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Molecular phylogenetic analyses reveal a new southern hemisphere oniscidean family (Crustacea : Isopoda) with a unique water transport system

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Abstract. A significant diversity of terrestrial oniscidean isopods was recently discovered in the subterranean 'calcrete islands' of Western Australia, but the species and higher-level systematic status of much of the fauna are currently uncertain. Here we focus on one group of species that was initially assigned to the genus *Trichorhina* (Platyarthridae), based on several shared characters, and investigate the phylogenetic relationships of these species to 21 oniscidean genera, including 13 known families, using *18S* rDNA sequence data. We then present phylogenetic analyses using *28S*-only and combined *18S*, *28S* rDNA and mitochondrial cytochrome *c* oxidase subunit I (*COI*) data for a more restricted sampling of taxa, and present results for a detailed morphological study of the antennae and other cephalic structures of exemplar taxa. Bayesian and maximum likelihood analyses of the extended *18S*-only, the *28S*-only and multi-gene datasets provide strong evidence for a distinct well-supported monophyletic group comprising the new Western Australian and one South American taxon. This clade is unrelated to all included members of Platyarthridae, which appears to be polyphyletic, and it forms a distinct group relative to other oniscidean families. Given these findings and the results of the morphological study, a new southern hemisphere oniscidean family, Paraplatyarthridae Javidkar & King, fam. nov. is erected based on *Paraplatyarthrus subterraneus* Javidkar & King, gen. & sp. nov. (type genus and species), and several undescribed taxa which occur in the arid (terrestrial and subterranean) regions of Western Australia and subtropical South America. Paraplatyarthridae is distinguishable from all other oniscidian families on a combination of character states including, among others, the presence of fan-like scale setae on the dorsal body, and the ventral second antenna with leaf-like scale setae and a furrow containing elongated hair-like capillary setae that form part of a water conducting system unique within Oniscidea. This study has important implications for the higher-level classification of oniscidean crustaceans and points to the need for a more detailed molecular phylogeny that includes a comprehensive sampling of southern hemisphere taxa.

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Introduction

Oniscidea (Crustacea : Malacostraca : Peracarida) is one of the largest isopod suborders, with over 3600 described species (*sensu* Schmalfuss 2003) classified into 33 families (Gruner *et al.* 1993). Species in this suborder are unique among crustaceans by being fully adapted to terrestrial life, with diagnostic features including the presence of complex water conducting systems (Verhoeff 1920; Hoese 1981, 1982; Wägele 1989). Although the monophyly of terrestrial isopods is supported by the results of morphological studies (Schmalfuss 1974, 1989), the relationships among oniscidean families and to their marine relatives are still poorly understood (Martin and Davis 2001). Crinocheta Legrand, 1946, comprising approximately 2750 species in 28 families, including Platyarthridae, is the largest and most diverse group (section) of oniscideans. They are considered to be monophyletic based on several synapomorphies associated with the musculature of the pleons and the structure of the male copulatory apparatus, marsupium, mouthparts and stomach (Schmidt 2008).

Platyarthridae, with over 120 nominal species, has been widely reported from tropical, subtropical and temperate regions, in substrates such as leaf litter, rotten wood and moist soil. It comprises seven genera: *Platyarthrus* Brandt, 1833 (Palaearctic except for one species from India); *Trichorhina* Budde-Lund, 1908 (Central and South America, Africa, Europe, Burma to Australia); *Lanceochaetus* Schmalfuss & Ferrara, 1978 (Africa); *Niambia*

Budde-Lund, 1904 (mostly Africa); *Gerufa* Budde-Lund, 1909 (Africa); *Echinochaetus* Ferrara & Schmalfuss, 1983 (Africa); and *Cephaloniscus* Ferrara & Taiti, 1989 (Malaysia). The family is generally recognised by having a two-jointed flagellum, the presence of modified scale setae on the dorsal segments, absence of pleopodal lungs, and an inability to roll up. However, the monophyly of the family is considered doubtful as at least some of these characters are thought to have evolved independently in other oniscidean groups (Ferrara and Taiti 1989; Mattern 2003; Schmidt 2003, 2008).

Despite several detailed morphological and preliminary molecular studies (reviewed by Schmidt 2008), the family continues to be poorly defined. Collinge (1943) suggested a distinct family, Trichorhinidae, for the genera *Trichorhina*, *Gedania* Budde-Lund, 1912 (now a synonym of *Trichorhina*), *Bathytropa* Budde-Lund, 1885 and *Calycuoniscus* Collinge, 1915, but this was not widely accepted. Vandel (1946) included *Trichorhina*, *Calycuoniscus*, *Labyrinthasius* Verhoeff, 1929 and *Platyarthrus* with two- or three-jointed second antennal flagellum in Squamiferidae Vandel, 1946 (=Platyarthridae), while later authors considered taxa with a two-jointed second antenna (*Trichorhina*, *Platyarthrus*) to comprise a distinct family, Platyarthridae. Taiti *et al.* (1992) proposed Bathytropidae Vandel, 1952 was a possible synonym of Platyarthridae due to a lack of explicit morphological differences, while Schmidt (2008) considered Bathytropidae to be an artificial assemblage of taxa. Schmidt (2003) redefined the Squamiferae to comprise Platyarthridae, Dubioniscidae Schultz, 1995 and Spelaeoniscidae Vandel, 1948 based on several synapomorphies including shell-shaped tergal scale setae with narrow bases, teeth on the outer endite of the first maxilla broader than half the apical margin, and fusion of the distal two articles of the maxilliped palps. Schmidt (2003, 2008) also proposed that Platyarthridae and Dubioniscidae were likely to be paraphyletic or polyphyletic, while Spelaeoniscidae constituted a monophyletic group with several autapomorphies.

Schmidt (2002) suggested a re-examination of *Trichorhina* species owing to incongruence in the position of noduli laterales, which are located close to the posterior margin of the tergites in *Trichorhina tomentosa* Budde-Lund, 1893 (type species of the genus), whereas they are distant from the posterior margin in other *Trichorhina* species. Schmidt (2002) also noted that as gland pores have been reported on the coxal plates of *Niambia* species, their classification within Platyarthridae also required reassessment. The only molecular studies so far undertaken (Mattern and Schlegel 2001; Mattern 2003) employed a single gene (*18S*) for a restricted group of taxa and lacked numerous critical species but, nonetheless, pointed to Platyarthridae being polyphyletic.

The retention of water to moisten the pleopods, which are the gas-exchange organs, and preventing water loss through physiological and morphological means, are critical adaptations in oniscideans to terrestrial life. In Platyarthridae, coxal plates and pleonal epimera (3–5) are enlarged, making individuals capable of sticking their bodies firmly to the substratum (typically referred to as the 'clinger' type; Schmidt 2002), except for some *Platyarthrus* species that are 'creepers' (S. Taiti, pers. comm.). This modification is thought to effectively decrease water loss through evaporation by enclosure of air between the ventral side of the body and the substrate. Another prominent morphological feature, well-developed in Oniscidea, is the water conducting system, which is considered a synapomorphy for the group (Verhoeff 1920; Hoese 1981, 1982; Wägele 1989). This system is composed of a series of interconnected channels of scale rows on the ventral side of the coxal plates, linking the bilateral maxilliped glands in the cephalothorax to an area on the first pair of pleopods, through which fluids (water and urine) are conducted via capillary action (Carefoot 1993). Hoese (1981, 1982) distinguished two types of water conducting systems within the Oniscidea: open and closed. In the open system (*Ligia*-type), which occurs in Ligiidae Leach, 1814, Tylidae Dana, 1852, section Synocheta Legrand, 1946 and Mesoniscidae Verhoeff, 1908, there are scale rows on pereopods 6 and 7 where water moves by capillary action to join the ventral scale row channels. In the closed system (*Porcellio*-type), present in most Crinocheta, water conduction involves the ventral channels as well as dorsal exoskeletal articulations that circulate fluid (urine) at each articulation from one ventral channel to the other. Despite numerous studies on the morphology and taxonomy of terrestrial isopods, the evolution of the water conducting system is poorly known.

In terrestrial isopods, respiration takes place primarily within the pleopods which comprise five pairs of biramous appendages (Schmidt and Wägele 2001). In putatively primitive forms of Oniscidea, respiration is performed via the pleopodal endopods which function similarly to gills (Verhoeff 1917, 1920; Hoese 1983; Kummel 1984). In Oniscidea adapted to terrestrial life, specialised structures, referred to as lungs or pseudotracheae, have evolved on the pleopod exopods. Major types of lungs include: (a) uncovered lungs, considered as the simplest type, which comprise a wrinkled respiratory area on the dorsal surface of the exopods; (b) partially covered lungs, where the rest of the pulmonary area develops into the walls of the exopods; and (c) covered lungs, which are internal tubuliform cuticular respiratory areas deeply wedged into the exopod walls and connected to the exterior via spiracles (Paoli *et al.* 2002). In species of Ligiidae, Platyarthridae and several other families, lungs are absent, and in Platyarthridae this is possibly a secondary loss (Mattern and Schlegel 2001; Schmidt and Wägele 2001). *Niambia* is the only representative of Platyarthridae containing a distinct respiratory area comprising uncovered lungs (e.g. Schmalfuss and Ferrara 1978; Ferrara and Taiti 1981; Taiti and Ferrara 1991, 2004). Recently, it has been suggested that lungs have evolved multiple times independently in oniscideans (Ferrara *et al.* 1994; Taiti *et al.* 1998) so their presence or absence, although likely to be phylogenetically informative at some level, may not indicate strict monophyly (Mattern and Schlegel 2001; Schmidt and Wägele 2001).

