THIRD GENUS OF PARASTENOCARIDID COPEPODS FROM AUSTRALIA SUPPORTED BY MOLECULAR EVIDENCE (COPEPODA, HARPACTICOIDA)

BY

T. KARANOVIC[1,2,4]) and S. J. B. COOPER[3,5])

[1]) Department of Life Sciences, Hanyang University, Seoul 133-791, Korea

[2]) IMAS, University of Tasmania, Hobart 7000, Tasmania, Australia

[3]) Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide SA 5000, Australia

ABSTRACT

A new species of parastenocaridid copepods was discovered in arid Western Australia, which could not be assigned to any of the currently known genera. Molecular evidence reveals only a remote phylogenetic relationship with two other Australian genera, *Parastenocaris* Kessler, 1913 and *Kinnecaris* Jakobi, 1972, and the pairwise distances among them are remarkably similar to those among three canthocamptid genera used as outgroups in our analysis. We interpret this as a very strong molecular signal in support of establishing a new genus for this Australian parastenocaridid, in addition to many morphological characters. The genus *Dussartstenocaris* n. gen. is currently monospecific, although we notice some morphological similarities between *Dussartstenocaris idioxenos* n. sp. and the Portuguese *Parastenocaris conimbrigensis* Noodt & Galhano, 1969. The two differ by the shape of the third leg exopod in male, a bifid exopodal spine being an autapomorphic feature of the new genus. The fifth legs both in male and female are an additional morphological character (or set of characters) that distinguish *Dussartstenocaris* from all 258 parastenocaridid species. The new genus differs from the other two Australian genera by at least ten major morphological characters each, seven of which are probably apomorphic features. This interesting parastenocaridid comes from a newly discovered locality in the Yilgarn region, with an unprecedented diversity of copepod crustaceans (up to seven species per single bore, and 22 morpho-species in total). This equals to 70% of the previously recorded diversity in the whole region, although the area investigated represents less than 3% of its surface. We also report on the first ever case of two sympatric parastenocaridids in Australia, which are a rare group on this continent. A very strong seasonal dynamics in this subterranean community was observed, and this is a novel concept for these ecosystems globally. A key to Australian species of Parastenocarididae Chappuis, 1940 is also included.

[4]) e-mail: Tomislav.Karanovic@utas.edu.au

[5]) e-mail: Steve.Cooper@samuseum.sa.gov.au

RÉSUMÉ

Une nouvelle espèce de copépode Parastenocarididae a été découverte en Australie-Occidentale aride, qui n'a pu être attribuée à aucun des genres aujourd'hui connus. Les données moléculaires révèlent une relation phylogénétique seulement éloignée avec deux autres genres australiens *Parastenocaris* Kessler, 1913 et *Kinnecaris* Jakobi, 1972, et les distances génétiques mesurées par paires sont remarquablement similaires à celles obtenues entre trois genres de Canthocamptidae utilisés comme « outgroups » dans notre analyse. Nous interprétons ceci comme un signal moléculaire très fort appuyant l'établissement d'un nouveau genre pour ce Parastenocarididae australien, s'ajoutant à plusieurs caractères morphologiques. Le genre *Dussartstenocaris* n. gen. est actuellement monospécifique, bien que nous ayons noté des similarités morphologiques entre *Dussartstenocaris idioxenos* n. sp. et l'espèce portugaise *Parastenocaris conimbrigensis* Noodt & Galhano, 1969. Les deux taxons diffèrent par la forme de l'exopodite de la troisième patte du mâle, une épine bifide de l'exopodite étant un caractère autopomorphique du nouveau genre. Les cinquièmes pattes chez le mâle comme chez la femelle, constituent un caractère morphologique supplémentaire (ou un ensemble de caractères) qui distingue *Dussartstenocaris* de tous les 258 espèces de Parastenocarididae. Le nouveau genre diffère des deux autres genres australiens par au moins dix caractères morphologiques majeurs chacun, dont sept sont probablement des caractères apomorphes. Cet intéressant Parastenocarididae provient d'une localité nouvellement découverte dans la région de Yilgarn, qui présente une diversité sans précédent de crustacés copépodes (jusqu'à sept espèces par prélèvement, et 22 morpho-espèces au total). Ceci équivaut à 70% de la diversité auparavant connue dans toute cette région, bien que la zone étudiée représente moins de 3% de sa surface. Nous mentionnons aussi le premier cas en Australie de deux Parastenocarididae sympatriques, ce groupe étant rare sur ce continent. De très fortes variations saisonnières ont été observées dans cette communauté souterraine, et ceci constitue un nouveau concept pour ces écosystèmes. Une clé d'identification des espèces australiennes de Parastenocarididae Chappuis, 1940 est aussi proposée.

INTRODUCTION

Parastenocarididae Chappuis, 1940 are a harpacticoid family highly specialized for life in continental groundwater, and almost exclusively restricted to this habitat (Galassi & De Laurentiis, 2004). They are, however, distributed on all continents except Antarctica and New Zealand (Karanovic, 2004), which is remarkable considering that stygofauna has a limited active dispersal potential and lacks resting stages that could be dispersed passively (Culver & Pipan, 2009). Because parastenocaridids have no marine relatives or modern pathways between different continents (Boxshall & Jaume, 2000), it has been postulated that they have a Pangean origin (Karanovic, 2006). In Australia, for example, Karanovic (2004) speculated that they started colonizing subterranean waters just after the Permo-Carboniferous glaciation, which spread throughout much of what will subsequently become the Gondwana supercontinent and covered the entire Australian plate (Frakes, 1999; Playford, 2003).

This makes it likely that present distributions of most parastenocaridids are a result of continental drift (Boxshall & Jaume, 2000), and thus an ideal group to study vicariance models in zoogeography. Unfortunately, no research has been done on their phylogeography so far. Vicariance has been considered to be a more acceptable hypothesis for explaining zoogeographic connections of freshwater subterranean faunas with disjunct distribution patterns (Boxshall & Jaume, 2000; Karanovic, 2004, 2005a, 2006; Karanovic & Ranga Reddy, 2005), while dispersal has been regarded traditionally as a better model for explaining recent disjunct distributions of marine and continental surface-water animals (Wilson, 1999; Reid, 2001; Waters & Roy, 2004; Waters & Craw, 2006; Karanovic, 2008). This view was never challenged seriously, although recent debate about New Zealand biogeography showed that we have unjustly underestimated recent long distance dispersal in favour of ancient vicariance (Sanmartin & Ronquist, 2004; Waters & Craw, 2006). Dispersal cannot be completely rejected even for some subterranean freshwater copepods with disjunct distributions (see Karanovic & Ranga Reddy, 2004), although this can sometimes be a consequence of anthropogenic translocation associated with early shipping activities (Karanovic, 2005b). This study offers the first insight into phylogenetic relationships of parastenocaridid copepods using modern molecular tools, even though only on the Australian continent. One of the aims was to stimulate similar research on other continental plates, which will help us to understand better the evolution and historical zoogeography of this interesting group of copepods.

The family is a monophyletic group within Harpacticoida, being easily distinguished by the sexual dimorphism in the third pair of swimming legs (Corgosinho et al., 2007). Modification of these legs in males into a grasping organ, that allows them to hold females during copulation (Glatzel, 1996), is one of the most important synapomorphies of the group (Martinez Arbizu & Moura, 1994), but many other morphological characters make it very easy to instantly recognize its members, both males and females: extremely vermiform habitus; seven-segmented antennula with very short first segment and longest second segment; antenna with allobasis and one-segmented exopod, latter with a single seta; one-segmented mandibular palp with two setae; maxillula without exopod or endopod; maxilla with two endites on coxa and one-segmented endopod, latter with two setae; maxilliped three-segmented, armed with a single apical spine; swimming legs with extremely conservative segmentation and armature formula (Boxshall & Halsey, 2004). In fact, such a great number of morphological characters are conservative in this family, that its generic di-

vision is a real nightmare (Reid, 1995; Galassi & De Laurentiis, 2004; Karanovic, 2005a; Schminke, 2010). Lang (1948) was very much aware of this, as he refrained from splitting the monogeneric family but rather subdivided the genus *Parastenocaris* Kessler, 1913 into eight species-groups for 31 of the 40 species known at that time. For this he mostly used characters of the male fourth leg endopod, and nine species were either known only as females or were insufficiently described. The system was coping rather well with a subsequent steady influx of newly described species from around the world, culminating in the decade between 1963 and 1972 when 75 new species were added (Schminke, 2010). New species groups were added by Noodt (1962, 1963, 1972a), mostly for the newly discovered and very diverse South American fauna, but it became apparent that the increasingly more complex system of species groups is not a reflection of true phylogenetic relationships, and many taxonomists were describing new taxa without even considering them. Jakobi (1969) described one of Noodt's groups as a new genus, and it was Jakobi (1972) who made the first effort to revise the family, by splitting it into 26 different genera (although only assigning 98 out of 155 known species). This system was strongly criticized by Schminke (1976), and was ignored for a long time by most subsequent taxonomists working on this group, all of them accepting only two of Jakobi's genera as valid (see Por & Hadel, 1986; Dussart & Defaye, 1990; Reid, 1995; Karanovic & Bobic, 1998; Ranga Reddy, 2001; Boxshall & Halsey, 2004; Galassi & De Laurentiis, 2004; Karanovic, 2005b, 2006; Cottarelli et al., 2006, 2007, 2008; Ranga Reddy & Defaye, 2007, 2009; Wells, 2007). Jakobi (1972), for example, divided the *brevipes*-group of Lang (1948) into 5 different genera, which was shown by Reid (1995) to be a group of very closely related species. She even showed that the type species of one new genus proposed by Jakobi is in fact a junior subjective synonym of the type species of *Parastenocaris*. Nevertheless, new genera were proposed for some unusual new members from South America (Dussart, 1979; Reid, 1994), Europe (Galassi & De Laurentiis, 2004), Africa (Schminke, 2009), and Asia (Cottarelli et al., 2010). Recently, some researchers (Corgosinho & Martinez Arbizu, 2005; Schminke, 2008; Corgosinho et al., 2010) started to revalidate and redefine some genera originally proposed by Jakobi (1972), as most of them remained valid and available names under the rules of the ICZN (1999), while at the same time synonymizing some others. Finally, Schminke (2010) listed all 258 valid species described until then in the family Parastenoarididae, provisionally accepted 27 genera as valid (accepting most of those described by Jakobi, although mainly listing just their type species as valid members), and subdivided the family into two subfamilies. As a result of the Principle

of Coordination, Parastenocaridinae Chappuis, 1940 has already (potentially) existed since 1940, with *Parastenocaris* as its type genus. In that respect, "Parastenocaridinae nov.", Schminke's (2010) most frequent way to refer to the taxon, is an error. Schminke does, however, correctly calls it "Parastenocaridinae Chappuis, 1940" in three places in his paper. On the other hand, he seems reluctant to call these two groups subfamilies, putting the word "subfamily" in quotes in the abstract and noting that such subgroups as he is proposing are "traditionally called subfamilies" (p. 344). Besides these instances, he does not use the term subfamily in the diagnosis section (pp. 361-362) or anywhere else. Still the above quoted notation on p. 344, together with the frequent notation "nov.", is enough to show that he is intentionally proposing a new taxon (Fontinalicaridinae) of subfamily rank (i.e., it is not some sort of informal or "provisional" or Phylocode-type unavailable taxon), and he explicitly designates its type genus. Therefore, we think, he has (barely) met the requirements for availability of new names. Due mostly to incomplete descriptions or absence of males, he was able to classify only 112 species to the genus level, leaving a majority of them in the genus *Parastenocaris*. Division of the genus *Parastenocaris* into *Parastenocaris* s. str. and *Parastenocaris* s. l., as first proposed by Galassi & De Laurentiis (2004) and adopted with a different meaning by Schminke (2010), has neither nomenclatural bearing nor phylogenetic justification as s. str. by definition must be part of s. l. Also, Schminke (2010) failed to define the two proposed subfamilies by a clear set of morphological synapomorphies, and our new genus could not be classified using this subdivision.

Compared to other continents, the diversity of parastenocaridid copepods is surprisingly low in Australia (Karanovic, 2004, 2006). It was Schminke (1981) who first reported a discovery of "four species belonging to three genera" of parastenocaridids from here, but unfortunately they all remain as yet undescribed. The first described species, *Parastenocaris solitaria* Karanovic, 2004, was reported from the Yilgarn region of Western Australia by Karanovic (2004), and only after three females collected from three different bore holes in Depot Springs pastoral station, some 65 km west of Leinster. Karanovic (2005a) described another two species from Western Australia: one from a cave near Margaret River (*Parastenocaris eberhardi* Karanovic, 2005), and the other from a bore in the Argyle Diamond Mine in the Kimberley region (*P. kimberleyensis* Karanovic, 2005). Both species were described after males and females. Because females of *P. eberhardi* were found to be very similar to those of the previously described *P. solitaria*, both species were assigned to the *minuta*-group of Lang (1948). Schminke (2008) moved them both into the redefined genus *Kinnecaris* Jakobi, 1972. *Parastenocaris kimberleyensis*, on

the other hand, is a member of the *brevipes*-group of Lang (1948), which was considered by Galassi & De Laurentiis (2004) and Schminke (2010) as *Parastenocaris* s. str., because the type species of the genus is a member of this group. Finally, Karanovic (2006) described one new species after both sexes from the Pilbara region in Western Australia: *Parastenocaris jane* Karanovic, 2006. This species also belongs to the *brevipes*-group. Given the size of the regions surveyed in Karanovic (2004, 2006), it is clear that parastenocaridids are indeed very rare in Australia. However, there is an interesting zoogeographical pattern emerging, with members of the genus *Parastenocaris* being present in the northern part of Western Australia (Pilbara and Kimberley regions) and *Kinnecaris* in the southern part of Western Australia (Yilgarn region and south-western Western Australia). New discoveries (all unpublished or in preparation) only confirm this subdivision. Two new species from the Pilbara region are awaiting description and both are closely related to *P. jane*, while five new short-range endemics from the Yilgarn region (two of them used for molecular analysis in this paper) are extremely similar to *Kinnecaris solitaria*, and can be only distinguished morphologically from each other by details in urosomal ornamentation and caudal rami shape. Although one has to assume that the zoogeography of some of the most ancient landscapes on earth would be very complex, what does not stop to amaze are the regional differences in stygofauna assemblages in Australia, and especially those between the neighbouring Pilbara and Yilgarn regions of Western Australia (see also Karanovic, 2006, 2008). The discovery that Australian regions have different relationships to other Gondwanan areas was already anticipated by Weston & Crisp (1994). Giribet & Edgecombe (2006) showed the importance of looking at small-scale patterns when inferring Gondwanan biogeography for terrestrial invertebrates. Karanovic (2006) proposed a "pulsating desert hypothesis" as a novel dynamic model that may explain some of the differences observed between these two neighbouring regions. Other, published (Karanovic, 2008, 2010; Karanovic & Hancock, 2009; Karanovic et al., 2011) and unpublished research (Karanovic, in prep.), done recently on subterranean waters in eastern Australia showed a similar dividing line between the stygofaunas of Queensland and New South Wales, although it is not quite clear yet where this boarder lies precisely. In short, copepods found in Queensland are more closely related to those the Western Australian Pilbara region, than the neighbouring New South Wales. A strong connection between the Pilbara region, tropical Queensland, and New Zealand was observed, which may even predate Gondwanan regionalism (Karanovic et al., 2011).

Thus, it was exciting to find a new lineage of parastenocaridids in Australia, which we describe below as a new genus. It was found sympatrically with a new species of *Kinnecaris* in the Yilgarn region. This is the first case of two parastenocaridid species in the same locality in Australia, which is a common phenomenon in most other parts of the world (see, for example, Noodt, 1962; Enckell, 1970; Ranga Reddy, 2001; Corgosinho et al., 2007). Given the conservative morphology in this family and the taxonomic confusion that surrounds many of its genera (see above), in addition to normal homoplasies that were found in many groups of subterranean animals (Humphreys, 2008), we decided to test its phylogenetic relationship with the other two Australian lineages using molecular tools.

