subterranean environments (Lefébure et al., 2007; Villacorta et al., 2008) and are an interesting phenomenon because they suggest a selective pressure on morphology that is not reflected in mtDNA markers. However, the supposed morphologically cryptic nature of the Sturt Meadows chiltoniid amphipod lineages (Bradford et al., 2010) was not upheld once detailed morphological examination had taken place; the lineages were only cryptic in so far as they are part of a largely unknown and undescribed fauna. Nevertheless, Chiltoniidae are particularly well known as a difficult group in terms of species-level mor-

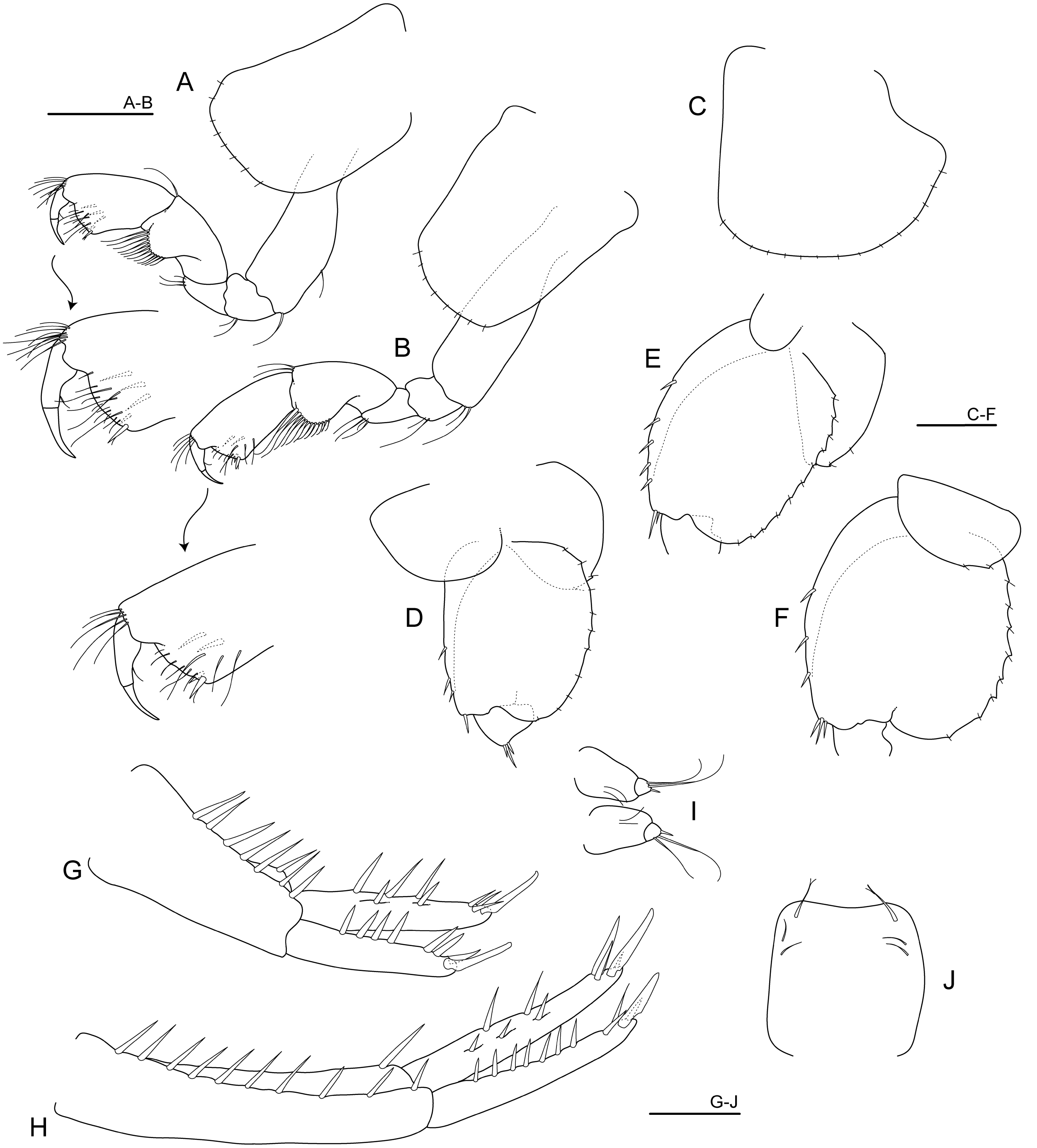

Fig. 14. *Yilgarniella sturtensis* King, n. sp. allotype female, 4.7 mm, WAM C49176. A, Gnathopod 1; B, Gnathopod 2; C, Pereiopod 4 (coxa); D, Pereiopod 5 (basis); E, Pereiopod 6 (basis); F, Pereiopod 7 (basis); G, Uropod 2; H, Uropod 1; I, Uropod 3; J, Telson. Scale bars: (A-B, C-F) 0.2 mm, (G-J) 0.1 mm.