In recent years, detailed surveys have identified a diverse assemblage of terrestrial oniscideans inhabiting subterranean voids above the water table in groundwater calcrete aquifers of central Western Australia. These calcretes act as isolated 'subterranean islands' for a variety of invertebrate species, including dytiscid water-beetles, amphipods, isopods and bathynellids (Cooper *et al.* 2002; Leys *et al.* 2003; Cooper *et al.* 2007, 2008; Guzik *et al.* 2008, 2009; Humphreys *et al.* 2009; King *et al.* 2012; Abrams *et al.* 2013). Numerous oniscideans recently collected from these calcretes were provisionally ascribed to

Trichorhina (Platyarthridae), but their identity has remained problematic. This study examines the systematic relationships of these Western Australian taxa, along with an apparently related undescribed South American species, using first a single (*18S*) gene phylogenetic analysis employing all available published oniscidean sequence data, followed by single *28S* and multigene analyses and morphological comparison that included detailed examination of their water conducting system on the ventral second antenna. The hypothesis that these southern hemisphere taxa can be phylogenetically assigned to Platyarthidae was tested with a broad sampling of representative taxa. Owing to the robust position of these Australian and South American species in the phylogeny, their significant divergence from all platyarthrid genera, and the unique morphology of their water conducting system (among other characters), a new family based on a new genus and species is described.

NB. Authors for all species and some higher group names referred to in the text are included in Appendix 1 along with abbreviations for genera; family names in inverted commas indicate they are likely to be non-monophyletic, as postulated in the references above.

Materials and methods

Collecting methods

Oniscidean species used in this study were collected using several techniques. Subterranean species from above groundwater calcretes in central Western Australia (WA) (Fig. 1*A*) were collected using slotted PVC pipes filled with sterilised leaf litter, which were left in non-lined mineral exploration boreholes (1–3 m underground) (Fig. 1*B*) for 6 to 12 months for colonisation by troglobionts. After recovery of the traps, their contents were sealed in zip lock bags and transported to the Western Australian Museum (WAM) for processing. Isopod samples were extracted from the leaf litter using Tullgren funnels and specimens were preserved in 100% ethanol. After transporting the preserved samples to the Adelaide laboratory, the specimens were kept in a –20° freezer.

Surface terrestrial isopod species were collected by hand from different habitats including arid and temperate regions of Western Australia. The sampling was carried out by searching under stones, rotten wood beside trees and shrubs and, specifically, in crevices of broken tree trunks, where they were found frequently. Specimens of *Ligia* sp. were also caught by hand from a rocky shore in Rapid Bay, South Australia (SA) while *Deto marina* was sampled from a sandy beach at Hallet Cove, SA. All specimens were preserved in 100% ethanol and kept in a –20° freezer prior to DNA extraction. Collection data, vouchers and accession numbers of species used both for molecular and morphological work are listed in Appendix 1. All voucher specimens are lodged either at the WAM or the South Australian Museum (SAM) (see Appendix 1). Some species used only for morphological studies (old material, DNA degraded) were kindly donated by colleagues (see 'Acknowledgements').

DNA extraction, sequencing and alignment

Three to six pereopods (except for male pereopod 7 which is important for morphological diagnosis) were dissected from animals preserved in 100% ethanol and rinsed in 10 mM Tris to remove alcohol prior to the extraction process. Total genomic DNA was isolated using a Gentra Puregene Tissue Kit (Qiagen, www.qiagen.com) according to the manufacturer's instructions (DNA purification from 5–10 mg fresh or frozen solid tissue) with the following minor modifications. For DNA precipitation, samples were centrifuged at 14 674*g* for 20 min and 5 min (the step containing 70% ethanol), respectively.

To obtain partial sequences from the mitochondrial cytochrome *c* oxidase subunit 1 (*COI*) gene, a ~677 bp portion was amplified using the universal primers LCO1490_t1 and HCO2198_t1 (designed by Robin M. Floyd at BOLD: The Barcode of Life Data system; see Table 1). Polymerase chain reaction (PCR) amplification of all *COI* sequences involved an initial denaturation at 95°C for 10 min and 34 subsequent cycles of 94°C for 45 sec, 48°C for 45 sec, 72°C for 1 min and a single final extension of 72°C for 10 min, followed by a 2-min hold time at 25°C. For the sequencing reactions, M13F and M13R primers were used (Messing 1983).

The nuclear *18S* rDNA gene is among the most frequently used markers for the reconstruction of deep phylogenetic relationships among arthropods and has been used previously for Oniscidea (Mattern 2003). Approximately 776 bp, including core and variable regions C1, V1, C2, V2 and C3 of the gene, were amplified using 18s1.2F and 18sb5.0 primers (Whiting 2002; Table 1). Polymerase chain reaction cycling conditions consisted of a 10-min initial denaturation at 95°C, 34 cycles for 45 sec at 94°C, 45 sec at 50°C, 1 min at 72°C and a single final elongation cycle of 6 min at 72°C.

The D1 to D3 region of the nuclear *28S* rDNA gene was amplified using universal primers 28srD1.2a and 28srd4.2b (Whiting 2002; Table 1). For those samples that failed to PCR-amplify, new internal primers G2281 and G2282 (Table 1) were designed for PCR and sequencing amplifications. Cycling conditions for the *28S* sequences consisted of one single cycle at 95°C for 10 min, 34 cycles at 94°C for 45 sec, 50°C (for universal primers) to 55°C (for internal primers) for 45 sec, 72°C for 1 min and a single step 6-min final extension of 72°C followed by an incubation stage at 25°C for 2 min.

All PCRs were carried out on either a Palm-Cycler thermal cycler (Corbett, CG1-96; Sydney, Australia) or Kyratec Supercycler thermal cycler (SC300; Brisbane, Queensland, Australia) using 25 µL reaction volumes consisting of 15.4–15.9 µL of nuclease-free molecular water, 5 µL of 5× Immolase PCR buffer (Bioline; Alexandria, NSW, Australia), 1 µL of each primer (5 µM for *COI* and *18S* primers, 10 µM for G2281 and G2282, 7 µM for 28srD1.2a and 5 µM for 28srd4.2b), 0.1 µL of Immolase DNA polymerase (5 U μL^{-1}) and 2–2.5 µL of ~1 µg mL^{-1} DNA. Polymerase chain reaction and sequencing products were cleaned up using multiscreen$_{384}$ filter plates (Millipore Corporation; Darmstadt, Germany) and a vacuum manifold using the manufacturer's protocol. Purified PCR products were sequenced in both directions using an ABI Prism Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems; Foster City, CA, USA) and analysed on an ABI 3700 DNA capillary sequencer. Sequences were edited in Geneious Pro version 5.6.4 (Biomatters Ltd, http://www.geneious.com/).

As the *18S* gene consists of regions with different rates of sequence divergence including slow-evolving (conserved regions) and fast-evolving (variable) regions, a four-step process was