MATERIAL AND METHODS

All specimens studied here were collected from subterranean waters by private environmental consulting agencies (Subterranean Ecology Pty Ltd., Outback Ecology Pty Ltd., and Bennelongia Pty Ltd.) and entrusted to the senior author for morphological identification. They resulted from various impact assessment and monitoring projects, primarily done for the mining industry. Most specimens were collected from or near proposed or existing mining sites, but due to the sensitivity of such data no further information about mining operations or plans will be given here. Locality data and number of specimens are listed for every species separately and all types are deposited in the Western Australian Museum (WAM), Perth. Some specimens were kept as vouchers by consulting agencies, but will be ultimately also deposited in the WAM. Two as yet undescribed species of the genus *Kinnecaris* Jakobi, 1972 (*K*. sp. 1 and *K*. sp. 2) came from the Yeelirrie calcrete, as does the new genus described here. *Parastenocaris jane* Karanovic, 2006 was collected from several bores near Newman in the Pilbara region of Western Australia, and was intended as an outgroup. The other three outgroups for our molecular phylogenies come from the family Canthocamptidae Brady, 1880: *Cletocamptus deitersi* (Richard, 1897) CO1 sequence data are available from GeneBank, *Australocamptus hamondi* Karanovic, 2004 was collected also in the Yeelirrie calcrete (bore line E, bore No 312), and *Elaphodella humphreysi* Karanovic, 2006 was collected from several bores near Newman in the Pilbara region of Western Australia.

Samples were collected with haul-nets (mesh size 50 or 150 μm) or a groundwater sampling pump from bores. Bores are holes mainly made by mining companies or agricultural enterprises for the purpose of water

monitoring and abstraction or mineral exploration. They are usually 50 to 200 mm in diameter and may be lined entirely, or in part, by PVC tubing (the casing). This tubing may be open only at the bottom, or it may be pierced at one or more levels by holes of various sizes ("slots"). The top may be securely capped or entirely open to the elements. Some bores record the water pressure at a given level in the aquifer (piezometers), while others, together with hand dug wells (ca. 1×1.5 m) equipped with windmills, provide water for pastoral use. Many of these features are derelict. Haul-nets are actually simple plankton nets of a different size suitable for the bore; the collar can range from 30 to 150 mm in diameter and is made of stainless steel. Weighed nets (using simple fishing leads, or more complicated brass intermediate collars) were lowered down into the bore with a bottle screwed on its distal part and then hauled through the water column, usually a number of times. Samples were preserved in the field in 100% ethanol, sorted in a laboratory under a dissecting microscope, and assigned a field number (every consulting agency has a different system of numbering its samples). Many bores established for hydrogeological work, mineral exploration, and water monitoring have prefixes or suffixes of relevance only to that drilling program. These codes are cited in the material examined for each species to aid specification of the location, although precise coordinates are also provided for each sample.

Specimens were dissected and mounted on microscope slides in Faure's medium, which was prepared following the procedure discussed by Stock & 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, making possible the observation of morphological details. During the examination water slowly evaporates and appendages eventually remained in a completely dry Faure's medium, ready for long term depositing. All line drawings were prepared using a drawing tube attached to a Leica MB2500 phase-interference compound microscope, with N-PLAN (5×, 10×, 20×, 40× & 63×, all dry) of PL FLUOTAR (100× oil) objectives. Specimens that were not drawn were examined in propylene glycol ($CH_3CH(OH)CH_2OH$) and, after examination, were again preserved in 100% ethanol. Photographs of whole specimens were taken in propylene glycol with a Leica DFC420 micro-camera attached to a Leica M205C dissecting microscope. The software package Leica Application Suite (LAS), Version 3.5.0, was used to create a multifocal montage image. Specimens for scanning electron micrography were dehydrated in progressive

TABLE I
Oligonucleotide primers[1]) used to PCR-amplify the 5′ end of CO1

Primer code	Primer sequence (5′-3′)	Designed by
LCO1490 (M414)	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (2004)
HCO2198 (M423)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (2004)
M1321	TRRNGAYGAYCARRTTTATAATGT	K. Saint
M1322	TCAAAATARRTGYTGRTAWARHAC	K. Saint
M1323	GAYGAYCARRTTTATAATGT	K. Saint

[1]) Three additional primers were also developed but were unsuccessful in PCR-amplifications of CO1.

ethanol concentrations, critical-point dried, coated in gold, and observed under a LEO 1525 microscope on the in-lens detector, with working distances between 5.9 and 6.1 mm and accelerating voltages of 5 or 10 kV.

Morphological terminology follows Huys & Boxshall (1991), except for 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 standardize the terminology for homologous appendages in different crustacean groups. Biospeleological terminology follows Humphreys (2000).

Specimens for molecular analysis were examined without dissecting under a compound microscope (objective 63× dry) in propylene glycol. After examination they were returned to 100% ethanol. DNA was extracted using the GENTRA method (Puregene) according to the manufacturer's protocol for fresh tissues. PCR-amplifications of a 623-bp fragment from the mitochondrial CO1 gene were generally carried out with the "universal" primers LCO1490 and HCO2198 (Folmer et al., 1994). The use of these primers, however, proved problematic in many cases and hence additional 'nested' primers were designed by Ms. Kathleen Saint (South Australian Museum) from preliminary copepod CO1 sequence data and used in combination with the Folmer et al. (1994) primers to improve the PCR-amplification efficiency (table I). An initial PCR-amplification used the combination LCO1490/HCO2198, then 1 μl of product was used to seed nested PCRs in the following combinations: M1323/HCO2198 or M1321/M1322 (see table I for codes). PCR-amplifications were carried out in 25 μl volumes containing, 4 mM $MgCl_2$, 0.20 mm dNTPs, 1× PCR buffer (Applied Biosystems), 6 pmol of each primer and 0.5 U of AmpliTaq Gold (Applied Biosystems). PCR amplification was performed under the following conditions: 94°C 9 min, then 34 cycles of 94°C 45 s; annealing 48°C 45 s; 72°C, 60 s; with a final

TABLE II
List of copepod specimens for which mtCO1 fragment was successfully amplified; see text for generic names and authors of the specific names

Code	Species	Region	Bore line	Bore number	Date	GenBank
7081a	*A. hamondi*	Yilgarn	E	312	13 Jan 2010	JN039160
7081b	*A. hamondi*	Yilgarn	E	312	13 Jan 2010	JN039163
7101	*P. jane*	Pilbara	–	FMGSM1386	24 Jan 2010	JN039164
7122	*A. hamondi*	Yilgarn	E	312	19 Mar 2010	JN039165
7315	*Kinnecaris* sp. 1	Yilgarn	L	LUNK1	14 Nov 2009	JN039162
7991	*E. humphreysi*	Pilbara	–	FMGSM1529	23 Jan 2010	JN039161
8110	*E. humphreysi*	Pilbara	–	FMGSM3644	02 Mar 2010	JN039166
8119	*E. humphreysi*	Pilbara	–	FMGSM3645	01 Mar 2010	JN039173
8310	*Kinnecaris* sp. 1	Yilgarn	L	Snake Well	18 Mar 2010	JN039167
8405	*D. idioxenos*	Yilgarn	P	YYHC0133A	20 Mar 2010	JN039168
8496	*Kinnecaris* sp. 2	Yilgarn	1	YYD22	15 Mar 2010	JN039169
8527	*A. hamondi*	Yilgarn	E	312	16 Mar 2010	JN039170
8536	*Kinnecaris* sp. 2	Yilgarn	F	YU2	17 Mar 2010	JN039171
8563	*Kinnecaris* sp. 2	Yilgarn	H	TPB33	18 Mar 2010	JN039172

elongation step at 72°C for 6 min. PCR products were purified using a vacuum plate method and sequencing was undertaken using the ABI prism Big Dye Terminator Cycle sequencing kit (PE Applied Biosystems, Foster City, CA). Sequencing was carried out on an ABI 3700 DNA analyser and sequences were edited and manually aligned in SeqEd version 1.0.3 (Applied Biosystems). For this study DNA was extracted and the CO1 fragment successfully PCR-amplified from 43 copepod specimens (table II).

Phylogenetic analyses of the CO1 sequence data were conducted with or without the use of outgroup taxa, and using a combination of different approaches to assess the robustness of the tree topology. A distance approach, using Neighbour Joining (NJ), and a Maximum Parsimony (MP) approach were conducted using the program PAUP* version 4.0b10 (PC program, Swofford, 2002). A Maximum Likelihood (ML) approach was conducted using the maximum likelihood (ML) program RAxML and the WEB-based RAxML "black box" (http://phylobench.vital-it.ch/raxml-bb/; Stamatakis et al., 2008) provided by the Vital-IT Unit of the Swiss Institute of Bioinformatics. The data were also analysed using a Bayesian Inference (BI) approach using MRBAYES v.3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). An HKY-85 (Hasegawa et al., 1985) distance model was used for the NJ analyses. MP analyses were conducted using a heuristic search option and default options (TBR branch swapping, ACCTRAN character state optimization), with the exception of using random stepwise addition repeated

100 times. NJ and MP bootstrap analyses (Felsenstein, 1985) were carried out using 1000 bootstrap pseudoreplicates, employing a heuristic search option as above with random input of taxa and "max trees" set to 100 for the MP bootstrap analysis. The ML analyses were conducted applying a General Time Reversible model and unequal variation at sites modelled using a Gamma distribution. Support for branches was estimated using the bootstrap option in RAxML, using 100 bootstrap pseudoreplicates.

The program MODELTEST (version 3.7; Posada & Crandall, 1998) with the Akaike Information Criterion was used to show that a General Time Reversible (GTR) model (Rodríguez et al., 1990), with a proportion of invariant sites (I) and unequal rates among sites (G) (Yang, 1996) was most appropriate for BI analyses. The BI analysis of CO1 data was carried out using default uninformative priors with four chains run simultaneously for five million generations in two independent runs, sampling trees every 500 generations. After this number of generations the final standard deviation of split frequencies had reduced to 0.0045 and the PSRF was $\sim$1.0 for all parameters, suggesting convergence had been reached. Assessment of effective sample sizes for each parameter estimate was determined using the program Tracer v1.4 (Rambaut & Drummond, 2007). The likelihood values converged to relatively stationary values after about 5000 generations. Trees from each MrBayes run were combined and a burnin of 5000 trees (25% of the total) was chosen, with a $>$50% posterior probability consensus tree constructed from the remaining 15 002 trees.

Average DNA sequence divergence within groups (morpho-taxa) and between groups was estimated using the program MEGA v. 4 (Kumar et al., 2008), with a composite likelihood distance applied under a HKY-85 model of DNA sequence evolution.

TAXONOMIC PART

Class MAXILLOPODA Dahl, 1956
Subclass COPEPODA H. Milne Edwards, 1840
Order HARPACTICOIDA G. O. Sars, 1903
Family PARASTENOCARIDIDAE Chappuis, 1940

Genus **Dussartstenocaris** n. gen.

Diagnosis. — Relatively large parastenocaridids, with body length in males and females relatively similar, from 475 to 512 μm. Habitus vermiform, almost seven times as long as wide in dorsal view, with greatest width at fourth

pedigerous (third free) somite both from dorsal and lateral view. Integument weakly sclerotized, very smooth, without any surface ornamentation except sensilla and pores, cephalothorax with hardly visible double dorsal window posteriorly (no integumental windows on other somites). Tergites and pleura of second and third pedigerous somites ornamented exactly as those of first pedigerous somite (which is fused to cephalothorax): with two pairs of dorsal sensilla, one pair of dorsolateral posterior sensilla, and one pair of lateral sensilla (one sensillum on each side); fourth pedigerous somite with sensilla only on posterior margin, but also eight in total; first three (two in female) urosomal somites with six posterior sensilla (genital somite in female with two additional lateral sensilla at middle); fourth urosomite (third in female) with ten posterior sensilla, while preanal somite without any surface ornamentation; anal somite with pair of dorsal sensilla and two pairs of pores (one dorsal, other ventral). Anal operculum wide and convex, ornamented with row of spinules on inner surface. Caudal rami 4.5 times as long as wide, 1.4 times as long as anal somite, almost cylindrical, armed with six armature elements (two lateral, one dorsal, and three apical); ornamented posteriorly with large cuticular pore ventrolaterally and row of spinules ventrally. dorsal seta inserted at 3/5 of ramus length; lateral setae thin and smooth, inserted close to each other at 1/3 of ramus length; inner apical seta small and smooth; middle apical seta strongest, without breaking plane and unipinnate; outer apical seta small but strong, without breaking plane and unipinnate. Antennula seven-segmented, with short aesthetascs on fourth and seventh segments; male with geniculation between third and fourth and fifth and sixth segments, small distal spiniform process on sixth segment, last two segments in line, and setal formula 0.6.5.3.1.1.8; female with no geniculation or processes, and with two setae less on second and one less on fourth segment. Swimming leg segmentation formula (exopod/endopod) as in majority of parastenocaridids 3/2, 3/1, 2/1, 3/1; inner distal corners of each exopodal segment with hyaline frills distally (except those of transformed third leg in male); first exopodal segments armed with single outer spine; last exopodal segments with armature formula 4.3.0.2 in male and 4.3.2.2 in female; middle exopodal segment absent in third leg, unarmed in other legs; endopod of first legs with unarmed and long first segment, second segment short and with two apical elements. Endopod of second leg armed with single apical seta, pointing inwards, ornamented with spinules both along distal and outer margins. Endopod of third leg in female short and unarmed, curved outwards, ornamented with several spinules on inner margin distally. Endopod of fourth leg in female long,

armed apically with strong spine, ornamented with spinules both along distal and inner margins. Third leg in male transformed into grasping organ; basis robust, armed with long outer seta, ornamented with diagonal row of large spinules close to outer margin, large cuticular pore on anterior surface, and with longitudinal row of short but stout spinules along inner margin; endopod about as long as largest spinules on basis; exopod with both segments fused; ancestral proximal segment about 2.9 times as long as wide, with chitinous bulb on distal part of inner margin, ornamented with one longitudinal row of spinules along outer margin proximally and two distally, armed subapically with strong, smooth and curved spine, twice as long as apophysis and fused basally to another small spine or chitinous process; ancestral distal segment (apophysis) very short and inflated distally, oriented inward, unornamented but armed with minute spine apically, which is fused to segment; large endopodal spine fits into lateral grooves of apophysis. Basis of fourth leg in male with four or five large spinules at base of endopod on anterior surface; endopod almost conical, curved slightly outwards, with apical spine fused basally to segment, serrulate along outer margin distally. Fifth legs very similar in male and female, simple rounded cuticular plates, distinct at base, divergent, and pointing outwards; ornamented with large cuticular pore basally, another small pore at outer base of minute apical spine, and with row of several large spinules along inner margin distally; armed with three smooth setae and one short spine along distal margin; outermost seta (ancestral basal one) longer than leg and about 4.3 times as long as middle seta; middle seta 1.7 times as long as innermost seta and 3.8 times as long as minute spine; two small setae and minute spine inserted on anterior surface and almost distally. Sixth legs in male very disproportionate in size, right one almost completely reduced, left one enlarged, forming single, smooth, large operculum covering gonopore; no ornamentation or armature. Sixth legs in female vestigial, fused into simple cuticular plate, covering gonopore, unornamented and unarmed.

Etymology. — The genus name is dedicated to the late Dr. Bernard Dussart, in memory of his contribution to our knowledge of freshwater copepods. His surname is prefixed to the existing generic name *Stenocaris*. Gender masculine.

Type and only species. — *Dussartstenocaris idioxenos* n. sp.

Dussartstenocaris idioxenos n. sp.
(figs. 1-6)

Type locality. — Australia, Western Australia, Yilgarn region, Yeelirrie pastoral station, bore line E, bore 312, 27.025132°S 119.691245°E.

Type material. — Holotype male dissected on one slide (WAM C37496). Allotype female dissected on one slide (WAM C37497). Other paratypes are: 19 males, 10 females, and eight copepodids in alcohol (WAM C37498), four males and three females on one SEM stub in toto coated in gold (WAM C37499), and one male and one female dissected on one slide each (WAM C37500 and C37501, respectively), all collected at type locality, leg. T. Karanovic & S. Callan, 19 March 2010, seLN7122. Topotypes: 12 males, five females, and five copepodids in alcohol, one male destroyed for mtCO1 DNA sequence (amplification unsuccessful), all collected at type locality, leg. T. Karanovic & G. Perina, 16 March 2010, seLN8527. Another topotype female in alcohol and one destroyed for mtCO1 DNA sequence (amplification unsuccessful), both collected at type locality, leg. T. Karanovic & S. Callan, 13 January 2010, seLN7081.