phological variability (Zeidler, 1997; King, 2009a, b; King and Leys, 2011) and it is possible that cryptic species will be found as more stygobitic species are sequenced and described. In these cases, it is likely that species boundaries will be defined primarily by genetic divergence levels and geographic isolation within a calcrete.

The provisional delineation of crustacean species using nucleotide distance measures has been variously set in attempts to qualify molecular data with traditional taxonomic descriptive methods. One method identified species level limits as 10 times the intra-population *COI* divergence level (Witt et al., 2006) and another as a static 0.16 substitutions per site for the *COI* gene, as measured by patristic distances but also more roughly by pairwise distance methods (Lefébure et al., 2006). The lineages observed within the Yilgarn calcretes largely meet both criteria for delimitation of species. Nucleotide distance measures have been found to be highly variable within Amphipoda, ranging from 4 to

25% for species levels (Hogg et al., 2006; Witt et al., 2008); when they have been used within descriptions of species and genera, they are often presented in conjunction with additional data (e.g. morphological, geographical) (King and Leys, 2011). As the availability of molecular data increases, the intrinsic value of distance measures for species delineation, and the corresponding evolution of morphological form, will be better determined.

Interpreting the evolutionary histories of calcrete invertebrates remains a complex task, dependent on variables that include individual life history strategies as well as localised geological and geographic events. Nonetheless, there has been recent evidence for speciation and adaptive evolution within the confines of a cave environment (Juan et al., 2010), as revealed by the presence of sister species (Leys et al., 2003; Leys and Watts, 2008) or monophyletic groups of subterranean taxa (Ribera et al., 2010). The phylogenetic reconstruction presented here shows that multiple discrete lineages exist in at least two Yilgarn calcretes sampled (Sturt Meadows with three lineages and Depot Springs with two). In the case of Sturt Meadows, at least two lineages (= *Scutachiltonia* and *Stygochiltonia*) are more closely related to taxa outside the calcrete, indicating that they evolved following multiple independent colonisation events from distinct ancestral species. This finding contrasts with reports of dytiscid beetle speciation occurring within an individual calcrete, resulting in two to three lineages of highly morphologically variable beetles (Leys and Watts, 2008). Differing life history strategies and subsequent niche partitioning are thought to have heavily influenced the speciation of these beetles (Leys and Watts, 2008; Bradford et al., 2010). Given their morphological distinctions, the Sturt Meadows amphipods may also be utilising habitats of differing structure and sediment size within the calcrete; with the availability of these habitats possibly influencing the dominance of *Y. sturtensis*.

The genera *Stygochiltonia* and *Yilgarniella* are likely to be wide spread and comprised of species from at least two different calcretes (Sturt Meadows and Depot Springs for *Stygochiltonia* and Sturt Meadows and Nambi for *Yilgarniella*), indicating past connections within each taxonomic group that are broader than current calcrete boundaries and for the latter case, that cross palaeo-drainage boundaries (see Fig. 1). Research on other crustacean groups within Yilgarn calcretes, crangonyctoid amphipods (Cooper et al., 2007) and bathynellacean syncarids (Parabathynellidae) (Guzik et al., 2008), has similarly provided evidence for isolated calcrete species within more broadly distributed genera, with the implication that ancestral taxa may have been more widely distributed during less arid times and were later confined to individual calcretes once aridification altered the landscape.

With evidence from Sturt Meadows of a unique invertebrate fauna, in comparison to nearby calcretes, and more specifically of multiple divergent lineages of amphipods in varying population proportions, it is likely that current estimates of stygobitic amphipod diversity are low. The evolutionary histories of individual calcretes are highly variable and so fine scale sampling is necessary to best determine invertebrate biodiversity across the Yilgarn region.

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