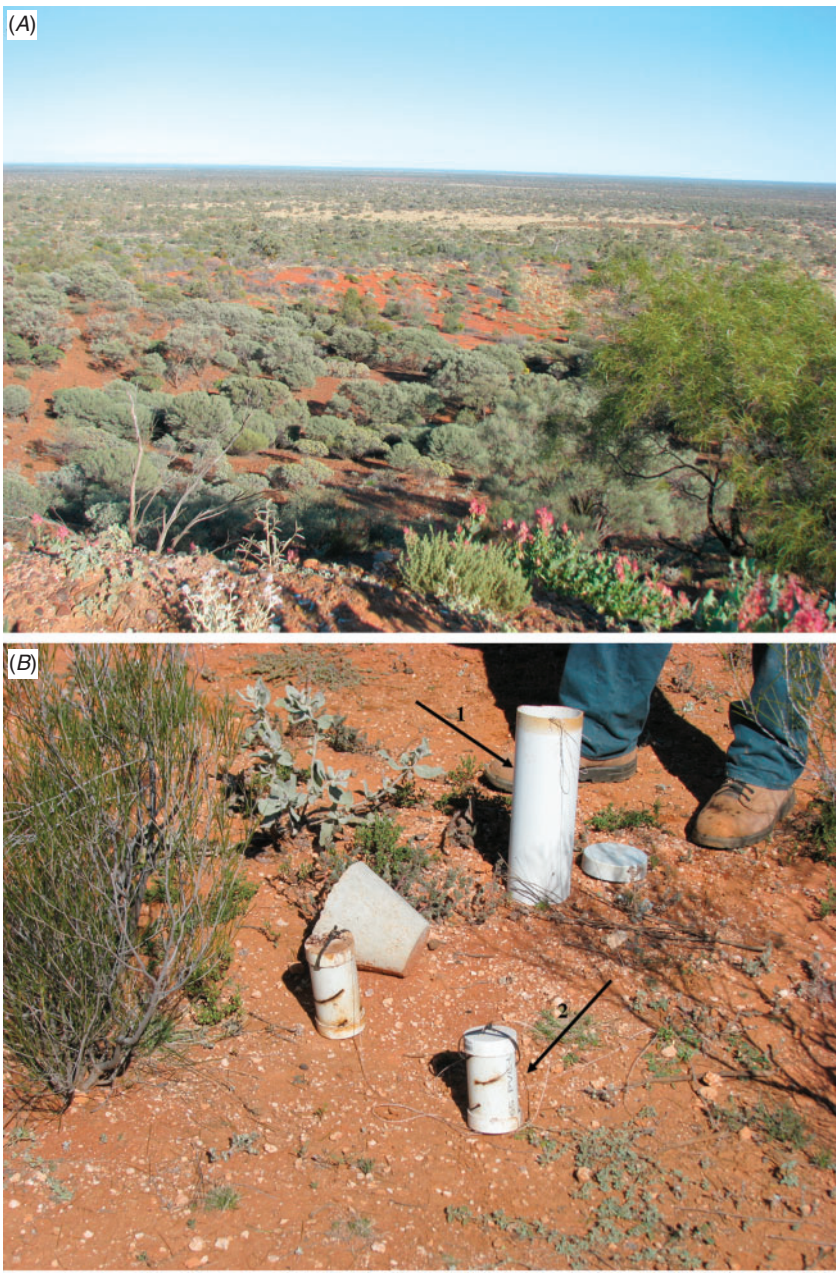

Fig. 1. (*A*) The landscape at Laverton Downs, Mt Windarra, Western Australia where *Paraplatyarthrus subterraneus* Javidkar & King, sp. nov. was collected; (*B*) troglofauna sampling method used in the study showing a PVC pipe used to stabilise the entrance of a bore hole, and a slotted trap filled with leaf litter that is lowered down a bore hole.

followed for alignment of the sequence data. The whole dataset was first aligned using ClustalW (gap opening cost of 9, gap extension cost of 3, an IUB cost matrix and free end gaps) within Geneious Pro 5.6.4, which led to the alignment of the conserved regions. Next the preliminary alignment was aligned to an annotated oniscidean *18S* alignment to identify the core (conserved) and variable regions (Mattern and Schlegel 2001). After identifying the conserved (for ease of use, C1, C2 and C3 nomenclature was used for conserved regions in the alignment) and variable (V1 and V2) regions, an additional regional alignment was applied for the variable regions using the Muscle alignment plug-in for Geneious Pro 5.6.4 (according to the default settings). In the last step the alignment was checked by eye and refined manually. In the *18S* alignment, considering that our dataset started at nucleotide position 52, there is a 19–21 bp gap (Fig. S1) in all sequences of Crinocheta and Synocheta in the C1 region relative to species of Diplocheta.

No gene positions or nucleotide regions were removed from the alignment. *COI* and *28S* alignments were generated

Table 1. Primers used for amplification of *COI*, *18S* and *28S* in the sampled oniscidean taxa
F, forwards; R, reverse

Primer	Direction	Gene	Sequence (5 –3)
LCO1490_t1	F	*COI*	TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG
HCO2198_t1	R	*COI*	CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA
M13F	F	*COI*	TGTAAAACGACGGCCAGT
M13R	R	*COI*	CAGGAAACAGCTATGAC
18s1.2F	F	*18S*	TGCTTGTCTCAAAGATTAAGC
18sb5.0	R	*18S*	TAACCGCAACAACTTTAAT
28srD1.2a	F	*28S*	CCCSSGTAATTTAAGCATATTA
28srd4.2b	R	*28S*	CCTTGGTCCGTGTTTCAAGACGG
G2281	F	*28S*	GSGATGCCGCGTWTGGGAGN
G2282	R	*28S*	TTCACCGTCBVAGAGGCCGT

in ClustalW with the same opening and extending gap penalties as for *18S*. The domain regions in the *28S* isopod alignment were identified manually using the annotated *28S* sequence for *Drosopohila melanogaster* (Tautz *et al.* 1988).

Taxon sampling and phylogenetic analyses

For the *18S*-only analyses, 47 ingroup taxa were included comprising 21 oniscidean genera belonging to 13 known families (Appendix 1). Exemplar species included nine subterranean and surface species from WA superficially similar to *Trichorhina tomentosa* and problematically ascribed to 'Platyarthridae', namely, taxa 1–9. In addition, the type species of their respective genera, *T. tomentosa* (from Brazil) and *Platyarthrus hoffmannsegii* (from Italy) were included, along with an undescribed taxon intially ascribed to *Trichorhina* (from Brazil; taxon 10), and two species of *Niambia* from Botswana (Africa) and Australia. As discussed above, although the monophyly of 'Platyarthridae' has been seriously questioned (Schmidt 2003, 2008), it has also been treated as synonymous with Trichorhinidae, hence the importance of including the type species of both the nominal genera (N.B. Schmidt (2003) largely based his description of Platyarthridae on *T. tomentosa*). Also, a number of additional oniscidean sequences were included in the *18S* analyses to expand the family and genus representation, and were either sequenced as part of this study or were obtained from the NCBI GenBank database. These included representatives of Armadillidae, Armadillidiidae, Cylisticidae, Detonidae, Ligiidae, Oniscidae, 'Philosciidae' (including *Philoscia muscorum*, *Haloniscus* spp. and an unknown genus and species from Australia, taxon 11), Porcellionidae, Stenoniscidae, Styloniscidae, 'Trachelipodidae' and Trichoniscidae (see Appendix 1). Four crustacean species available on GenBank for *18S*, *Gammaracanthus lacustris*, *Orchestia* sp. (Amphipoda), *Heterocarpus* sp. (Decapoda) and *Brevisomabathynella magna* (Syncarida) were selected as outgroups.

The phylogenetic analyses of the *COI* data comprised 32 species representing nine known families, the *28S*-only data included 19 species representing nine families, while the combined data (*COI*, *18S* and *28S*) included 19 species representing eight known families (Appendix 1). We were unable to obtain samples from several families, but the representation of taxa was sufficient for assessment of inter-relationships of the Australian taxa to key oniscidean families. No likely outgroups were available on GenBank for all three genes and so *Sphaeroma serratum* (Isopoda) was selected and sequenced for *COI*, *18S* and *28S* as part of this study. This taxon is a representative of the suborder Flabellifera and hence an appropriate outgroup for all members of the suborder Oniscidea. All Genbank accession numbers are included in Appendix 1. Additional platyarthrid species, *Platyarthrus aiasensis* (Italy), *P. costulatus* (Italy) and *Trichorhina anophthalma* (Portugal), could not be included as their DNA was too degraded, but they were used for morphological examination.

In order to estimate the best nucleotide substitution model for the presumed data partitions, MrModeltest 2.3, which is a modified version of Modeltest 3.6 (Posada and Crandall 1998), under an Akaike information criterion (AIC) (Posada and Buckley 2004) framework, was used. Nucleotide models were selected for all data subsets; GTR+I+G (Rodríguez *et al*. 1990; Yang 1996) for 1st (1) and 2nd (2) codon positions of *COI*, combined 1st–2nd *COI* codon positions (1,2), full *COI* (1,2,3) and combined *COI–18S–28S* (1,2,3,4,5,6), HKY+G (Hasegawa *et al.* 1985; Yang 1996) for *COI* 3rd position (3), SYM+I+G (Zharkikh 1994; Yang 1996) for full *18S* (4,5), while SYM+G (Zharkikh 1994; Yang 1996) and GTR+G (Rodríguez *et al.* 1990; Yang 1996) were chosen for the *18S* core components C1-C2-C3 (4) and *18S* expansion elements V1-V2 (5), respectively. A GTR+G (Rodríguez *et al.* 1990; Yang 1996) model was found to be the most appropriate for *28S* (6) and combined *18S–28S* (4,5,6). Genetic algorithm for rapid likelihood inference (GARLI) 2.0-win (Zwickl 2006), which performs phylogenetic searches using the maximum likelihood (ML) criterion, was used to examine the best partitioning scheme for the dataset. Eleven different partitions of *COI* first, second and third base codon positions, core and variable regions of *18S* (C1-C2-C3, V1-V2) and *28S* were examined to calculate the lnL and AIC index for each partition (Table S1).

To run individual partitioned models of ML for each scheme the GARLI configuration file was set for two independent search replicates and all parameters were unlinked (except for partition 1 in which data subsets were assumed to be a single dataset). The subset-specific rate multiplier was set to vary over data subsets, and other settings of the GARLI configuration file were according to the defaults. The likelihood scores of the two independent runs were computed and the greater likelihood score was chosen for calculation of AIC scores. The AIC score of each partitioning scheme was calculated as AIC = 2× (#parameters – lnL) and the

lowest value was chosen as the best score. Partition number 11 (P11), which treats each subset separately, showed the highest ML score (–18126.42588) and lowest AIC value (36362.85176), and was selected as the best partition scheme for phylogenetic analyses.