Other material examined. — Two females and three copepodids in alcohol, one female destroyed for mtCo1 DNA sequence (amplification successful), Australia, Western Australia, Yilgarn region, Yeelirrie pastoral station, bore line P, bore YYHC0133A, 27.017769°S 119.646068°E, leg. T. Karanovic & S. Callan, 20 March 2010, seLN8405.

One male in alcohol, Australia, Western Australia, Yilgarn region, Yeelirrie pastoral station, bore line A, Wirraway Bore, 27.097464°S 119.760755°E, leg. P. Bell & S. Callan, 15 November 2009, seLN7332.

Description. — Male (based on holotype and several paratypes). Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding caudal setae), from 483 to 512 μm (503 μm in holotype). Preserved specimen colourless. Nauplius eye absent. Body composed of prosome (consisting of cephalothorax and three free pedigerous somites (first pedigerous fused to cephalothorax)), and urosome (consisting of fifth pedigerous somite, genital somite, four abdominal somites, and caudal rami). Habitus (figs. 1A, 5A) cylindrical and very slender, without any demarcation between prosome and urosome; prosome/urosome ratio 1.25; greatest width at fourth pedigerous (third free) somite both from dorsal and lateral view. In fact, middle region looks somewhat swollen, from third pedigerous to second urosomal somite. Body length/width ratio about 6.8; cephalothorax 0.95 times as wide as genital somite. Free pedigerous somites without any expansions laterally or dorsally, all connected by well developed arthrodial membranes. Hyaline fringes of all somites smooth, very narrow and hard to distinguish from arthrodial membranes, especially dorsally. Integument weakly sclerotized, very smooth, without any surface ornamentation except sensilla and pores (no spinules or cuticular pits); cephalothorax with hardly visible (fig. 5A) double dorsal window posteriorly (smaller window with thinner integument inside bigger one), without any integumental windows on other somites. Pleural areas of cephalothorax and free pedigerous somites (fig. 5A, B, C) not well developed, cephalic appendages and coxae of swimming legs clearly exposed in lateral view. Rostrum small, membranaceous, not demarcated at base, ornamented with two large dorsal sensilla, linguiform, almost reaching distal margin of first antennular segment, about twice as long as wide.

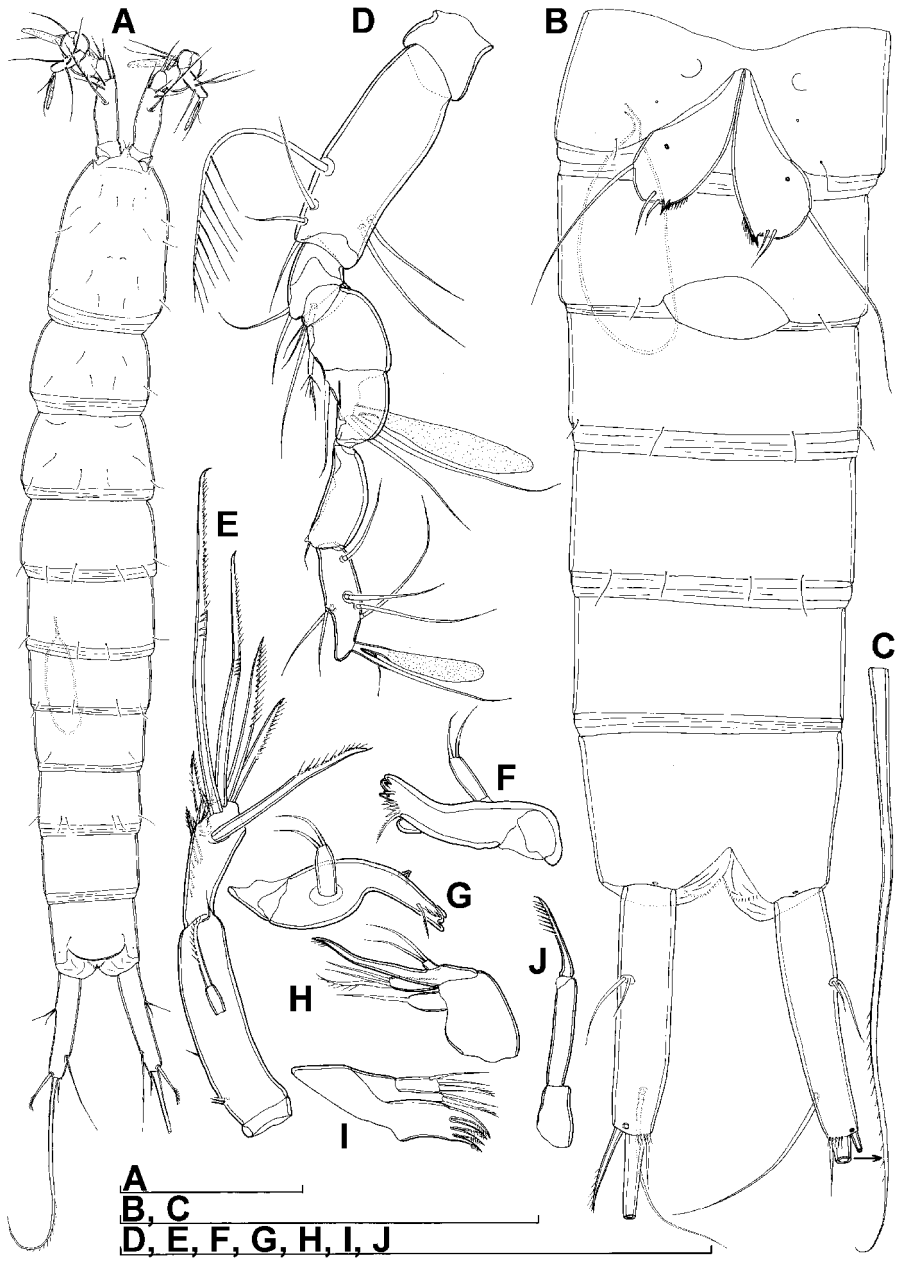

Fig. 1. *Dussartstenocaris idioxenos* n. sp. A, paratype male I; B–J, holotype male. A, habitus dorsal view, integumental window on cephalothorax not illustrated; B, urosome, ventral view; C, principal caudal seta; D, antennula, dorsal view; E, antenna, lateral (outer) view; F, mandibula, anterior view; G, mandibula, postero-median view; H, maxilla, anterior view; I, maxillula, posterior view; J, maxilliped, anterior view. Scale bars = 100 μm.

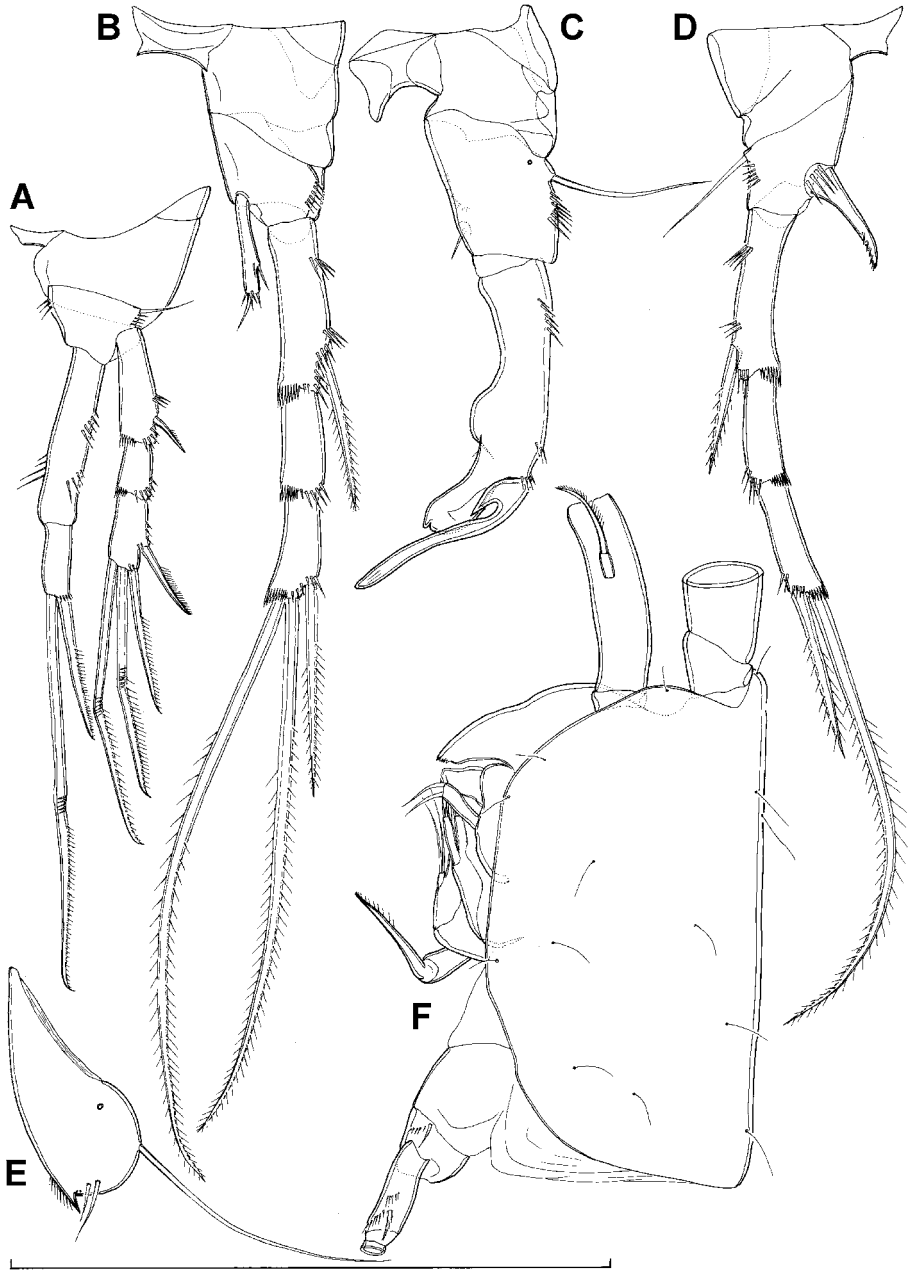

Fig. 2. *Dussartstenocaris idioxenos* n. sp. A–E, holotype male; F, paratype male I. A, first swimming leg, anterior view; B, second swimming leg, anterior view; C, third swimming leg, anterior view; D, fourth swimming leg, anterior view; E, fifth leg, anterior view; F, cephalothorax, lateral view, integumental window not illustrated. Scale bar = 100 μm.

Cephalothorax (figs. 1A, 2F, 5A, B) about 1.5 times as long as wide in dorsal view; representing 19% of total body length. Surface of cephalic shield ornamented with eight large sensilla in posterior half (posterior to cuticular window, corresponding to fused first pedigerous somite), and 18 sensilla in anterior half (six dorsal, two lateral on each side, and four on each side at ventral margin of pleura.

Tergites and pleura of second and third pedigerous somites (fig. 1A) ornamented exactly as those of first pedigerous somite: with two pairs of dorsal sensilla (one at midlength more widely spaced than posterior pair), one pair of dorsolateral posterior sensilla, and one pair of lateral sensilla (one sensillum on each side); midlength pair of dorsal sensilla more widely spaced on third pedigerous somite. Fourth pedigerous somite with sensilla only on posterior margin, but also eight in total.

Fifth pedigerous somite (fig. 1A, B) ornamented with three pairs of sensilla on posterior margin (one dorsal, one dorsolateral, and one ventrolateral), and one pair of very small cuticular pores at base of fifth legs (fig. 5D).

Genital somite (fig. 1A, B) ornamented with three pairs of sensilla on posterior margin (one dorsal, one dorsolateral, and one ventrolateral), about twice as wide as long, with single, large, completely formed and longitudinally placed spermatophore visible inside; spermatophore about 1.5 times as long as genital somite and can be placed either on left or on right side. Third urosomite (fig. 1A, B) also ornamented with six posterior sensilla, but dorsal pair more widely spaced, and lateral pair not visible from dorsal view. Fourth urosomite (fig. 1A, B) ornamented with 10 posterior sensilla, four dorsally, two laterally (one on each side) and four ventrally. Fifth urosomal (preanal) somite without any surface ornamentation.

Anal somite (fig. 1A, B) ornamented with pair of large dorsal sensilla at base of anal operculum, pair of minute cuticular pores dorsolaterally close to posterior margin, and pair of slightly larger cuticular pores ventrally, at base of caudal rami (see also fig. 6C). Anal operculum (fig. 1A) well developed, unornamented on outer surface, ornamented with row of spinules on inner surface, with convex and smooth distal margin, not reaching posterior end of anal somite, representing 67% of somite's width. Anal sinus widely open, with two diagonal rows of minute spinules on ventral side and transverse row of spinules on dorsal side (below anal operculum).

Caudal rami (figs. 1A-C, 5A, 6C) about 4.5 times as long as greatest width (dorsal view) and almost 1.4 times as long as anal somite, cylindrical (slightly narrowing towards distal end), divergent, with space between them

more than 1.5 times one ramus width; armed with six armature elements (two lateral, one dorsal, and three apical). Ornamentation consists of large cuticular pore ventrolaterally close to posterior margin, and row of spinules along posterior margin ventrally. Dorsal seta slender and smooth, inserted somewhat closer to inner margin at about 3/5 of ramus length, about 0.8 times as long as caudal ramus, triarticulate basally. Lateral setae thin and smooth, inserted close to each other at 1/3 of ramus length. Proximal lateral seta placed more dorsally, about 1.7 times as long as distal minute one, and about 0.3 times as long as ramus. Inner apical seta small, smooth, inserted more ventrally, about 0.2 times as long as ramus. Middle apical seta strongest, without breaking plane, unipinnate distally, about 5.6 times as long as outer apical seta and 0.24 times as long as whole body. Outer apical seta small, also without breaking plane and unipinnate, about 0.35 times as long as ramus.

Antennula (figs. 1D, 6D, E) slightly longer than cephalothorax, seven-segmented but third segment somewhat subdivided with surface sutures on inner and ventral sides, prehensile and usually strongly digeniculate, unornamented. First segment very short, while second longest. Geniculation between third and fourth and between fifth and sixth segments. Distal anterior corner of seventh segment protrudes as small spiniform process; another spiniform process present on fifth segment basally, at base of inner seta (see fig. 6E). Broad aesthetasc on fourth segment almost reaching tip of appendage, fused basally to slightly longer seta. Much shorter apical aesthetasc on seventh segment, fused basally to two setae. Setal formula: 0.6.5.3.1.1.8. All setae slender and almost all with pore on tip; proximalmost seta on second segment uniplumose distally (fig. 1B), and proximalmost seta on fourth segment with tuft of spinules at midlength (fig. 6E); all other setae smooth. Largest seta on second segment and all free setae on seventh segment biarticulate basally.

Antenna (fig. 1E) relatively stout and long, composed of coxa, allobasis, one-segmented endopod, and one-segmented exopod. Coxa very short, unornamented. Allobasis about 3.6 times as long as wide, unarmed, ornamented with two short rows of large spinules along anterior surface. Endopod about 2.5 times as long as wide, with surface frill subdistally, ornamented with few large spinules along anterior surface, armed laterally with two bipinnate spines (proximal somewhat shorter) and apically with five strong and unipinnate elements (two geniculate). Exopod minute, cylindrical, about 2.4 times as long as wide, unornamented but armed with single unipinnate apical seta, which is about 3.6 times as long as segment.

Mandibula (figs. 1F, G, 6B) with narrow cutting edge on elongated coxa, armed with two complex teeth ventrally (both tricuspidate), one unipinnate

seta dorsally, and several smaller teeth and/or spinules in between. Palp one-segmented, cylindrical, about three times as long as wide, unornamented, and armed apically with two smooth and subequal setae, each with pore on tip.

Maxillula (figs. 1I, 6B) with relatively small praecoxa, arthrite rectangular, about 1.4 times as long as wide from lateral view, unornamented but armed with four apical elements (probably three spines and one strong seta), each with a tuft of basally fused spinules at distal end, forming little scoops. Coxal endite armed with one smooth and one unipinnate setae apically (both with pore on tip), somewhat shorter than praecoxal arthrite. Basis slightly shorter than coxal endite, armed with one subapical smooth seta and two apical setae (one smooth, other unipinnate), all of about same length. Endopod and exopod absent (fused to basis without trace).

Maxilla (figs. 1H, 6B) unornamented, composed of syncoxa, basis, and one-segmented endopod. Syncoxa with two endites; proximal one armed apically with single smooth seta; distal endite longer than proximal one, armed apically with two smooth and one strong unipinnate seta; all setae with pore on tip. Basis drawn out into strong claw, without seta at base, with tuft of basally fused spinules distally, forming small scoop, and single pore on ventral surface close to base of scoop (pore not visible with light microscope). Endopod represented by minute but distinct segment, armed with two smooth subequal apical setae, both with pore on tip.

Maxilliped (figs. 1J, 6B) with stout and short syncoxa, unarmed and ornamented with two spinules at base on outer margin; basis slender, about five times as long as wide and 1.7 times as long as syncoxa, unornamented and unarmed; endopod represented by short curved claw, swollen at base as indication of ancestral one-segmented endopod, ornamented with row of spinules along concave side distally, about 0.8 times as long as basis.

First swimming leg (fig. 2A) with smooth praecoxa, coxa, and intercoxal sclerite; intercoxal sclerite small, trapezoidal; praecoxa small, triangular; coxa large, quadriform. Basis much smaller than coxa, pentagonal, ornamented with few large spinules at base of outer spine and few large spinules along inner margin proximally. Exopod three-segmented, armed with one outer spine on first segment and four elements on third segment (two outer spines and two apical geniculate setae); ornamented with few large spinules along outer margin on all segments and with frills on inner distal corners of first and second segments. Endopod two-segmented, about as long as exopod; first segment reaching slightly beyond distal margin of second exopodal segment, about 4.6 times as long as wide, unarmed, ornamented with two short rows of large

spinules along outer margin and one long on inner margin; second segment armed apically with long geniculate seta and much shorter spine; endopodal geniculate seta 1.6 times as long as entire endopod and 1.4 times as long as larger geniculate exopodal seta; all armature elements on ultimate endopodal and exopodal segments strongly unipinnate along outer concave margin.

Second swimming leg (fig. 2B) with smooth praecoxa, coxa, and intercoxal sclerite. Praecoxa very small and triangular, but intercoxal sclerite much larger than in first leg, also with concave distal margin. Basis unarmed, ornamented with row of spinules on outer margin. Exopod three-segmented, ornamented with large spinules along outer margin, and with hyaline frills on each segment distally on inner side; first segment armed with single outer spine; second segment unarmed; third segment armed with three long elements (probably outer spine and two apical setae), innermost one about 1.5 times as long as exopod; all exopodal armature bipinnate. Endopod one-segmented, cylindrical and slender, about six times as long as wide, reaching middle of first exopodal segment, ornamented with two large spinules on outer margin, several spinules along apical margin; armed apically with one smooth minute seta, about 0.4 times as long as segment.

Third swimming leg (figs. 2C, 5C, 6A) with smooth praecoxa, coxa, and intercoxal sclerite. Praecoxa larger than in second leg. Basis robust, armed with long outer seta and ornamented with diagonal row of large spinules close to outer margin, large cuticular pore on anterior surface and close to outer basal seta, and with longitudinal row of stout spinules along inner margin, reaching base of endopod. Endopod minute but distinct segment, about as long as largest spinules on outer margin, unornamented and armed apically with smooth seta basally fused to segment. Exopod with both segments fused; ancestral proximal segment about 2.9 times as long as wide, with chitinous bulb on distal part of inner margin, ornamented with one longitudinal row of spinules along outer margin proximally and two distally, armed subapically with strong, smooth and curved spine, twice as long as apophysis and fused basally to another small spine or chitinous process; ancestral distal segment (apophysis) very short and inflated distally, oriented inwards, unornamented but armed with minute spine apically, which fused to segment; complex tridimensional structure of apophysis and large endopodal spine allow the latter to fit into lateral grooves of former.

Fourth swimming leg (figs. 2D, 5C, 6A) with smooth praecoxa, coxa, and intercoxal sclerite. Praecoxa relatively large, while coxa and intercoxal sclerite smaller than in second or third leg. Basis relatively large, armed with slender

and smooth outer seta, ornamented with four or five large spinules at base of endopod, and one longitudinal row of smaller spinules along outer margin. Exopod three-segmented, ornamented with few large spinules along outer margin on all segments, and with hyaline frills distally on inner side; first segment with concave inner margin, armed with single outer spine; second segment unarmed; third segment armed with outer spine and very long and strong apical seta; apical seta 1.2 times as long as entire exopod and 2.6 times as long as outer spine. Endopod one-segmented, about 0.6 times as long as first exopodal segment, claw-like, curved inwards, armed with single unipinnate apical element, fused to segment basally.

Fifth leg (figs. 2E, 5D, 6B) simple rounded cuticular plate, ornamented with single large cuticular pore basally, another small pore at outer base of minute apical spine, with row of seven or eight large spinules along inner margin distally, and with small spinule at base of outer seta posteriorly (hardly visible from anterior surface); armed with three smooth setae and one short spine along distal margin; outermost seta longest, 1.1 times as long as entire leg and about 4.3 times as long as middle seta; middle seta 1.7 times as long as innermost seta and 3.8 times as long as minute spine; two small setae and minute spine inserted on anterior surface and almost distally. Fifth legs distinct at base, with very small space between them, and pointing outwards. Outermost seta probably ancestral basal one; distal two setae and small spine probably ancestral endopodal armature; spinule at base of basal seta posteriorly possibly remnant of exopodal armature or ornamentation.

Sixth legs (figs. 1B, 6B) very disproportionate in size, right one almost completely reduced, left one enlarged, forming single, smooth, large operculum covering gonopore, which represents 40% of genital somite's width; no ornamentation or armature.

Female (based on allotype and several paratypes). Body length, excluding caudal setae, from 475 to 508 μm (498 μm in allotype). Habitus (fig. 3A), ornamentation of prosomites, colour, and nauplius eye similar to male, except genital and first abdominal somite fused into double somite and middle part slightly less inflated.

Genital double somite (fig. 3A, B) about as long as wide (note: urosome slightly compressed in fig. 3B), without any trace of subdivision except for two ancestral lateral sensilla at middle; additionally ornamented with six posterior sensilla (two dorsal, two ventral and two lateral). Genital complex (fig. 3A) occupying anterior ventral half of genital double somite; single genital aperture and copulatory pores closed off by fused vestigial sixth legs;

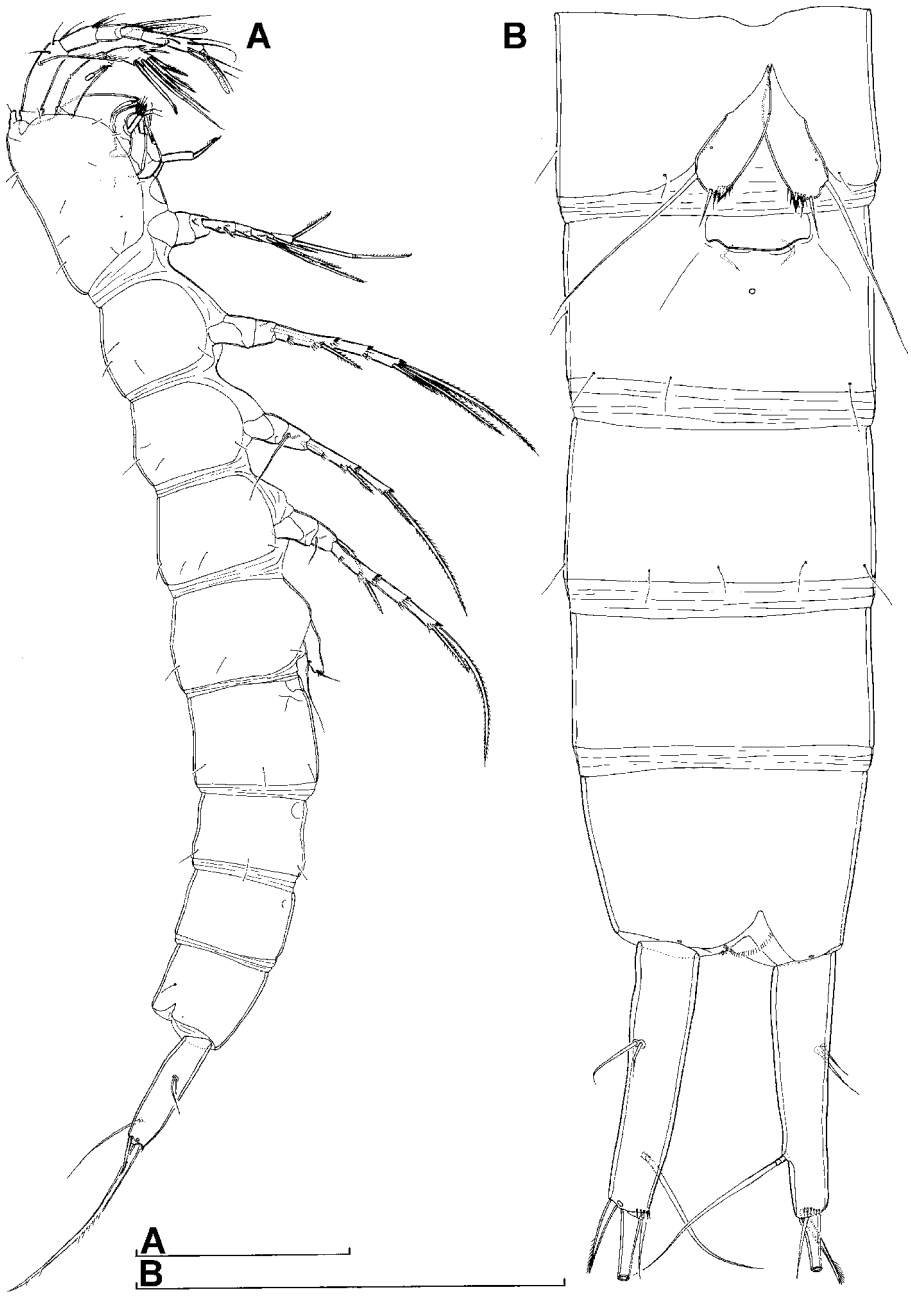

Fig. 3. *Dussartstenocaris idioxenos* n. sp., allotype female. A, habitus, lateral view, integumental window on cephalothorax not illustrated; B, urosome, ventral view. Scale bars = 100 μm.

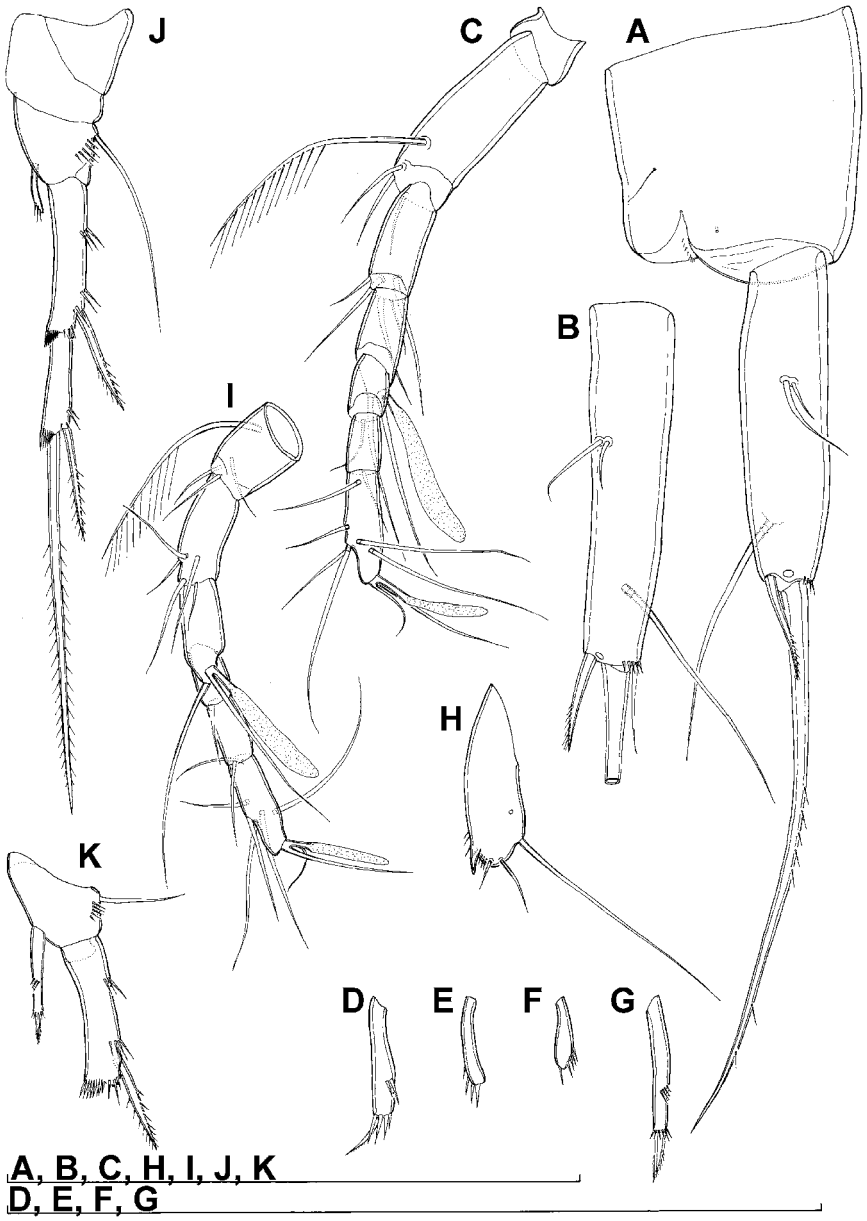

Fig. 4. *Dussartstenocaris idioxenos* n. sp. A–H, allotype female; I–K, paratype female I. A, anal somite and right caudal ramus, lateral view; B, right caudal ramus, ventral view; C, antennula, dorsal view; D, endopod of left second swimming leg, anterior view; E, endopod of right third swimming leg, posterior view; F, endopod of left third swimming leg, posterior view; G, endopod of right fourth swimming leg, anterior view; H, fifth leg, anterior view; I, antennula without first and part of second segment, ventral view; J, third swimming leg, anterior view; K, fourth swimming leg, anterior view. Scale bars = 100 μm.

seminal receptacles small, hard to distinguish from internal tissue and gut content; copulatory duct very short and weakly sclerotized. Third, fourth (preanal), and fifth (anal) urosomal somites similar to those of male (figs. 3B, 4A, 6F).

Caudal rami (figs. 3B, 4A, B, 6F) similar to those of male but slightly shorter in proportion to anal somite and slightly wider at middle from lateral view; ornamentation and armature same as in male.

Antennula (fig. 4C, I) seven-segmented, unornamented, approximately as long as cephalothorax, with broad aesthetasc on fourth segment, almost reaching tip of appendage and more slender apical aesthetasc on seventh segment, which is fused basally to two apical setae; both aesthetascs more slender than in male; setal formula: 0.4.5.2.1.1.8. Most proximal seta on second segment uniplumose, all other setae smooth and most with pore on tip. Largest seta on second segment and all free setae on seventh segment biarticulating basally. Length ratio of antennular segments, from proximal end, 1 : 3.3 : 1.8 : 1.7 : 1 : 1 : 2.1.

Antenna, mandibula, maxillula, maxilla, maxilliped, first swimming leg, second swimming leg (fig. 4D), and exopod of fourth swimming leg (fig. 4K) all similar to male. Note: endopod of second leg (fig. 4D) also ornamented with two large spinules at 2/3 on outer margin as in male.