For individual *18S* and combined *COI–18S–28S* data, both Bayesian inference (BI) and ML analyses were undertaken, while for individual *COI* and *28S* data, only BI analyses were performed. MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) was employed for BI analyses, with posterior probabilities to examine the robustness of the nodes. The P11 partitioning scheme, with separate unlinked models for each of the six data partitions, was used for the BI analysis of the combined data. All parameters were unlinked and the rates were allowed to vary over the subsets. Two independent runs with four different chains were run simultaneously for five million generations, subsampling trees every 100 generations. In this BI analysis, the final standard deviation of split frequencies reached 0.08% and PSRF values for all parameters were 1.0, suggesting convergence had occurred. For each independent MrBayes run, a 25% burn-in, which equated to 12 500 samples, was discarded from the 50 001 samples obtained during the analysis (37 501 samples were included). To further assess convergence to the stationary distribution, the software package Tracer version 1.5 (Rambaut and Drummond 2003) was used. The effective sample size (ESS) for all parameters of the combined runs (runs 1 and 2) were between 1047 (TL) and 60 732 (alpha2). A 50% majority rule BI consensus tree was constructed from the remaining trees and visualised using the program FigTree version 1.3.1 (Rambaut 2009). Individual BI analyses were also performed on *18S* (partitioned by core and variable regions, each with separate nucleotide substitution models), using a similar analysis to that given above (10 million generations, 25% burn-in, unlinked models and rates variable over subsets) and *28S* data (one million generations and a 25% burn-in value). The single BI analysis converged on stationary distributions with standard deviations of split frequencies of 0.21% for *18S* and 0.86% for *28S*. The AWTY program (Wilgenbusch *et al.* 2004) was used to further explore the convergence of Markov chain Monte Carlo (MCMC) chains in the BI phylogenetic analyses. The results for both individual and combined datasets, including the cumulative split frequencies, and the bivariate plot of split frequencies for both first and second runs of simulations, showed tree topologies had been adequately sampled and convergence had been reached in each case.

Maximum likelihood analyses were conducted using PhyML version 3.1 (Guindon *et al*. 2010) for the *18S* data and GARLI (Zwickl 2006) for the combined data, the latter using the P11 model (six partitions with separate models of evolution) that was found to give the lowest AIC score (Table S1) and the approach as given above. The PhyML analysis was conducted using a single GTR+G+I (Rodríguez *et al.* 1990; Yang 1996) model for *18S*. Robustness of the ML trees was assessed using bootstrap proportions, estimated from 200 pseudoreplicates (PhyML) or 500 pseudoreplicates (GARLI).

Scanning electron microscopy

In order to dry specimens prior to scanning electron microscopy (SEM), depending on whether they were fresh or preserved in 100% ethanol, two different methods were used. As most of the samples were brittle and susceptible to being damaged during any handling, an electric point drier was not used. Freshly collected samples were fixed for 24 h in EM fixative (4% paraformaldehyde and 1.25% glutaraldehyde in PBS, plus 4% sucrose, Ph 7.2). Samples were washed in a buffer (PBS + 4% sucrose) for 5 min then post-fixed in 2% O_sO_4 (osmium tetroxide) for 1 h. Scanning electron microscopy samples were then dehydrated using 70% ethanol (two changes of 10 min each), 90% ethanol (2 changes of 10 min each) and 100% ethanol (three changes of 15 min each). Samples were placed in a 1 : 1 solution of HMDS (hexamethyldisilazane) and 100% ethanol for 10 min and then transferred to 100% HMDS for two changes of 10 min each. After removing the HMDS, samples were air dried (all the treatments were done under a fume hood). Specimens that had been preserved in 100% ethanol were placed in small petri dishes with a 1 : 1 solution of HMDS and 100% ethanol for 15 min. The samples were immersed in 100% HMDS for three changes of 15 min, 15 min and 20 min, respectively. Scanning electron microscopy samples were then air dried. All dried samples and associated body parts were mounted on metal stubs using paper sticks and coated with carbon and gold. A Philips XL20 or Philips XL40 instrument at Adelaide Microscopy (The University of Adelaide, SA) was used to examine specimens.

Results

Individual and combined phylogenetic analyses

The 50% majority rule posterior probability *18S* tree from the BI analysis (Fig. 2) resolved Crinocheta as monophyletic (posterior probability (pp) = 1.0), but 'Platyarthridae' (represented by *Niambia* spp. 1 and 2, *Platyarthrus hoffmannseggii*, *P. schoeblii* and *T. tomentosa*) and 'Philosciidae' (represented by *Haloniscus* spp. 1–4 and *Philoscia muscorum*) were polyphyletic. Three families, Armadillidiidae, Armadillidae and Porcellionidae, represented by multiple exemplars were resolved as monophyletic (pp = 1.0), as were the Synocheta (Styloniscidae and Trichoniscidae) (pp = 0.96) represented by *Haplophthalmus danicus*, *Hyloniscus riparius*, *Styloniscus* sp. and *Trichoniscus pusillus*. However, the Diplocheta (Ligiidae) represented by two species each of *Ligia* and *Ligidium* formed a paraphyletic grade at the base of the tree, although this arrangement was not well supported (pp = 0.65). Also there was evidence that the Trachelipodidae (represented by *Porcellium fiumanum*, *Protracheoniscus politus* and *Trachelipus ratzeburgii*) was polyphyletic. However, a likely long-branch effect that was noticeable in the *T. ratzeburgii* + Porcellionidae part of the tree, which may be due to an expanded V2 region of *18S* that was difficult to align among the taxa, casts some doubt on the relationships among these taxa.

A separate monophyletic clade (pp = 0.95), which comprised the Australian taxa (taxa 1–9) and one South American species (taxon 10), was highly divergent from all included 'Platyarthridae', but was sister to the single Stenoniscidae + *Haloniscus* spp. 1–4 (pp = 0.94), with *T. tomentosa* being sister to these, albeit with low support (pp = 0.6). The Australian taxa 1–9 were sister to the South American species (pp = 1.0).

Results from the *18S*-only ML analysis were almost the same as those for the BI tree although the bootstrap support values for a

Fig. 2. The 50% majority rule posterior probability tree from the Bayesian inference (BI) analysis of *18S* using 47 ingroup species of 13 known families. Families in inverted commas are not monophyletic. Outgroups not shown. The first numbers adjacent to nodes are BI posterior probabilities, followed by maximum likelihood bootstrap values (only those greater than 50% are shown). Abbreviations for generic names are given in Appendix 1.

number of nodes were relatively low. Importantly, the same relationships were resolved for taxa 1–10 as sister to Stenoniscidae + *Haloniscus* although *T. tomentosa* as sister to these was equivocal, and 'Platyarthridae' was again polyphyletic.

The topology of the individual BI analysis for *COI* was poorly resolved for most of the deeper nodes and so it is not discussed any further. However, the individual *28S* BI tree (Fig. S2) provided further support for the monophyly of taxa 1–10 from Australia and South America (pp = 0.99). All platyarthrid species formed a distinct inclusive clade also containing *Armadillidium vulgare* (Armadillidiidae; pp = 0.95), with the latter showing a poorly supported sister lineage relationship to *T. tomentosa* (pp = 0.69), and a sister relationship between *P. hoffmannseggii* and *Niambia* spp. (pp = 0.97).

The BI and ML trees for the combined *COI*, *18S* and *28S* data (Fig. 3) were identical and showed a very similar topology to that for the *18S* BI tree, with the exception that *D. marina* (Detonidae) was sister to *Troglarmadillo* spp. 1 and 2 (Armadillidae) (pp = 1.0; bp = 77%) rather than to all other Crinocheta, and *T. tomentosa* was sister to *A. vulgare* (Armadillidiidae) + 'Platyarthridae' rather than to taxa 1–10 + *Haloniscus* spp. ('Philosciidae') + Stenoniscidae (pp = 0.98; bp = 70%). Support levels for nodes were increased by combining the three genes and applying a 6-partitioned model-based scheme (P11; Table S1). 'Platyarthridae' was again polyphyletic, with *Niambia* spp. + *P. hoffmannseggii* (pp = 1.00; bp = 80%), and *A.* vulgare (Armadillidiidae) forming a monophyletic group (pp = 1.00; bp = 96%) to the exclusion of *T. tomentosa*. Again, taxa 1–10 were monophyletic (pp = 1.0; bp = 100%) and sister to Stenoniscidae + *Haloniscus* spp. (pp = 1.0; bp = 51%), as in the *18S* tree, albeit with low bootstrap support. Further, Crinocheta was monophyletic (pp = 1.0; bp = 81%) and sister to the single Synocheta included (*Styloniscus* sp.) (pp = 0.99; bp = 80%), while the single Diplocheta included (*Ligia* sp.) was sister to Crinocheta + Synocheta (pp = 1.00).

Structure of the water conducting system on the second antennae

A previously unreported structure, a furrow, exists on the ventral second antenna of all members of taxa 1–10. This furrow contains elongated modified hair-like setae which likely act as capillary setae (Fig. 6*A*, *B*). A similar furrow was not found in *Trichorhina tomentosa* (Fig. 4*A*), *T. anophthalma* (Fig. 4*C*), *P. hoffmannsegii* (Fig. 4*B*), *P. aiasensis* (light microscopy) or *P. costulatus* (light

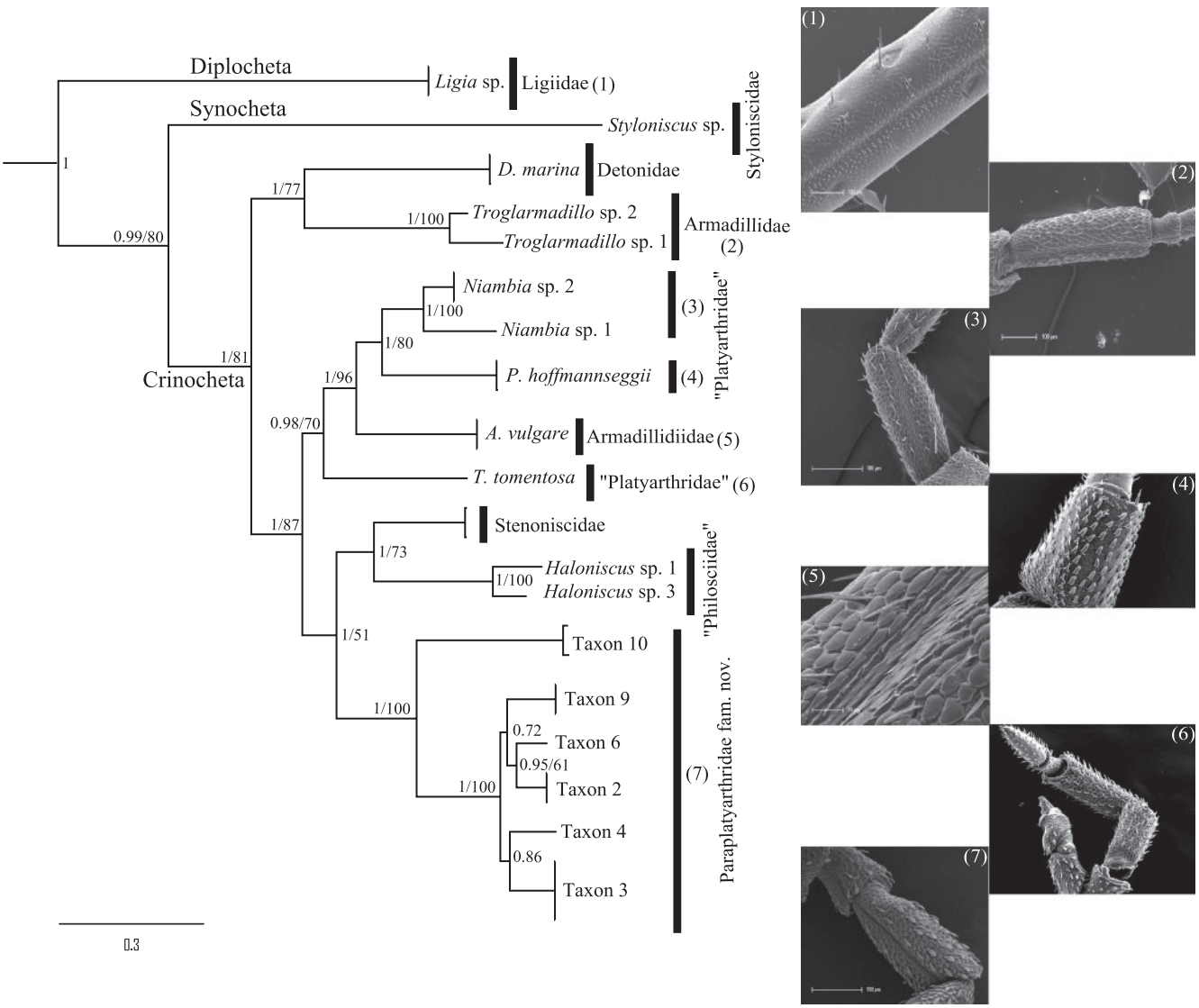

Fig. 3. The 50% majority rule posterior probability tree from a Bayesian inference (BI) analysis for *COI*, *18S* and *28S* sequence data combined, using 19 ingroup species belonging to eight known oniscidean families. Families in inverted commas are not monophyletic. Outgroups not shown. The first numbers adjacent to nodes are BI posterior probabilities, followed by maximum likelihood bootstrap values (only those greater than 50% are shown). Abbreviations for generic names are given in Appendix 1.

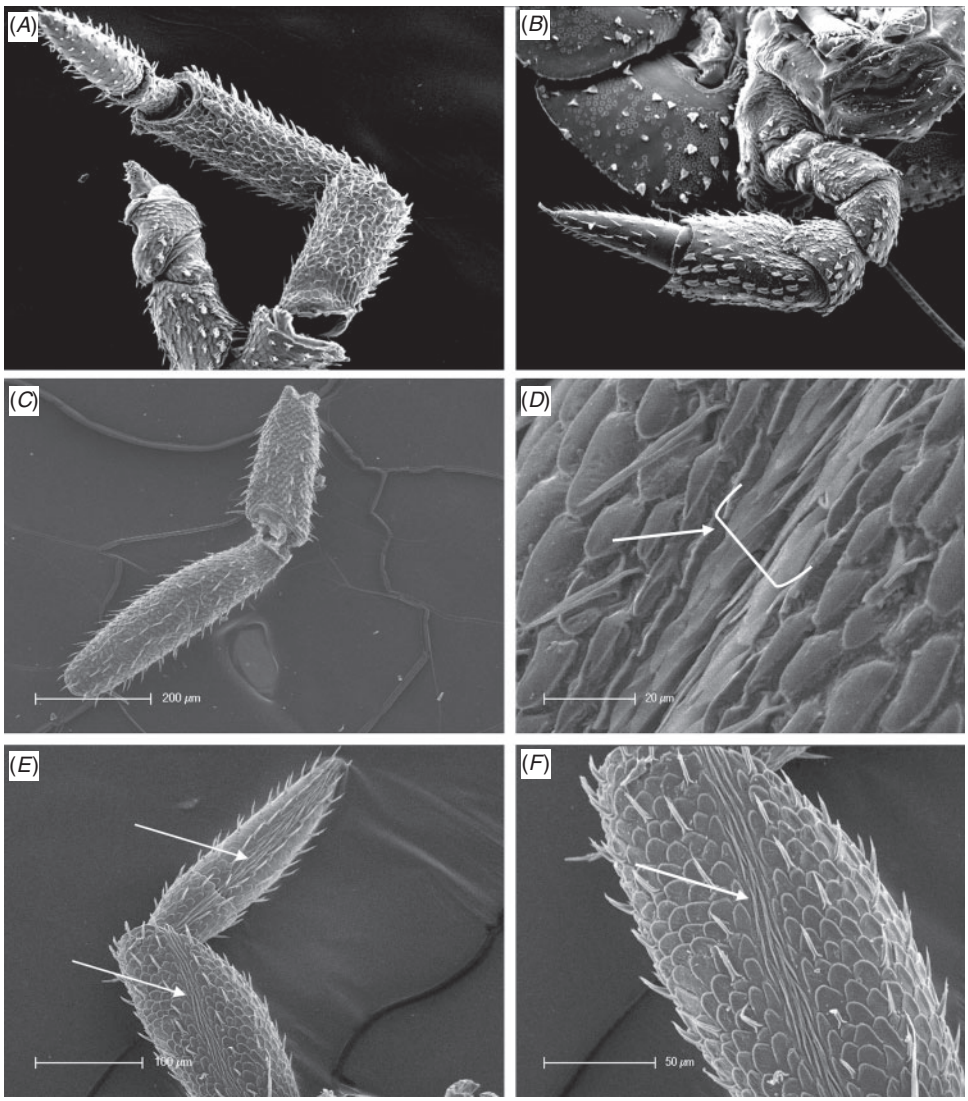

Fig. 4. Morphology of the second antenna water conducting system: (*A–C*) capillary system not present: (*A*) *Trichorhina tomentosa* ('Platyarthridae'); (*B*) *Platyarthrus hoffmannseggii* ('Platyarthridae'); (*C*) *Trichorhina anophthalma* ('Platyarthridae'). (*D*) *Armadillidium vulgare* (Armadillidiidae) showing position of capillary furrow (arrowed). (*E*, *F*) Capillary furrow on *Niambia* sp. ('Platyarthridae'); (E) capillary system with setae but lacking a furrow (arrowed); (*F*) close-up of capillary setae (arrowed).

microscopy). In addition, in all representatives of taxa 1–10 the ventral second antenna peduncle possesses scale setae relatively similar to those occurring on the dorsal body (Fig. 6*A*). Further, the distal article of the antennal flagellum possesses tightly packed elongated setae, which are directed towards the apical cone (Fig. 6*C*, *D*). In *Niambia* the capillary system on the second antennae comprises elongate tightly packed setae, but no furrow is present (Fig. 4*E*, *F*).

No specific antennal capillary furrow occurs in *Porcellionides pruinosus* (Porcellionidae) (Fig. 5*E*) except for longitudinal depressions along the second antenna, which do not seem to be homologous with the capillary furrows in other oniscideans. In *A. vulgare* (Armadillidiidae) the furrow is wider, consisting of two rows of scale-like setae (not elongated), separated by stout pointed setae medially. In addition, the cuticular scales on the second antenna are sub-rectangular and do not overlap (Fig. 4*D*). The capillary furrow on the ventral second antenna in *Orthometopon dalmatinum* (Trachelipodidae) is distinct in that the capillary setae are elongated and arranged in three rows: the setae on the medial row are straight, whereas the two rows either side include elongate setae which bend inwards over the medial row. The furrow on the flagellum is not as deep as that on the peduncle and includes capillary setae which are set upwards (Fig. 5*A*, *B*). *Trachelipus cavaticus* (Trachelipodidae) possesses a different type of capillary furrow which is not demarcated by cuticular scales laterally, but possesses relatively thickened setae that are not tightly packed (Fig. 5*C*, *D*). In *Ligia*, a furrow occurs on the ventral second antenna, but it lacks any setae (Fig. 5*F*).

Based on the results of the phylogenetic analyses above (Figs 2, 3), and the presence of an apparently uniquely

structured water conducting system in taxa 1–10, we suggest that there is ample evidence to recognise these taxa as a new family based on the following description of a new genus and species from Western Australia.