Third swimming leg (fig. 4E, F, J) with large praecoxa, smooth coxa and intercoxal sclerite. Basis ornamented with row of large spinules near outer margin, armed with very long and smooth outer seta, which is about as long as first exopodal segment. Exopod two-segmented, ornamented with large spinules along outer margin, both segments with hyaline frills distally on inner side; first segment armed with single outer spine; second with outer spine and apical strong seta; all elements bipinnate. Endopod one-segmented, small, curved outwards, unarmed, and ornamented with three to five large spinules along inner margin distally; hardly reaching 1/4 of first exopodal segment in length.

Fourth swimming leg (fig. 4G, K) without spiniform processes on basis. Endopod one-segmented, ornamented with four spinules at 3/5 on inner margin, and transverse apical row of five or six spinules at base of apical spine, which is distinct at base, bipinnate, and about 0.35 times as long as endopod. Exopod similar to that of male.

Fifth leg (fig. 4H) very similar to that of male, but slightly more elongated, with narrower distal part, larger innermost spine, and more spinules along inner margin.

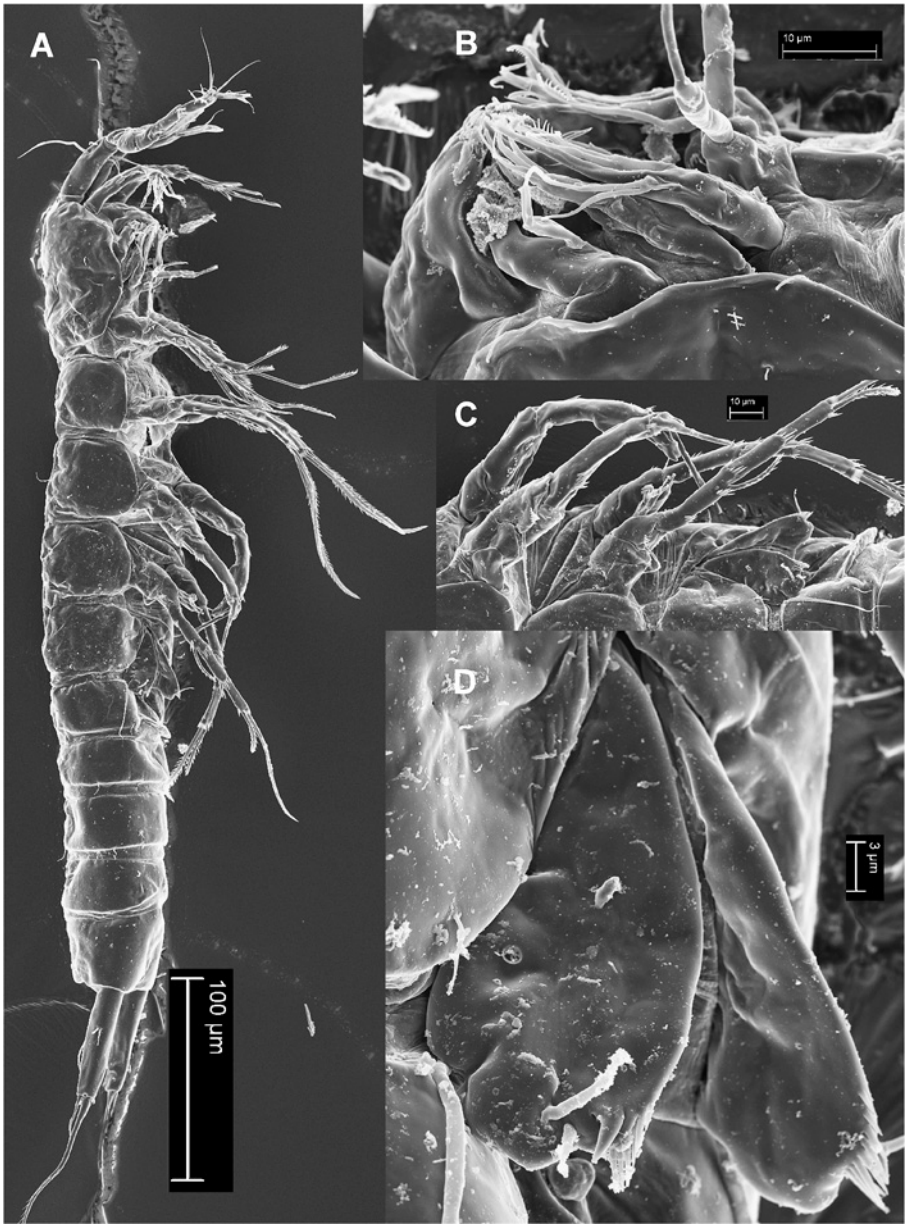

Fig. 5. *Dussartstenocaris idioxenos* n. sp., paratype male II, scanning electron micrographs. A, habitus, ventro-lateral view; B, oral appendages, ventro-lateral view; C, third to sixth legs, ventro-lateral view; D, fifth legs, ventro-lateral view. Scale bars: A = 100 μm; B, C = 10 μm; D = 3 μm.

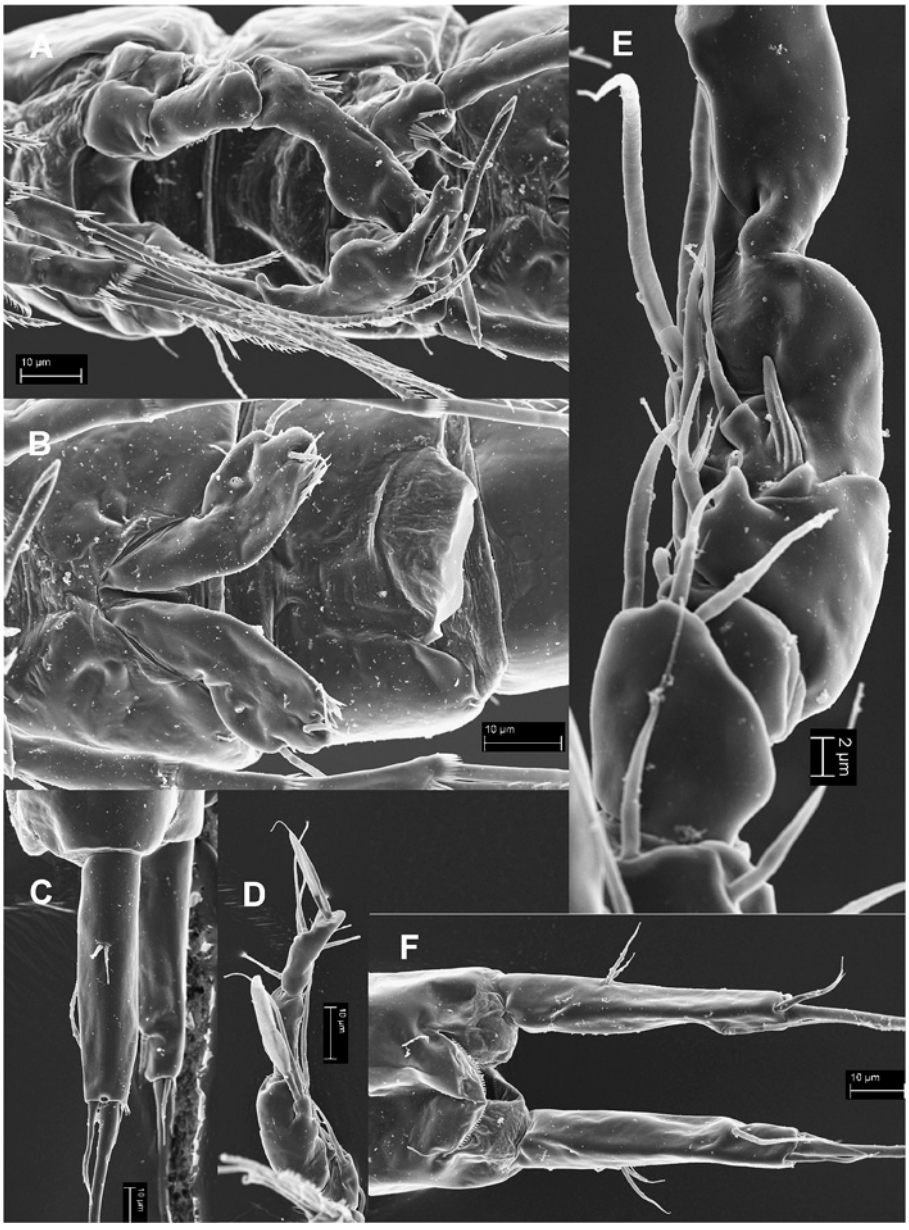

Fig. 6. *Dussartstenocaris idioxenos* n. sp., scanning electron micrographs. A, B, D, paratype male III; C, paratype male II; E, paratype male IV; F, paratype female II. A, third swimming legs and endopods of fourth swimming legs, anterior view; B, fifth and sixth legs, anterior (ventral) view; C, caudal rami, lateral view; D, distal part of antennula, ventral view; E, middle part of antennula, anterior view; F, anal somite and caudal rami, dorsal view. Scale bars: F = 2 μm; all others = 10 μm.

Sixth legs (fig. 3B) vestigial, fused into simple cuticular plate, covering gonopore, unornamented and unarmed.

Etymology. — The species name (idioxenos = close friend, Gr.) is a noun in apposition to the generic name, and refers to the fact that this new species lives sympatrically with another parastenocaridid, which is the first record of this kind for Australia.

Variability. — Body length of males ranges from 483 to 512 μm (501 μm average; n = 19), while that of females ranges from 475 to 508 μm (495 μm average; n = 10). The number of large spinules at the base of the fourth leg endopod in the male can be four or five (figs. 2D, 6A). The endopod of the third leg in the female varies in size and slightly in shape, too (fig. 4E, F, J). The dorsal cuticular windows on the cephalothorax can be hardly visible in some specimens, and especially under a light microscope (figs. 1A, 2F, 3A, 5A). The small spiniform processes at the base of the basal seta on the fifth leg (figs. 2E, 4H, 5, D, 6B) vary in size and are hardly visible from the anterior surface.

MOLECULAR RESULTS

DNA was extracted and the CO1 fragment successfully PCR-amplified from 14 copepod specimens (table II) using a nested combination of primers as given in table I. The edited CO1 sequences were imported into the program MEGA v. 4 (Kumar et al., 2008) and aligned using CLUSTAL W. All sequences were translated into protein using MEGA and were shown to have no evidence of stop codons indicative of non-functional copies of CO1. BLAST analyses of GenBank revealed that the sequences obtained are copepod in origin and not contaminants, and one of the GenBank CO1 sequences (#AF315010.1) from the species *Cletocamptus deitersi* (Richard, 1897) was included in our phylogenetic analyses.

Average pairwise distances between morpho-taxa were found to be very high, with the lowest divergence (13.3%) between *Kinnecaris* sp. 1 and *Kinnecaris* sp. 2 (table III). Such high divergence values are indicative of distinct species by comparison with other crustaceans (Lefébure et al., 2006). The highest divergences within morpho-taxa were those between two populations of *Elaphoidella humphreysi* Karanovic, 2006, being 4.3%. Those between two different populations of *Kinnecaris* sp. 1 and three populations of *Kinnecaris* sp. 2 were 0.2% and 0.3%, respectively. These are all indicative

TABLE III

Average pairwise NJ distances (P-distance model) among CO1 sequences between each morpho-species (lower diagonal) and within morpho-species (diagonal). Note: average pairwise distances using the Tamura–Nei model were from 15 to 32% higher between species, while they were almost the same within species. See text for generic names and authors of species names

Species	1	2	3	4	5	6	7
1. *A. hamondi*	0.000						
2. *C. deitersi*	0.248	–					
3. *D. idioxenos*	0.251	0.291	–				
4. *E. humphreysi*	0.199	0.285	0.255	0.043			
5. *Kinnecaris* sp. 1	0.242	0.276	0.221	0.283	0.002		
6. *Kinnecaris* sp. 2	0.219	0.272	0.226	0.290	0.133	0.003	
7. *P. jane*	0.244	0.273	0.220	0.292	0.199	0.196	–

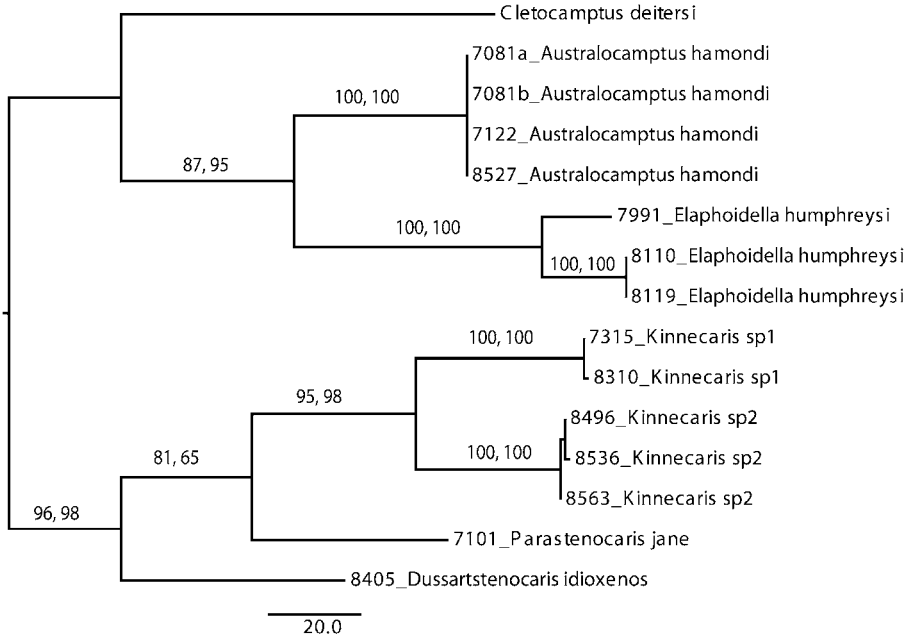

Fig. 7. A combined Neighbour Joining (NJ) and Maximum Parsimony (MP) cladogram based on mtCO1 DNA data from copepod specimens of the Yeelirrie calcrete and the Pilbara region. The values on the branches are MP bootstraps to the left and NJ bootstraps to the right, all from 1000 pseudoreplicates. The MP tree was a single one, with a length of 511 steps. Note only a remote relationship between the new genus and two other parastenocaridid representatives from Australia: *Kinnecaris* Jakobi, 1972 and *Parastenocaris* Kessler, 1913. The cladogram is drawn to scale and specimen numbers correspond to those in table II.

of interspecific variability. The pairwise distances between *Dussartstenocaris idioxenos* n. sp. and both *Parastenocaris jane* Karanovic, 2006 and two new *Kinnecaris* Jakobi, 1972 species are in excess of 22%, indicating only a remote relationship. In fact, they are even larger than those between the two Australian canthocamptid genera analysed here: *Elaphoidella* Chappuis, 1929 and *Australocamptus* Karanovic, 2004 (19.9%).

All analyses (fig. 7) supported the presence of at least seven genetically divergent lineages, each supported with very high bootstrap values. The tree topology did not differ depending on the methods used, which shows robustness of our data for this analysis. Only support values for Neighbour Joining (NJ) and Maximum Parsimony (MP) have been presented on fig. 7 and are discussed here. Two families formed two separate clades with a bootstrap support of 96% in MP analyses and 98% in NJ analyses. All lineages have a support of >81% in MP analyses, while all except one have a support of >95% in NJ analyses. The lineage with the weakest support (81% in MP and 65% in NJ analyses) is that suggesting a sister relationship of *D. idioxenos* and two other parastenocaridid genera. This lack of support is likely to be the result of the low phylogenetic resolution of the COI gene in basal nodes of the trees, possibly due to saturation at third codon positions.

DISCUSSION

This is the first ever attempt at a molecular analysis of parastenocaridid copepods, and our amplification success rates were very low with universal primers of Folmer et al. (1994). That is why we used additional 'nested' primers (table I), designed from preliminary copepod CO1 sequence data, in combination with universal primers to improve the PCR-amplification efficiency. Even then our success rates were about 50% on average. This may be due to the very small size of specimens and correspondingly low amount of DNA isolate, but it is probably also because we are yet to find an optimal procedure and combination of primers for this group.