Systematics

Order **ISOPODA** Latreille, 1817

Suborder **ONISCIDEA** Latreille, 1802

Section **CRINOCHETA** Legrand, 1946

Family **PARAPLATYARTHRIDAE** Javidkar & King, fam. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:D519F5A2-4D0E-4C66-8721-E6FAE39B197E

Type genus. *Paraplatyarthrus* Javidkar & King, gen. nov.

Diagnosis

Dorsal body entirely covered with fan-like scale setae from cephalothorax to pleotelson. Second antenna ventrally with leaf-like scale setae and a furrow containing elongated hair-like capillary setae that form part of the water conducting system; flagellum two-jointed. Head with postfrons and profrons fused. Maxilla 1 outer endite with 4 + 4 to 5 teeth, outer four teeth with one comparatively shorter stout tooth.

Description

Body length 2.5–5.5 mm from the anterior part of cephalothorax to pleotelson tip. Included species of the 'clinger type' with large coxal plates and pleon epimera. Dorsal body covered with fan-like scale setae from cephalothorax to pleotelson (Fig. 6*E*, *F*). Body pigmentation, depending on lifestyle, variable from fully

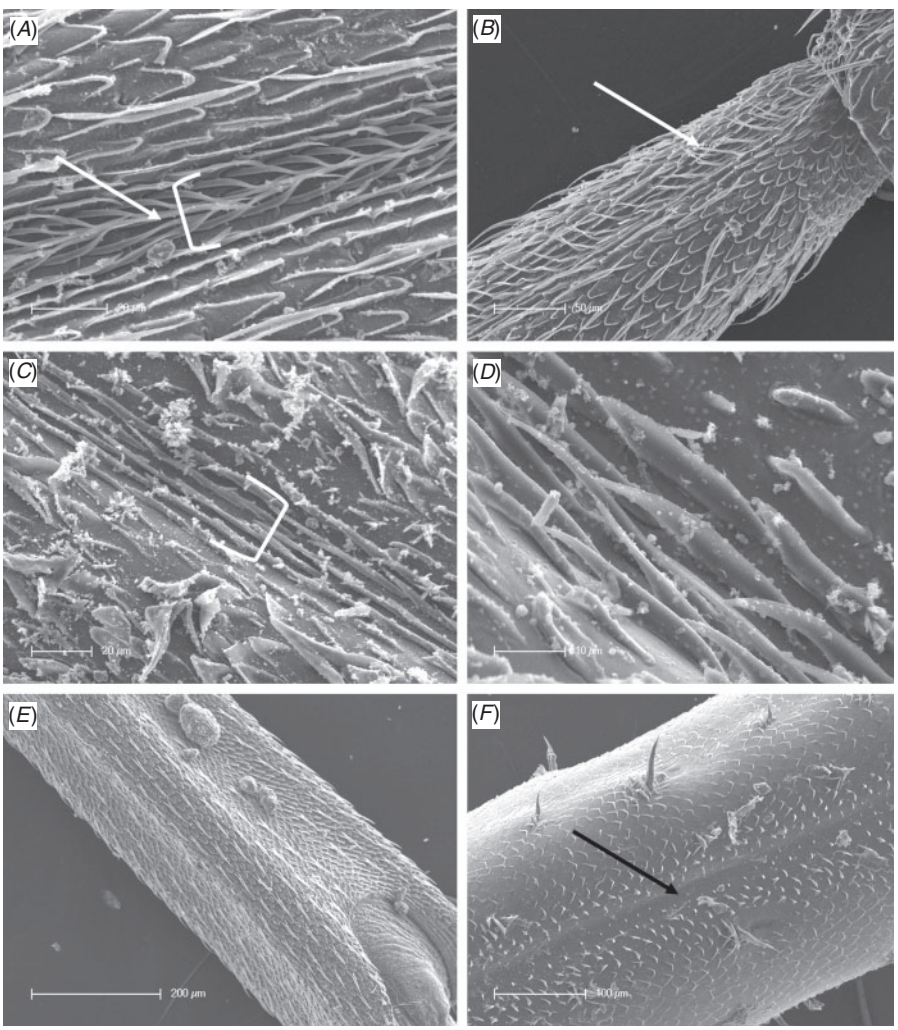

Fig. 5. Morphology of the second antenna capillary system. (*A*) *Orthometopon dalmatinum* ('Trachelipodidae') showing capillary furrow on the peduncle (arrowed); (*B*) *Orthometopon dalmatinum* showing capillary setae on the flagellum (arrowed); (*C*) *Trachelipus cavaticus* ('Trachelipodidae') showing furrow on the peduncle and associated capillary setae (bracket); (*D*) *T. cavaticus*, close-up of the furrow and thickened setae; (*E*) *Porcellionides pruinosus* (Porcellionidae), which lacks a furrow on the peduncle; (*F*) *Ligia* sp. (Ligiidae), showing a furrow on the peduncle (arrowed) but lacking capillary setae.

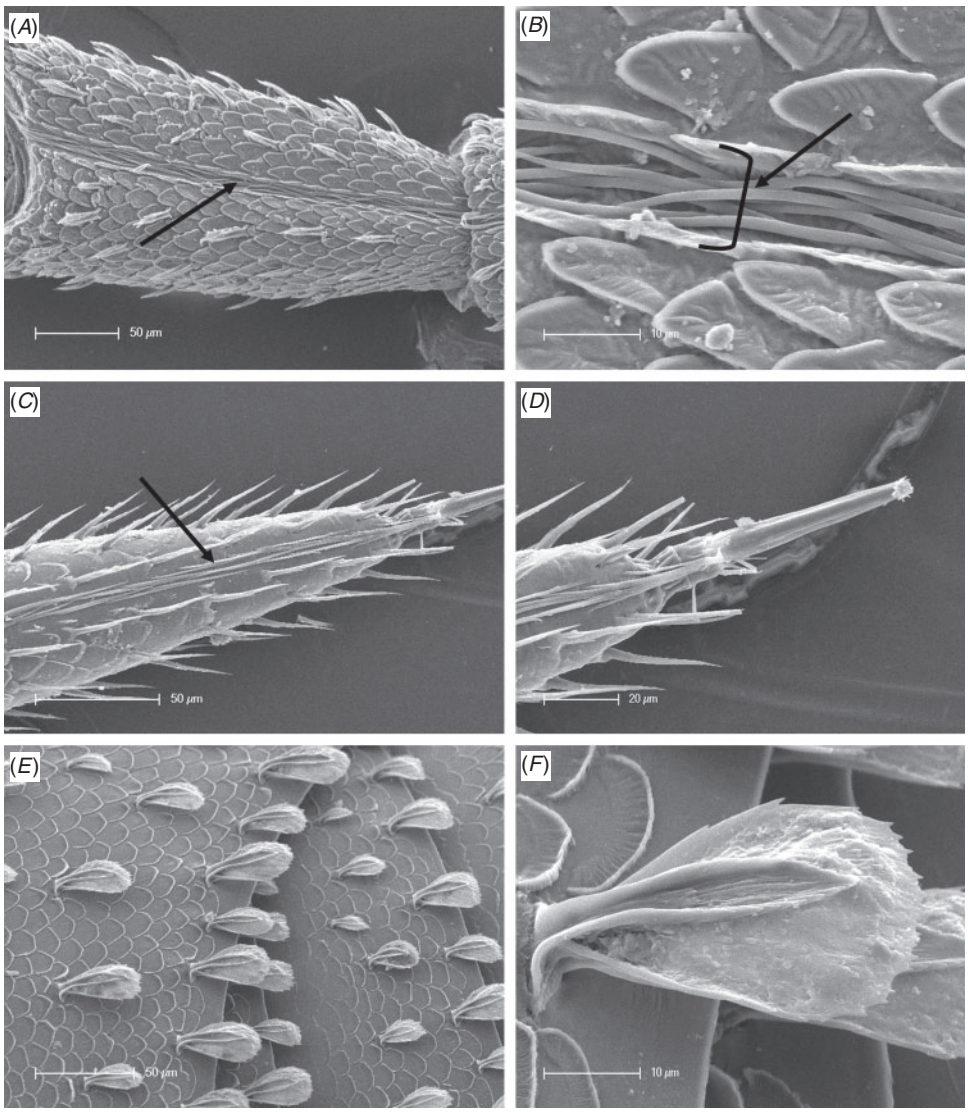

Fig. 6. (*A–F*) *Paraplatyarthrus* sp. (taxon 1, specimen from Mt Morgans, Western Australia), morphology of the second antenna. (*A*) Capillary furrow on the peduncle showing leaf-like scale setae scattered around the furrow (arrowed); (*B*) close-up of the capillary furrow and the associated elongated hair-like capillary setae (arrowed); (*C*) distal article of the flagellum showing the tightly packed setae (arrowed); (*D*) apical cone of the flagellum; (*E*) dorsal scale setae; (*F*) close-up of one scale seta and crescent-shaped cuticle.

pigmented in surface species to completely pale in troglobitic species.

Cephalic lateral lobes present from small to comparatively enlarged forms. Supra-antennal line present in form of lines of compressed cuticular scales (Fig. 7*B*). Frontal line absent. Postfrons completely fused with profrons (Fig. 7*C*). Number of ommatidia variable from maximum of seven in surface species to eyeless in subterranean species (Fig. 8*A*, *B*). First antenna three-jointed (Fig. 7*A*). Second antenna flagellum two-jointed, dorsal and lateral sides with simple setae, ventral side with hair-like setae lying along each other towards top (Fig. 6*C*), the apical cone short with longitudinal sutures and lateral setae at basal part, top of cone circular with very small setae in a circle (Fig. 6*D*); ventral side of second antennal peduncle with leaf-like scale setae and a distinct furrow containing modified elongate hair-like setae which forms part of the water conducting system (Figs 6*A*, *B*, 7*E*).

Mandibles with one to two plumose setae on hairy lobe and one stout plumose seta between lobe and pars molaris; pars molaris on both mandibles with a tuft of plumose setae. Maxilla 1 outer endite with outer group of four simple large teeth, one smaller than the other but stout, inner group with a combination of four to five more slender cleft and simple teeth; inner endite with two stout plumose setae (Fig. 9*C*). Maxilla 2 either with suture (line) delimiting lobes or suture absent or vestigial, inner lobe with thick sensilla on distal margin, outer lobe with very fine small setae from apical margin towards subapical area (Fig. 9*D*). Maxilliped basal article of palp with

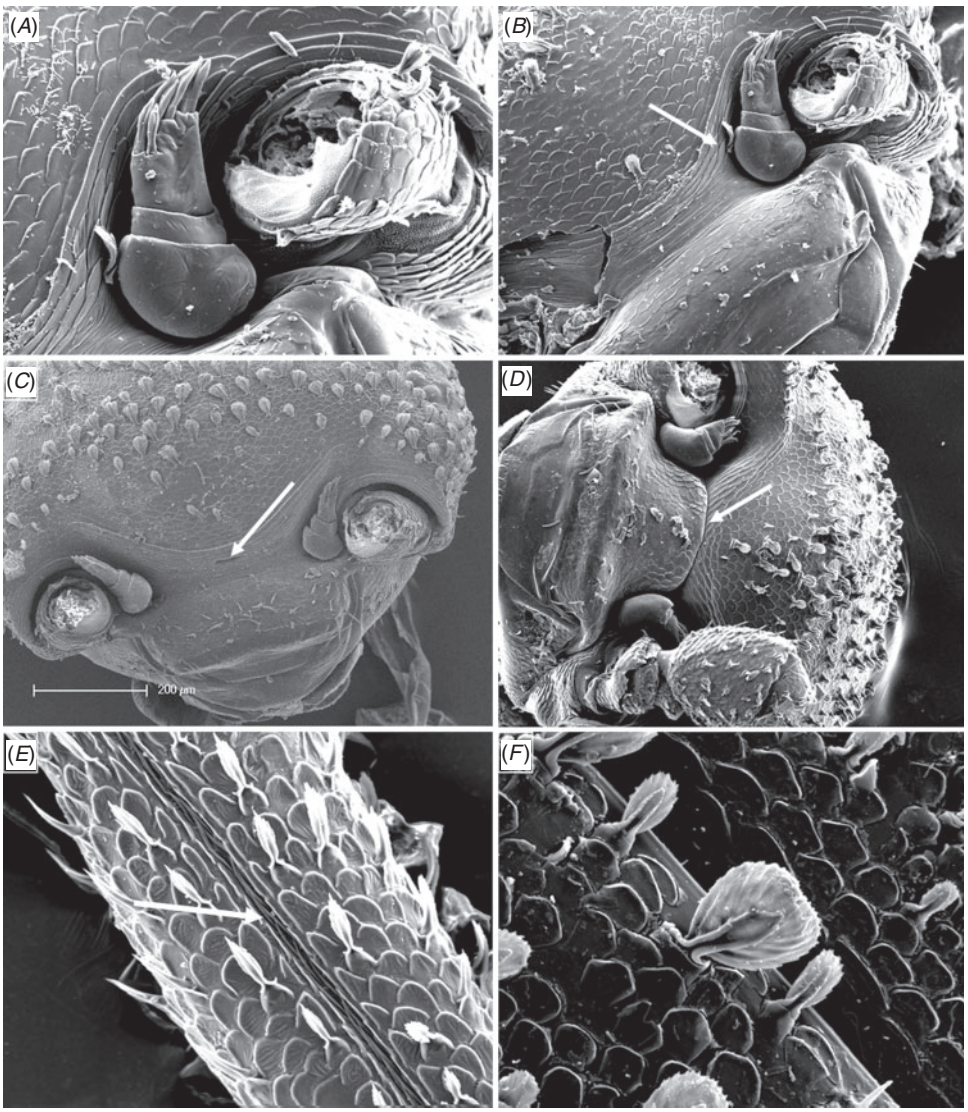

Fig. 7. (*A*, *B*) *Paraplatyarthrus subterraneus* Javidkar & King, sp. nov., paratype, sex unknown. (*A*) Close-up of first antenna (voucher BES15525.20); (*B*) supra-antennal line (arrowed) (voucher BES15525.20); (*C*) *Paraplatyarthrus* sp. (taxon 1, specimen from Mt Morgans, Western Australia), postfrons fused to the profrons (arrowed); (*D*) *Trichorhina tomentosa* ('Platyarthridae') showing the postfrons obviously delimited from the profrons (arrowed); (*E*, *F*) South American paraplatyarthrid taxon 10. (*E*) The occurrence of capillary furrow and the associated hair-like capillary setae (arrowed) plus leaf-like scale setae on the second antenna peduncle; (*F*) scale setae with ribbed structure.

two large setae; distal articles of palp fused with simple tufts of setae on inner side.

Noduli laterales present. Tergites 2–7 epimera with a fine suture originating from posterior epimera and extending towards anterior end.

Pereopods 1–7 with both simple and large serrate setae on inner side of propodus, carpus and merus; ischium, merus and carpus with few large serrate setae on outer apical part; dactylus with a long simple seta, outer claw straight or sickle-shaped, inner claw simple and situated at base of the outer claw.

Pleopods with no dorsal respiratory fields. Male pleopod 1 endopodite straight, much longer than exopodite, dorsal spermatic furrow narrow. Genital papilla ventral sheath triangular with pointed or rounded tip; ventral sheath surpassed by a long lobe with genital orifices most probably situated at apical corners. Male pleopod 2 endopodite slender and longer than that of pleopod 1. Pleopod 2–5 exopodites with both marginal simple and serrate setae on inner side.

Uropod exopodite dorsoventrally flattened, exceeding pleotelson; endopodite laterally flattened.

Etymology

The family name is derived from the name of the type genus, *Paraplatyarthrus*.

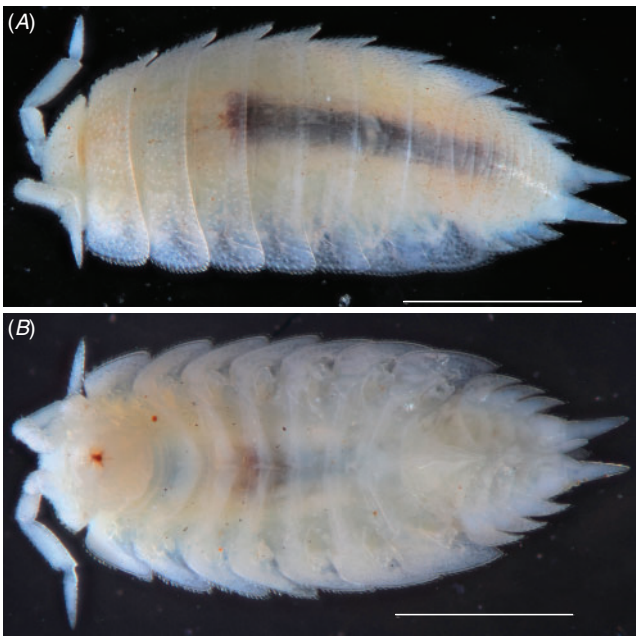

Fig. 8. *Paraplatyarthrus subterraneus* Javidkar & King, sp. nov., paratype ♂ (WAM C53627), habitus showing depigmented body typical of subterranean oniscideans: (*A*) dorsal view; (*B*) ventral view. Scale bars = 1 mm.

Distribution

Known from terrestrial and subterranean habitats in arid and temperate regions of Western Australia and subtropical South America.

Remarks

This new family is based on the results of the molecular phylogeny (Figs 2, 3) and several diagnostic morphological characters evident from examination of taxa 1–10 comprising both surface and subterranean taxa (Fig. 8*A*, *B*). Paraplatyarthridae is distinguishable from all other oniscidean families by the combination of characters above. It is superficially similar to *T. tomentosa* as a representative of 'Platyarthridae' (Schmidt 2003), but the latter lacks a capillary furrow on the second antenna and scale setae on the ventral side of the antenna (Fig. 4*A*), compared with the unique antennal furrow which is developed in all Paraplatyarthridae. An antennal furrow is also lacking in all *Platyarthrus* species examined. The postfrons is clearly delimited from the profrons in *T. tomentosa* (Fig. 7*D*), while in paraplatyarthrid species it is fused (Fig. 7*C*). In all paraplatyarthrids there are also four outer teeth including one stout smaller tooth on the outer endite of the first maxilla (Fig. 9*B*), whereas in 'Platyarthridae' the short stout tooth is absent. *Trichorhina anophthalma* from Portugal (not included in the molecular phylogeny due to degraded DNA), which also lacks a capillary furrow on the second antenna (Fig. 4*C*), has prominent delimitation of the postfrons from the profrons, and three outer teeth present on the apical margin of the first maxilla outer endite. Therefore, based on these characters *T. anophthalma* is clearly a member of *Trichorhina*. In addition, all paraplatyarthrid species have a supra-antennal line, while it is absent in true members of *Trichorhina* (i.e. those congeneric with *T. tomentosa*). In paraplatyarthrid species the supra-antennal line is weaker than that of *Niambia* species in which it is solid.