Our choice of outgroup taxa was limited by the amount of sequences available on GenBank and the accessibility of freshly collected harpacticoid material, but we considered members of the family Canthocamptidae G. O. Sars, 1906 as suitable candidates. They are the dominant harpacticoid family of freshwater habitats (although there are some marine representatives), occurring in most aquatic environments and on all continents (Boxshall & Halsey,

2004). Just like parastenocaridids, some large canthocamptid genera are almost cosmopolitan in distribution, and they are successful invaders of subterranean waters (Galassi, 2001; Reid, 2001). The genus *Australocamptus* Karanovic, 2004 is an Australian endemic, while the genus *Elaphoidella* Chappuis, 1928 is the second largest copepod genus, recorded from all continents, and a very successful invader of subterranean habitats (Karanovic, 2006). Just like in Australia is the case with *Kinnecaris* Jakobi, 1972 and *Parastenocaris* Kessler, 1913: the former having been recorded from the Yilgarn region and further south in Western Australia (Karanovic, 2004), while the latter is known from the Pilbara region (Karanovic, 2006) and the Kimberleys (Karanovic, 2005a). Rather conveniently, *Kinnecaris* and *Australocamptus* were collected sympatrically from the Yeelirrie calcrete (as was the new genus described here), while *Parastenocaris jane* Karanovic, 2006 and *Elaphoidella humphreysi* Karanovic, 2006 occurred also sympatrically in an area near Newman in the Pilbara region.

The topology of the cladogram that resulted from our phylogenetic analysis (fig. 7) did not differ depending on the method used, which shows that our data are very robust (i.e., phylogenetically informative), despite a relatively short segment of the mtCO1 gene. Both families were recognized as separate clades, with very high bootstrap support values. Each genus was also recognized as a well defined clade. The pairwise distances between *Parastenocaris* and *Kinnecaris* (from 19.6 to 19.9%) are remarkably similar to those between *Elaphoidella* and *Australocamptus* (19.9%). *Dussartstenocaris* n. gen. is only remotely related to two other Australian parastenocaridid genera, with pairwise distances in excess of 22% (table III; fig. 7). In our view, this is a very strong molecular signal in support of establishing a new genus for this Australian parastenocaridid, in addition to many morphological characters.

The transformed male third legs, armature of other swimming legs, as well as armature and segmentation of the mouth appendages place *Dussartstenocaris idioxenos* n. sp. unquestionably in the family Parastenocarididae Chappuis, 1940 (see the introduction section for a set of characters that defines this family). It can, however, be distinguished from all other parastenocaridids by its characteristic fifth legs, which are very similar in males and females, divergent, rounded, with two endopodal setae and a small spiniform process moved to the anterior surface, and a row of spinules along the inner margin distally. Using the system of species groups of Lang (1948) *D. idioxenos* should be placed in the *minuta*-group, just like two described and five undescribed Australian *Kinnecaris*, based on the shape of basis and endopod of the fourth leg in

TABLE IV

Major morphological differences between three Australian parastenocaridid genera. Cuticular windows are those on abdominal somites. Note that several as yet undescribed species of both *Kinnecaris* Jakobi and *Parastenocaris* Kessler have been examined. Autapomorphic features of *Dussartstenocaris* n. gen. are given in bold.

Characters	Genera		
	Dussartstenocaris	*Kinnecaris*	*Parastenocaris*
Lateral cuticular windows	Absent	Present	Absent
Dorsal cuticular windows	Absent	Absent	Present
Lateral caudal setae	2	3	2
Position of lateral caudal setae	**Anterior**	Posterior	Posterior
Exopodal setae on leg 5	2	3	2
Inner bulb on male leg 3	**Present**	Absent	Absent
Exopodal spine on male leg 3	**Complex**	Simple	Simple
Basal processes on male leg 4	Absent	Absent	Present
Spinules on inner margin of leg 5	**Present**	Absent	Absent
Spiniform distal process of male leg 5	**Small**	Large	Absent
Spiniform distal process of female leg 5	**Small**	Large	Large
Endopod of female leg 3	**Linguiform**	Spiniform	Spiniform
Long setules on male endopod of leg 4	Absent	Absent	Present

the male (figs. 2D, 6A). In both, the endopods are simple cylindrical or conical structures, with several spinules at the base. This shows how simplistic groupings, based on a single character, can result in the establishment of paraphyletic taxa, and this was the reason why Lang (1948) did not recognize his groups as separate genera. In our view, this represents a plesiomorphic character state. However, a closer inspection of this structure in *Dussartstenocaris* and *Kinnecaris* reveals some significant differences, both in the shape of the endopod and the position of the basal spinules. The latter are, for example, much closer to the base of the endopod in *Dussartstenocaris* than in *Kinnecaris*. Many other morphological characters, though, distinguish these two genera, the major ones being listed in table IV, where we also outlined some of the most important autapomorphies of the new genus. Interestingly, the number of shared major morphological features between *Dussartstenocaris* and *Parastenocaris* (four) is even slightly greater than between *Dussartstenocaris* and *Kinnecaris* (three; see table IV), which is remarkably similar to the phylogenetic signal in the mtCO1 data. This is encouraging for future morphological and molecular revisions of this notoriously problematic family (see the introduction section).

Morphological comparison of our new genus with other known parastenocaridids from around the world reveals that it really does not have a close relative. Notoriously inaccurate and/or incomplete descriptions of many species,

as well as those based on only one sex, make the comparison of many morphological details very hard or impossible (Galassi & De Laurentiis, 2004; Schminke, 2010), which is also one of the main obstacles for any phylogenetic analysis of this family (Karanovic, 2006; Corgosinho et al., 2010).

Inflated fifth legs can be found in some South American genera, such as *Murunducaris* Reid, 1994 (see Corgosinho et al., 2008), *Forficatocaris* Jakobi, 1969 (see Noodt, 1963, 1972b; Reid, 1982), and *Potamocaris* Dussart, 1979 (see Dussart, 1979; Reid, 1991). In all three genera, however, there is a strong sexual dimorphism in the shape of the fifth legs, as well as in their ornamentation and even armature. Female fifth legs are much simpler, while male fifth legs are elaborate tri-dimensional structures, much more complex and looking very different from those in *Dussartstenocaris*. It would be possible to imagine evolution of these complex structures from a *Dussartstenocaris*-like ancestor, but we think that any similarity here is a result of convergent evolution. All three South American genera, for example, have three lateral setae on the caudal rami, which has to be a plesiomorphic character. Fifth legs somewhat similar to those in *Dussartstenocaris* were illustrated for the following three species: *Parastenocaris fontinalis meridionalis* Rouch, 1990 from France (Rouch, 1990), *Parastenocaris silvana* Cottarelli, Bruno & Berera, 2000 from Corsica (Cottarelli et al., 2000), and *Parastenocaris reidae* Cotarelli, Bruno & Berera, 2007 from Italy (Cottarelli et al., 2007). These similarities, however, are only superficial, as *P. silvana* and *P. reidae* both have four well developed setae on the fifth legs, while the inner distal spine is much more produced in *P. f. meridionalis* and the spinules on the inner margin are located in the proximal part. All three species also have three lateral setae on the caudal rami (inserted very close to the posterior margin in *P. silvana* and *P. reidae* as an additional difference), and a very different apical part of the third leg exopod in the male. We notice many morphological similarities between *D. idioxenos* and the Portuguese *Parastenocaris conimbrigensis* Noodt & Galhano, 1969, especially in the shape and armature of the caudal rami, third leg in the female, and to a lesser extent the fifth and fourth legs in both sexes (cf. Noodt & Galhano, 1969). The two differ markedly by the shape of the third leg exopod in the male, with a bifid exopodal spine being an autapomorphic feature of the new genus. At this stage, we refrain from including *P. conimbrigensis* into the genus *Dussartstenocaris*, although it is possible that the two have a closer phylogenetic relationship than to any other parastenocaridid, because without doing a phylogenetic analysis we cannot be sure that their morphological similarities are not in fact plesiomorphic character states in a larger

group of species. The Portuguese species differs from *D. idioxenos* in the actual shape of the fifth legs (inner distal spine/spinulose process being longer in the former, with inner margin being straight, and many more spinules along it) presence of dorsal integumental windows on abdominal somites, ornamented coxa of the male fourth leg, and endopodal spine on the fourth leg endopod in male clearly articulating at base. Curiously, both species have the inner margin of the fourth leg endopod in females and the outer margin of the second leg endopod in both sexes ornamented with spinules almost in the same spot, but these may be plesiomorphic character states. Most parastenocaridid species descriptions are not detailed enough to be able to check these characters reliably throughout the family. Both other Australian genera lack these spinules (Karanovic, 2004, 2005a, 2006), but these differences were not listed above (table IV) among the major distinguishing characters. Finally, we will mention here the African *Kinnecaris lyncaea* (Cottarelli & Bruno, 1994), which has a somewhat similar fourth leg basis and endopod in the male (Cottarelli & Bruno, 1994), just like the Australian species of *Kinnecaris*, but differs by the shape of the anal operculum, shape and armature of the caudal rami, fifth legs, and the third leg in the male. This shows quite clearly that shape and ornamentation of the fourth leg endopod and basis cannot be used alone to define monophyletic lineages in this family. As mentioned in the introduction section, Schminke (2010) failed to define the two proposed subfamilies by a clear set of morphological synapomorphies, and our new genus could not be easily classified using this subdivision. While the shape of the female third leg endopod and caudal rami, for example, would suggest its placement in the Fontinalicaridinae Schminke, 2010, the shape of the male fourth leg is more similar to that commonly observed in the Parastenocaridinae Chappuis, 1940. Clearly, these two subfamilies will have to be better defined, and tested with both morphological and molecular phylogenetic analyses, before they can be more widely accepted. In our view, the subdivision of this ancient family into only two clades is overly simplistic.

The new genus comes from a newly discovered locality (fig. 8) in the Yilgarn region (not surveyed in Karanovic, 2004), which revealed an unprecedented diversity of copepod crustaceans. Up to seven species per single bore in a single sampling event were quite normal (fig. 9), but this number was going as high as ten when counting all sampling occasions from a single locality. Yeelirrie area, which contains one of the largest calcretes in the uppermost reaches of its palaeochannel, is only about 75 km long and less than 10 km wide, but the surface area of suitable habitats (calcrete) is probably less than

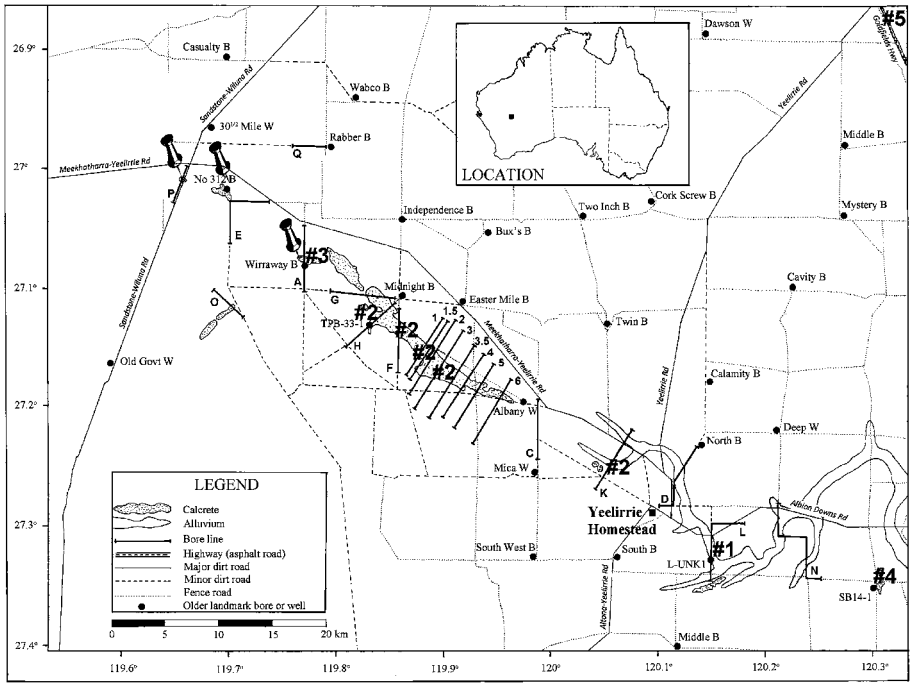

Fig. 8. Distributional ranges of *Dussartstenocaris idioxenos* n. sp. (pins) and five undescribed short range endemics from the genus *Kinnecaris* Jakobi, 1972 (#1-5) in the area investigated (#1-4 from the Yeelirrie palaeochannel; #5 from a parallel palaeochannel, in the vicinity of Lake Way). Map of the area investigated shows only some of the sampling localities (bores and wells); all others lie on the following 21 bore lines (from north-west to south-east): P, Q, O, E, A, G, H, F, 1, 1.5, 2, 3, 3.5, 4, 5, 6, C, K, D, L, and N. The water flow in the palaeochannel is also in this direction. Upper inset shows the location of the area in Australia. Scale bar: 20 km.

100 km^2 (see fig. 8). Using morphological methods we were able to distinguish 22 different species and subspecies, from six copepod families. Mitochondrial DNA sequence data from cytochrome C oxidase subunit one (CO1) confirmed all these and revealed three additional potentially cryptic species. This equals to 70% of the previously recorded diversity in the whole Yilgarn region, and this region was relatively well surveyed (Karanovic, 2004). The harpacticoid genus *Schizopera* G. O. Sars, 1905 is especially rich, encompassing nine different species in this calcrete (almost twice the number of species previously known from this whole region), and up to four species in a single sampling bore. This is usually associated with a remarkable size differentiation (see fig. 9), comparable only to that previously observed in some dytiscid beetles (Leys & Watts, 2008). However, both morphological and molecular data confirm that other major groups in this calcrete are much less diverse (C. Watts,

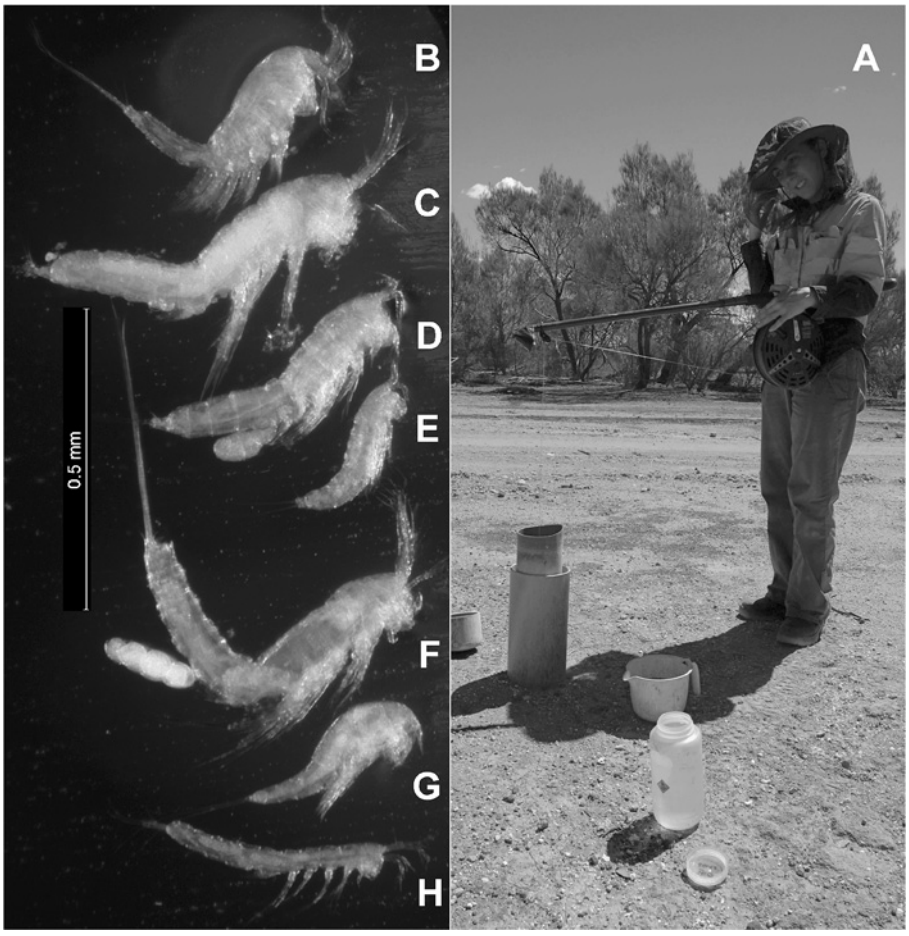

Fig. 9. Seven sympatric copepod species from bore YYD22 (bore line 1) in Yeelirrie, showing remarkable size differentiation. A, Ms. Giulia Perina sampling from the bore; B, *Halicyclops* sp., adult male; C, *Schizopera* sp. 1, adult female; D, *Schizopera* sp. 2, ovigerous female; E, *Schizopera* sp. 3, adult female; F, *Nitocra* sp., ovigerous female; G, *Pseudectinosoma* sp., adult female; H, *Kinnecaris* sp., adult female. Scale bar: 500 μm for all copepods.

S. Eberhard & T. Finston, pers. comm.; T. Karanovic, pers. observ.), containing the usual number of one to three species, which would suggest a different age and colonization history for different groups.

Arid Western Australia, and especially the Yilgarn region, is well known for its numerous isolated calcrete aquifers that lie along palaeodrainage channels, and range in diameter from tens of kilometres to hundreds of meters (Humphreys, 2008). Highly porous and carbonate rich sediments here represent an ideal habitat for various groups of stygofauna (aquatic subterranean

fauna), including dytiscid beetles, amphipods, isopods, bathynellids, ostracods, and copepods. Previous genetic and morphological studies suggested that individual calcretes are equivalent to closed island habitats, which have been isolated for millions of years (Cooper et al., 2008). The majority of the stygobitic species evolved within individual calcretes following independent colonization by epigean ancestors (Guzik et al., 2008). The diversity of the stygofauna is mostly dependent on the size of the calcrete, and typically includes one to three species from each major group, most of them endemic to that site. For example, in Karanovic (2004) five new *Schizopera* species were described, but each species was restricted to a single calcrete, or a group of neighbouring calcretes, and they were all allopatric. Although the sampling effort in Karanovic (2004) was very low in any given calcrete, some recent copepod identifications done for various environmental agencies in other Yilgarn calcretes were very thorough, and they did not indicate any exceptional diversity (mostly unpublished data). Reconstructed phylogenies from mtCO1 sequence data also revealed that both multiple colonizations and explosive radiations are responsible for the unprecedented copepod diversity in Yeelirrie. We think this is partly due to the geographical position of this calcrete, in the uppermost reaches of its palaeochannel, with more freshwater habitats than in lower parts, and more complex freshwater/saline-water interactions. Another important factor is the physical complexity of this calcrete, which through space and time probably acted more like a subterranean archipelago, rather than an island. Several more publications that deal with this interesting place and fauna are in preparation, but here we just want to mention one other preliminary discovery. Genetic divergence between different populations of some widely distributed *Schizopera* species is very interesting, as its most plesiomorphic population appears further down the palaeochannel (bore SB14MT), the next one is further upstream (line L), while the most terminal clade lives most upstream in the palaeochannel (largest patch of calcrete, i.e., lines 1, 2, 3, and 3.5; fig. 8). This probably reflects its colonization path, from a marine ancestor that was invading fresh waters and dispersing slowly and actively upstream. This pattern was observed in some other taxa of marine origin, like the genus *Nitocra* Boeck, 1865. Morphological and some molecular evidence would suggest a different colonization history in the genus *Kinnecaris*, where the most plesiomorphic form (sp. 3, fig. 8) lives in the uppermost reaches of the palaeochannel, and the trend in the caudal rami elongation and denser somite ornamentation is obvious down the channel. This makes perfect sense, as parastenocaridids are copepods of freshwater origin, and one can easily imagine dispersal of this

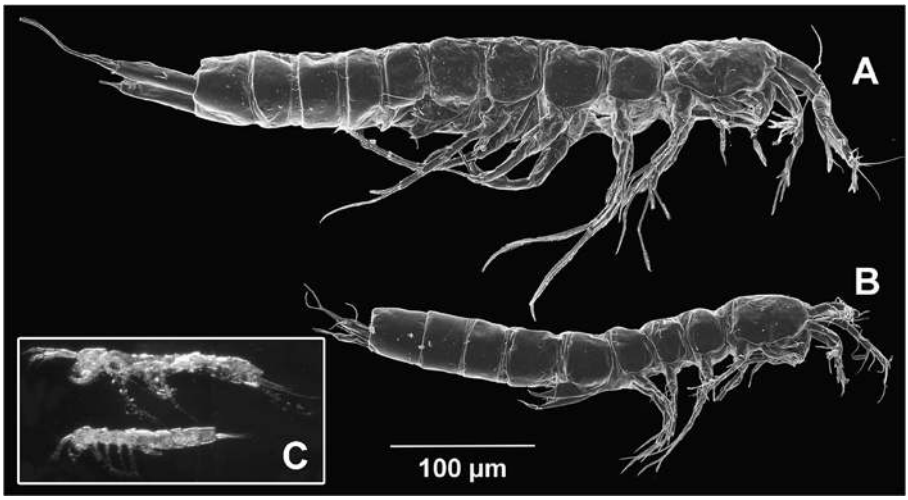

Fig. 10. Scanning electron micrographs of two sympatric parastenocaridids from Wirraway bore (line A), showing significant difference in size: A, *Dussartstenocaris idioxenos* n. sp., adult male; B, *Kinnecaris* sp., adult female; C, same two specimens under a dissecting microscope, showing highly transparent cuticula and most internal tissues. Scale bar: 100 μm for both.

group down the palaeochannel during periods of increased rainfall, which then evolve into separate species during periods of increased aridity. Two different colonization paths along this palaeochannel, one upstream for copepods of marine origin and the other downstream for freshwater representatives, may also be a mechanism that is partly responsible for this unusual species richness, as more species create more ecological niches and more opportunities for new colonizations.

Interestingly, the distributional ranges of *Dussartstenocaris* and *Kinnecaris* overlap only slightly (on line A), and there is a marked size difference between the two species (fig. 10), suggesting a potential niche-partitioning with a minimal competition for resources. The former lives in the upper reaches of the palaeochannel, in relatively fresh water, while the latter seems to have been adapted to waters of increased salinity, although were do not know the precise habitat of any taxon in a given bore, and it may be that *Kinnecaris* inhabits only the first few centimeters of the water column, where the water is relatively fresh. However, the present distributional data may be an indication that we may expect more species of the genus *Dussartstenocaris* to be discovered in the uppermost reaches of other palaeochannels in the Yilgarn region.

It is almost common knowledge that stygobitic animals exibit a reduction or loss of eyes and pigments, have enhanced non-optic sense organs, and species that are inhabiting interstitial spaces are most often vermiform (Culver et al.,

1995). It is also a widely accepted view that many convergent physiological adaptations occur, especially lower metabolic rates and loss of circadian periodicity and seasonal dynamics (Gibert et al., 1994; Langecker, 2000). They also lack resting stages, have fewer young, and are longer lived than their surface relatives (Coineau, 2000). Studies of subterranean animals in Europe have revealed, for example, that embryonic development in the single egg of a bathynellid can take up to nine months (Coineau, 2001). Studies on population dynamics and seasonal variability of stygobitic copepods in France and Slovenia (Lescher-Moutoué, 1973; Pipan & Brancelj, 2003, 2004) confirmed a generally accepted view that these ecosystems are indeed very stable, slow to recover, and intrinsically vulnerable to anthropogenic effects (Culver & Pipan, 2009). This notion was applied to Australian subterranean environments uncritically (Humphreys, 2001, 2008), although with some puzzling observations concerning the long persistence of stygofauna in subterranean habitats through geological eras and massive climatic changes. That is why we were so surprised to see pronounced differences in our stygofauna survey results in Yeelirrie in different months, despite very stable environmental conditions. Although most of the results are still awaiting publication, and in this paper we are only discussing one harpacticoid genus, it may be interesting to mention some observations.

One species of *Schizopera* was collected from the same bore (SB14-1) on three separate occasions, once in March 2009 and twice in March 2010, but was absent in January 2010, although we tried very hard to collect samples for DNA analysis. In fact, in January 2010 all harpacticoids were absent from this bore, and they included another new species of *Schizopera*, and as yet undescribed new species from each of the following three genera: *Kinnecaris*, *Nitocra*, and *Pseudectinosoma* Kunz, 1935. In January 2010, the only copepod in the bore SB14-1 was *Halicyclops eberhardi* De Laurentiis, Pesce & Humphreys, 2001. This bore was no exception, as many other localities produced very few or no harpacticoid specimens, despite an enormous sampling effort and the very small changes in the water level and salinity when compared to our field trip in November 2009. There were no significant rain events in Yeelirrie between January and March 2010, and the water level was even slightly lower and salinity generally slightly higher across the area. It is easy to imagine our surprise when we discovered an amazing diversity and density of copepods in the March 2010 sampling round. This would imply very strong seasonal dynamics in this subterranean community, which is a novel concept for these ecosystems. Interestingly, and this may be just a coincidence, at the

end of this sampling round there was a massive rain event in the area, which made some roads unusable and prevented us from taking the last few planned samples. It was like subterranean copepods (and other stygofauna) had an ability to predict the incoming rain and hatched to make the most of the incoming food input. Although just a speculation, this phenomenon is certainly worth further investigation. If proven reliable, it can be potentially used to forecast significant rain events in these arid regions, which are so irregular and hard to predict (Beard, 1976; Holmgren et al., 2006).

As the number of know parastenocaridids in Australia increases, we think a simple key to the species may be beneficial for further studies and specimen identification.

KEY TO AUSTRALIAN SPECIES OF PARASTENOCARIDIDAE

1. Male fifth leg with long inner distal corner; both sexes with lateral cuticular windows on urosomal somites 2
– Male fifth leg without any or with very small inner distal corner; cuticular windows on urosomal somites either absent or dorsal 3
2. Fifth leg with large cuticular window . . . . . *Kinnecaris eberhardi* (Karanovic, 2005)
– Fifth leg without any cuticular windows . . . . . *Kinnecaris solitaria* (Karanovic, 2004)
3. Basis of fourth leg in male with large chitinous processes; dorsal and lateral setae on caudal rami in both sexes inserted at about same level in posterior half 4
– Basis of fourth leg in male without chitinous processes; dorsal seta on caudal rami in both sexes inserted in posterior half, lateral setae in anterior half . . . . . *Dussartstenocaris idioxenos* n. gen. et sp.
4. Exopodal spine on male third leg about as long as apophysis; urosomal somites in both sexes with dorsal cuticular windows . . . . . *Parastenocaris jane* Karanovic, 2006
– Exopodal spine on male third leg about half as long as apophysis; urosomal somites in both sexes without cuticular windows . . . . . *Parastenocaris kimberleyensis* Karanovic, 2005

ACKNOWLEDGEMENTS

The authors are deeply indebted to Dr. Stefan Eberhard, Subterranean Ecology Pty Ltd., for entrusting the morphological identification of this material to the senior author and the molecular analysis to the junior author. Ms. Renate Walter, from the Zoologisches Museum Hamburg, is kindly acknowledged for her help in preparation of the SEM photographs. The following staff of Subterranean Ecology Pty are acknowledged for their help in the field, as well as for sorting the samples: Dr. Erich Volschek, Ms. Giulia Perina, Ms. Natalie Krawczyk, Mr. Peter Bell, and Mr. Shae Callan. Dr. Tessa Bradford and Ms. Kathleen Saint (South Australian Museum) did

all the sequencing, and Ms. Kathleen Saint is also greatly acknowledged for developing nested primers that were used in combination with universal primes for mtCO1 gene amplification. Financial support to the senior author came from Brain Pool and NIBR grants (both in Korea) and Subterranean Ecology Pty Ltd., while the necessary facilities were provided by the Zoologisches Museum in Hamburg and Hanyang University in Seoul. Financial support for mtCO1 sequencing was also provided by Subterranean Ecology.

REFERENCES

BEARD, J. S., 1976. Vegetation survey of Western Australia, Murchison: 1-141. (University of Western Australia Press, Perth).

BOXSHALL, G. A. & S. H. HALSEY, 2004. An introduction to copepod diversity, **1-2**: 1-966. (The Ray Society, London).

BOXSHALL, G. A. & D. JAUME, 2000. Making waves: the repeated colonization of freshwater by copepod crustaceans. Advances in Ecological Research, **31**: 61-79.

COINEAU, N., 2001. Syncarida. In: C. JUBERTHIE & V. DECU (eds.), Encyclopaedia Biospeologica, **3**: 863-876. (Societé de Biospéologie and: Académie Roumanie, Moulis and Bucharest).

COOPER, S. J. B., K. M. SAINT, S. TAITI, A. D. AUSTIN & W. F. HUMPHREYS, 2008. Subterranean archipelago: mitochondrial DNA phylogeography of stygobitic isopods (Oniscidea: *Haloniscus*) from the Yilgarn region of Western Australia. In: A. D. AUSTIN, S. J. B. COOPER & W. F. HUMPHREYS (eds.), Subterranean connections: biology and evolution in troglobiont and groundwater ecosystems. Invertebrate Systematics, **22**: 195-203.

CORGOSINHO, P. H. C. & P. MARTÍNEZ ARBIZU, 2005. Two new interstitial species of *Remaneicaris* Jakobi (Copepoda, Harpacticoida, Parastenocarididae) from the Ribeirão do Ouro River, Brazil, with a redefinition of the genus. Senckenbergiana Biologica, **85**: 147-162.

CORGOSINHO, P. H. C., P. MARTÍNEZ ARBIZU & E. N. DOS SANTOS-SILVA, 2007. Three new species of *Remaneicaris* Jakobi, 1972 (Copepoda, Harpacticoida, Parastenocarididae) from the Ribeirão do Ouro River, Minas Gerais, Brazil, with some remarks on the groundpattern of the Parastenocarididae. Zootaxa, **1437**: 1-28.

— —, — — & — —, 2010. Revision of *Brasilibathynellocaris* Jakobi, 1972 (Copepoda: Harpacticoida: Parastenocarididae) with redefinition of the genus. Zoological Journal of the Linnean Society, London, **159**: 527-566.

CORGOSINHO, P. H. C., P. MARTÍNEZ ARBIZU & J. W. REID, 2008. Revision of the genus *Murunducaris* (Copepoda: Harpacticoida: Parastenocarididae), with descriptions of two new species from South America. Journal of Crustacean Biology, **28**: 700-720.

COTTARELLI, V. & M. C. BRUNO, 1994. *Parastenocaris luncaea* n. sp. and *Stygoelaphoidella africana* n. sp. from interstitial continental waters of Sierra Leone. Ricerche Biologiche in Sierra Leone, parte IV. Accademia Nazionale dei Lincei, Rend., **267**: 97-109.

COTTARELLI, V., M. C. BRUNO & R. BERERA, 2000. *Parastenocaris corsica* sp. nov. and *Parastenocaris silvana* sp. nov., first Parastenocarididae from groundwater of Corsica (Copepoda, Harpacticoida). Crustaceana, **73**: 345-364.

— —, — — & — —, 2006. A new species of *Parastenocaris* from Mindoro Island, Philippines: *Parastenocaris distincta* sp. nov. (Crustacea: Copepoda: Harpacticoida: Parastenocarididae). Zootaxa, **1368**: 57-68.

— —, — — & — —, 2007. Interstitial harpacticoids from groundwater in Tuscany (central Italy): *Parastenocaris reidae* sp. nov., *Nitocrella ensifera* sp. nov., and notes on the morphology of *Parastenocaris* cf. *glacialis* Noodt (Crustacea: Copepoda). Italian Journal of Zoology, **74**: 83-99.

— —, — — & — —, 2008. Two new species of *Parastenocaris* (Copepoda, Harpacticoida, Parastenocarididae) from groundwater of Sardinia and Sicily. Crustaceana, **81**: 537-555.

— —, — — & — —, 2010. First record of Parastenocarididae from Thailand and description of a new genus (Copepoda: Harpacticoida). Journal of Crustacean Biology, **30**: 478-494.

CULVER, D. C., T. KANE & D. W. FONG, 1995. Adaptation and natural selection in caves: the evolution of *Gammarus minus*: 1-223. (Harvard University Press, Cambridge).

CULVER, D. & T. PIPAN, 2009. The biology of caves and other subterranean habitats: 1-256. (Oxford University Press, Oxford).

DUSSART, B. H., 1979. Algunos copepodos de America del Sur. Publicación Ocasional, Museo Nacional de Historia Natural, Santiago, **30**: 1-13.

DUSSART, B. & D. DEFAYE, 1990. Répertoire mondial des crustacés copépodes des eaux intérieures, III. Harpacticoïdes. Crustaceana, (Supplement) **16**: 1-384. (Brill, Leiden).

ENCKELL, P. H., 1970. Parastenocarididae (Copepoda Harpacticoida) from Ceylon. Arkiv för Zoologi, **22**: 545-556.

FELSENSTEIN, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783-791.

FOLMER, O., M. BLACK, W. HOEH, R. LUTZ & R. VRIJENOEK, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, **3**: 294-299.

FRAKES, L. A., 1999. Evolution of Australian environments. Flora of Australia (2^{nd} ed.), **1**: 163-203.

GALASSI, D. M. P., 2001. Groundwater copepods: diversity patterns over ecological and evolutionary scales. In: R. M. LOPES, J. W. REID & C. E. F. DA ROCHA (eds.), Developments in hydrobiology, **156**, Proceedings of the Seventh International Conference on Copepoda, held in Curitiba, Brazil, 25-31 July 1999. Hydrobiologia, **453/454**: 227-253.

GALASSI, D. M. P. & P. DE LAURENTIIS, 2004. Towards a revision of the genus *Parastenocaris* Kessler, 1913: establishment of *Simplicaris* gen. nov. from groundwaters in central Italy and review of the *P. brevipes*-group (Copepoda, Harpacticoida, Parastenocarididae). Zoological Journal of the Linnean Society, London, **140**: 417-436.

GIBERT, J., D. L. DANIELOPOL & J. A. STANFORD, 1994. Groundwater ecology: 1-571. (Academic Press, London).

GIRIBET, G. & G. D. EDGECOMBE, 2006. The importance of looking at small-scale patterns when inferring Gondwanan biogeography: a case study of the centipede *Paralamyctes* (Chilopoda, Lithobiomorpha, Henicopidae). Biological Journal of the Linnean Society, London, **89**: 65-78.

GLATZEL, T., 1996. Mating behaviour of the groundwater copepod *Parastenocaris phyllura* Kiefer, 1938 (Copepoda: Harpacticoida). Contributions to Zoology, Amsterdam, **66**: 103-108.

GUZIK, M., K. M. ABRAMS, S. J. B. COOPER, W. F. HUMPHREYS, J.-L. CHO & A. D. AUSTIN, 2008. Phylogeography of the ancient Parabathynellidae (Crustacea: Bathynellacea) from the Yilgarn region of Western Australia. In: A. D. AUSTIN, S. J. B. COOPER & W. F. HUMPHREYS (eds.), Subterranean connections: biology and evolution in troglobiont and groundwater ecosystems. Invertebrate Systematics, **22**: 205-216.

HASEGAWA, M., H. KISHINO & T. YANO, 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution, **22**: 160-174.

HOLMGREN, M., P. STAPP, C. R. DICKMAN, C. GRACIA, S. GRAHAM, J. R. GUTIÉRREZ, C. HICE, F. JAKSIC, D. A. KELT, M. LETNIC, M. LIMA, B. C. LÓPEZ, P. L. MESERVE, W. B. MILSTEAD, G. A. POLIS, M. A. PREVITALI, M. RICHTER, S. SABATE & F. A. SQUEO, 2006. Extreme climatic events shape arid and semiarid ecosystems. Frontiers in Ecology and the Environment, **4**: 87-95.

HUELSENBECK, J. P. & F. RONQUIST, 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics, **17**: 754-755.

HUMPHREYS, W. F., 2000. Background and glossary. In: H. WILKENS, D. C. CULVER & W. F. HUMPHREYS (eds.), Ecosystems of the world, **30**, Subterranean ecosystems: 3-14. (Elsevier, Amsterdam).

— —, 2006. Aquifers: the ultimate groundwater-dependent ecosystems. Australian Journal of Botany, **54**: 115-132.

— —, 2008. Rising from Down Under: developments in subterranean biodiversity in Australia from a groundwater perspective. In: A. D. AUSTIN, S. J. B. COOPER & W. F. HUMPHREYS (eds.), Subterranean connections: biology and evolution in troglobiont and groundwater ecosystems. Invertebrate Systematics, **22**: 85-101.

HUYS, R. & G. A. BOXSHALL, 1991. Copepod evolution: 1-468. (The Ray Society, London).

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE, 1999. International code of zoological nomenclature (4th ed.): i-xxix, 1-306. (International Trust for Zoological Nomenclature, London).

JAKOBI, H., 1969. *Forficatocaris noodti* n. gen., n. sp. (Copepoda, Harpacticoidae) aus brasilianischem Limnopsammal. Crustaceana, **17**: 231-238.

— —, 1972. Trends (Enp.P4 Mänchen) innerhalb der Parastenocarididen (Copepoda Harpacticoidea). Crustaceana, **22**: 127-146.

KARANOVIC, T., 2004. Subterranean Copepoda from arid Western Australia. Crustaceana Monographs, **3**: i-vi, 1-366. (Brill, Leiden).

— —, 2005a. Two new subterranean Parastenocarididae (Crustacea, Copepoda, Harpacticoida) from Western Australia. Records of the Western Australian Museum, **22**: 353-374.

— —, 2005b. Two new genera and three new species of subterranean cyclopoids (Crustacea, Copepoda) from New Zealand, with redescription of *Goniocyclops silvestris* Harding, 1958. Contributions to Zoology, Amsterdam, **74**: 223-254.

— —, 2006. Subterranean Copepods (Crustacea, Copepoda) from the Pilbara Region in Western Australia. Records of the Western Australian Museum, **70** (suppl.): 1-239.

— —, 2008. Marine interstitial Poecilostomatoida and Cyclopoida (Copepoda) of Australia. Crustaceana Monographs, **9**: i-vi, 1-331. (Brill, Leiden).

— —, 2010. First record of the harpacticoid genus *Nitocrellopsis* (Copepoda, Ameiridae) in Australia, with descriptions of three new species. International Journal of Limnology, **46**: 249-280.

KARANOVIC, T. & M. BOBIC, 1998. Two new species of Copepoda Harpacticoida from east Serbia (Balkan Peninsula): *Parastenocaris serbica* sp. n. and *Bryocamptus* (*R.*) *borus* sp. n. Crustaceana, **71**: 171-184.

KARANOVIC, T., S. M. EBERHARD & A. MURDOCH, 2011. A cladistic analysis and taxonomic revision of Australian *Metacyclops* and *Goniocyclops*, with description of four new species and three new genera (Copepoda, Cyclopoida). Crustaceana, **84** (1): 1-67.

KARANOVIC, T. & P. HANCOCK, 2009. On the diagnostic characters of the genus *Stygonitocrella* (Copepoda, Harpacticoida), with descriptions of seven new species from Australian subterranean waters. Zootaxa, **2324**: 1-85.

KARANOVIC, T. & Y. RANGA REDDY, 2004. First record of *Phyllognathopus bassoti* Rouch, 1972 from India, with remarks on the family Phyllognathopodidae Gurney, 1932 (Crustacea, Copepoda, Harpacticoida). International Journal of Limnology, **40**: 121-132.

— — & — —, 2005. First *Haplocyclops* Kiefer (Crustacea, Copepoda) from Indian subterranean waters: the most reduced free-living cyclopoid. International Journal of Limnology, **41**: 83-92.

KUMAR, S., J. DUDLEY, M. NEI & K. TAMURA, 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics, **9**: 299-306.

LANG, K., 1948. Monographie der Harpacticiden, **1-2**: 1-1682. (Nordiska Bokhandeln, Lund).

LANGECKER, T. G., 2000. The effect of continuous darkness on cave ecology and cavernicolous evolution. In: H. WILKENS, D. C. CULVER & W. F. HUMPHREYS (eds.), Ecosystems of the world, **30**, Subterranean ecosystems: 135-157. (Elsevier, Amsterdam).

LEFÉBURE, T., C. J. DOUADY, M. GOUY & J. GIBERT, 2006. Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimination. Molecular Phylogenetics and Evolution, **40**: 435-447.

LESCHER-MOUTOUÉ, F., 1973. Sur la biologie et l'écologie des copépodes cyclopides hypogés (Crustacés). Annales de Spéléologie, **28**: 429-674.

LEYS, R. & C. H. WATTS, 2008. Systematics and evolution of the Australian subterranean hydroporine diving beetles (Dytiscidae), with notes on *Carabhydrus*. In: A. D. AUSTIN, S. J. B. COOPER & W. F. HUMPHREYS (eds.), Subterranean connections: biology and evolution in troglobiont and groundwater ecosystems. Invertebrate Systematics, **22**: 217-225.

MARTÍNEZ ARBIZU, P. & G. MOURA, 1994. The phylogenetic position of the Cylindropsyllinae Sars (Copepoda, Harpacticoida) and the systematic status of the Leptopontiinae Lang. Zoologische Beiträge, **35**: 55-77.

NOODT, W., 1962. Limnisch-subterrane Copepoden der Gattung *Parastenocaris* Kessler aus Mittelamerika. In: E. TITSCHACK & H.-W. KOEPCKE (eds.), Beiträge zur neotropischen Fauna, **2**: 223-248. (VEB Gustav Fischer Verlag, Jena).

— —, 1963. Subterrane Crustaceen der zentralen Neotropis. Zoologischer Anzeiger, **171**: 114-147.

— —, 1972a. Drei neue *Parastenocaris* aus Kolumbien (Crustacea Copepoda), 1. Mitteilung über kolumbianische Grundwasser-Crustaceen. Studies on Neotropical Fauna, **7**: 101-112.

— —, 1972b. Brasilianische Grundwasser-Crustacea, 1. Studien an den Gattungen *Parastenocairs* Kessler and *Forficatocaris* Jakobi aus der Serra do Mar von São Paulo (Copepoda Harpacticoidaea). Crustaceana, **23**: 76-99.

NOODT, W. & M. H. GALHANO, 1969. Studien an Crustacea subterranea (Isopoda, Syncarida, Copepoda) aus dem Norden Portugals. Publicações do Instituto de Zoologia "Dr. Augusto Nobre" Faculdade de Ciências do Porto, **107**: 1-75.

PIPAN, T. & A. BRANCELJ, 2003. Fauna of epikarst-Copepoda (Crustacea) in percolation water of caves in Slovenia. Annales Series Historia Naturalis, Koper, **13**: 223-228.

— — & — —, 2004. Distribution patterns of copepods (Crustacea: Copepoda) in percolation water of the Postojnska Jama cave system (Slovenia). Zoological Studies, **43**: 206-210.

PLAYFORD, P., 2003. The Permo-Carboniferous glaciations of Gondwana: its impact on Western Australia. Western Wildlife, **7**: 1-5.

POR, F. D. & V. F. HADEL, 1986. Two new species of *Attheyella* (Copepoda: Harpacticoida: Canthocamptidae) from bromeliads of the Serra da Juréia (São Paulo, Brazil). Journal of Crustacean Biology, **6**: 777-788.

POSADA, D. & K. A. CRANDALL, 1998. Modeltest: testing the model of DNA substitution. Bioinformatics, **14**: 817-818.

RAMBAUT, A. & A. J. DRUMMOND, 2007. Tracer: MCMC Trace Analysis Package.

RANGA REDDY, Y., 2001. Discovery of Parastenocarididae (Copepoda, Harpacticoida) in India, with the description of three new species of *Parastenocaris* Kessler, 1913, from the River Krishna at Vijayawada. Crustaceana, **74**: 705-733.

RANGA REDDY, Y. & D. DEFAYE, 2007. Parastenocarididae (Crustacea, Copepoda, Harpacticoida) of India: description of *Parastenocaris mahanadi* n. sp., and redescription of *P. curvispinus* Enckell, 1970 from hyporheic habitats. Zootaxa, **1580**: 1-26.

— — & — —, 2009. Two new Parastenocarididae (Copepoda, Harpacticoida) from India: *Parastenocaris muvattupuzha* n. sp. from a river and *P. kotumsarenis* n. sp. from a cave. Zootaxa, **2077**: 31-55.

RODRÍGUEZ, F., J. F. OLIVER, A. MARÍN & J. R. MEDINA, 1990. The general stochastic model of nucleotide substitutions. Journal of Theoretical Biology, **142**: 485-501.

REID, J. W., 1982. *Forficatocaris schadeni*, a new copepod (Harpacticoida) from central Brazil, with keys to species of the genus. Journal of Crustacean Biology, **2**: 578-587.

— —, 1991. The neotropical genus *Potamocaris* Dussart (Copepoda: Harpacticoida: Parastenocarididae), with description of two new species. Journal of Crustacean Biology, **11**: 463-472.

— —, 1994. *Murunducaris juneae*, new genus, new species (Copepoda: Harpacticoida: Parastenocarididae) from wet campo in central Brazil. Journal of Crustacean Biology, **14**: 771-781.

— —, 1995. Redescription of *Parastenocaris brevipes* Kessler and description of a new species of *Parastenocaris* (Copepoda: Harpacticoida: Parastenocarididae) from the U.S.A. Canadian Journal of Zoology, **73**: 173-187.

— —, 2001. A human challenge: discovering and understanding continental copepod habitats. Hydrobiologia, **453/454**: 201-226.

RONQUIST, F. & J. P. HUELSENBECK, 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, **19**: 1572-1574.

ROUCH, R., 1990. Deux nouvelles *Parastenocaris* (Copépodes, Harpacticoïdes) des Pyrénées. Annales de Limnologie, **26**: 19-28.

SANMARTIN, I. & F. RONQUIST, 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. Systematic Biology, **53**: 216-243.

SCHMINKE, H. K., 1976. Systematische Untersuchungen an Grundwasserkrebsen — eine Bestandsaufnahme (mit der Beschreibung zweier neuer Gattungen der Familie Parabathynellidae, Bathynellacea). International Journal of Speleology, **8**: 195-216.

— —, 1981. Perspectives in the study of the zoogeography of interstitial Crustacea: Bathynellacea (Syncarida) and Parastenocarididae (Copepoda). International Journal of Speleology, **11**: 83-89.

— —, 2008. First record of groundwater fauna from Papua New Guinea: *Kinnecaris* Jakobi, 1971 redefined (Copepoda, Harpacticoida, Parastenocarididae), and description of a new species. Crustaceana, **81**: 1241-1253.

— —, 2009. *Monodicaris* gen. n. (Copepoda, Harpacticoida, Parastenocarididae) from West Africa. Crustaceana, **82**: 367-378.

— —, 2010. High-level phylogenetic relationships within Parastenocarididae (Copepoda, Harpacticoida). Crustaceana, **83**: 343-367.

STAMATAKIS, A., P. HOOVER & J. ROUGEMONT, 2008. A rapid bootstrap algorithm for the RAxML web-servers. Systematic Biology, **75**: 758-771.

STOCK, J. H. & J. C. VON VAUPEL KLEIN, 1996. Mounting media revisited: the suitability of Reyne's fluid for small crustaceans. Crustaceana, **69**: 749-798.

SWOFFORD, D. L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods), Version 4.0b10. (Sinauer Associates, Sunderland, Massachusetts).

WATERS, J. M. & D. CRAW, 2006. Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. Systematic Biology, **55**: 351-356.

WATERS, J. M. & M. S. ROY, 2004. Out of Africa: the slow train to Australasia. Systematic Biology, **53**: 18-24.

WELLS, J. B. J., 2007. An annotated checklist and keys to the species of Copepoda Harpacticoida. Zootaxa, **1568**: 1-872.

WESTON P. H. & M. D. CRISP, 1994. Cladistic biogeography of waraths (Proteaceae: Embothrieae) and their allies across the Pacific. Australian Systematic Botany, **7**: 225-249.

WHITE, M. E., 1994. After the greening: the browning of Australia: 1-288. (Kangaroo Press Pty Ltd., New South Wales, Australia).

YANG, Z., 1996. Among-site rate variation and its impact on phylogenetic analyses. Trends in Ecology and Evolution, **11**: 367-372.

First received 1 December 2010.
Final version accepted 3 January 2011.