Based on this assessment we propose that the new family comprise two genera: *Paraplatyarthrus*, gen. nov., described below, which is restricted to Australia and is represented by taxa 1–9; and a separate genus postulated to be endemic to South America (native forest, Porto Alegre, Belém Novo, Brazil), represented by taxon 10 which is sister to *Paraplatyarthrus*. However, we refrain from formal description of the South American genus until more material becomes available (N.B. it is currently only known from a few female specimens) and South American oniscideans, particularly species described under *Trichorhina*, can be examined in more detail.

Genus ***Paraplatyarthrus*** Javidkar & King, gen. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:0BED19D0-9BF0-401A-AADB-84719AEC9DDC

Type species. *Paraplatyarthrus subterraneus* Javidkar & King, sp. nov.

Diagnosis

Smooth fan-like scale setae covering the body (Fig. 6*F*). Maxilliped endite with two small arrow-like setae on distal margin. Pereonal tergite 7 with two noduli laterales on each side (four on the whole pereonite), pereonal tergites 1–6 with one nodulus lateralis on each side (two on the whole pereonite).

Etymology

The name of the genus is derived from the prefix 'para' meaning 'near' and 'Platyarthrus' due to its general morphological similarity to *Trichorhina* and *Platyarthrus*.

Distribution

Known from terrestrial and subterranean habitats in arid and temperate regions of Western Australia.

Remarks

Paraplatyarthrus species possess two prominent arrow-like setae on the outer apical margin of the maxilliped endite which are absent in all other oniscidean species examined including the South American paraplatyarthrid species (taxon 10) (i.e. not recognisable under light microscopy). The presence or absence of these specialised setae is likely a good diagnostic trait to separate *Paraplatyarthrus* and the South American species. However, it will be essential to examine additional South American species to confirm this. Moreover, *Paraplatyarthrus* and the South American paraplatyarthrid species (taxon 10) can also be separated on the structure of the scale setae on the tergites (smooth (Fig. 6*F*) in *Paraplatyarthrus*, ribbed (Fig. 7*F*) in taxon 10), and the number of noduli laterales on the pereonal tergite 7 (two on each side in *Paraplatyarthrus*, one on each side in taxon 10). Given these differences and the level of divergence between *Paraplatyarthrus* species and South American taxon 10 in the molecular analysis (Figs 2, 3), we propose that the latter species represents a distinct genus, as discussed above. Below we describe a single taxon (taxon 6 in Figs 2, 3) as the type species of the genus, but are preparing a comprehensive

Fig. 9. (*A–E*) *Paraplatyarthrus subterraneus* Javidkar & King, sp. nov., holotype ♂: (*A*) right mandible; (*B*) maxilla 1 outer endite; (*C*) maxilla 1 inner endite; (*D*) maxilla 2; (*E*) maxilliped (note the arrow-like setae on the apical outer corner of the endite). Scale bars = 0.1 mm.

revision of the genus as a separate study, to be published elsewhere.

Paraplatyarthrus subterraneus Javidkar & King, sp. nov.

(Figs 7–11)

http://zoobank.org/urn:lsid:zoobank.org:act:AC6980E5-CB80-455F-AB00-4C3C7C3B36CC

Material examined

Holotype. ♂, WAM C53623 (BES15525.19), Laverton Downs Windarra calcrete, Eastern Murchison region, WA, Australia: 28.50282°S,

122.17726°E. 13.vii.2010, W. F. Humphreys and S. J. B. Cooper, deposited in the WAM.

Paratypes. 5♂ including WAM C53624 (BES15525.24), WAM C53625 (BES15525.16), WAM C53626 (BES15525.12), WAM C53627 (BES15525.2), WAM C53628 (BES15525.3); 1♀ WAM C53629 (BES15525.4); BES15525.20 held on SEM stubs (sex unknown); same locality and collectors as holotype, all deposited in the WAM.

Description

Male (holotype)

Body length 3.3 mm (range for paratypes 3–3.8 mm). No pigmentation, the whole body pale and eyeless, typical of a true troglobitic form (Fig. 8*A*, *B*). A single scale seta present at top of supra-antennal line in middle (Fig. 7*B*). Cephalon lateral lobes present but not enlarged. Fan-like scale setae on dorsal body serrate at top of sheath; cuticular scales either crescent-shaped or curved and pointed at top. Medial article of antenna 1 shortest, distal article longest, bearing four pairs of aesthetascs, each with a very fine longitudinal suture medially (Fig. 7*A*). Basal article of antenna 2 flagellum about 1/3 length of distal article.

Left mandible pars molaris with tuft of about seven plumose setae; hairy lobe attached to lacinia mobilis bearing two plumose setae, top covered with a few small fine setae. Right mandible pars molaris with tuft of about eight plumose setae; one plumose seta on hairy lobe, very small fine setae around base of plumose seta (Fig. 9*A*); lacinia mobilis coronate. Maxilla 1 outer endite with an outer group of teeth covering about 60% of marginal area, comprising inner group of four bifurcate teeth and one simple tooth (Fig. 9*B*); apical outer corner of inner endite with two very fine setae close to each other (Fig. 9*C*). Maxilla 2 apically

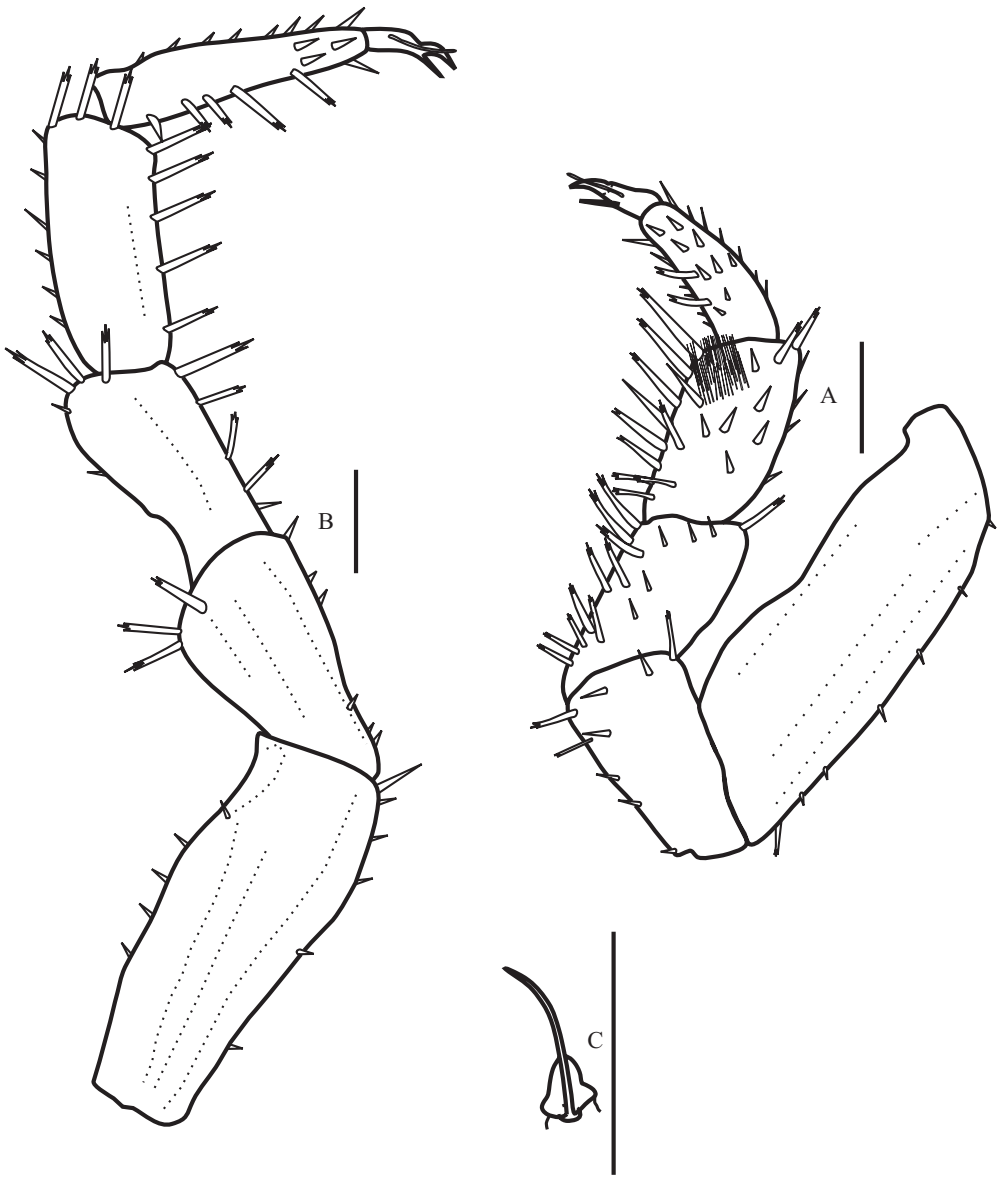

Fig. 10. *Paraplatyarthrus subterraneaus* Javidkar & King, sp. nov., holotype ♂: (*A*) pereopod 1; (*B*) pereopod 7. Scale bars = 0.1 mm. Paratype ♀: (*C*) nodulus lateralis (tergite 5) and structure of the sheath. Scale bar = 0.05 mm.

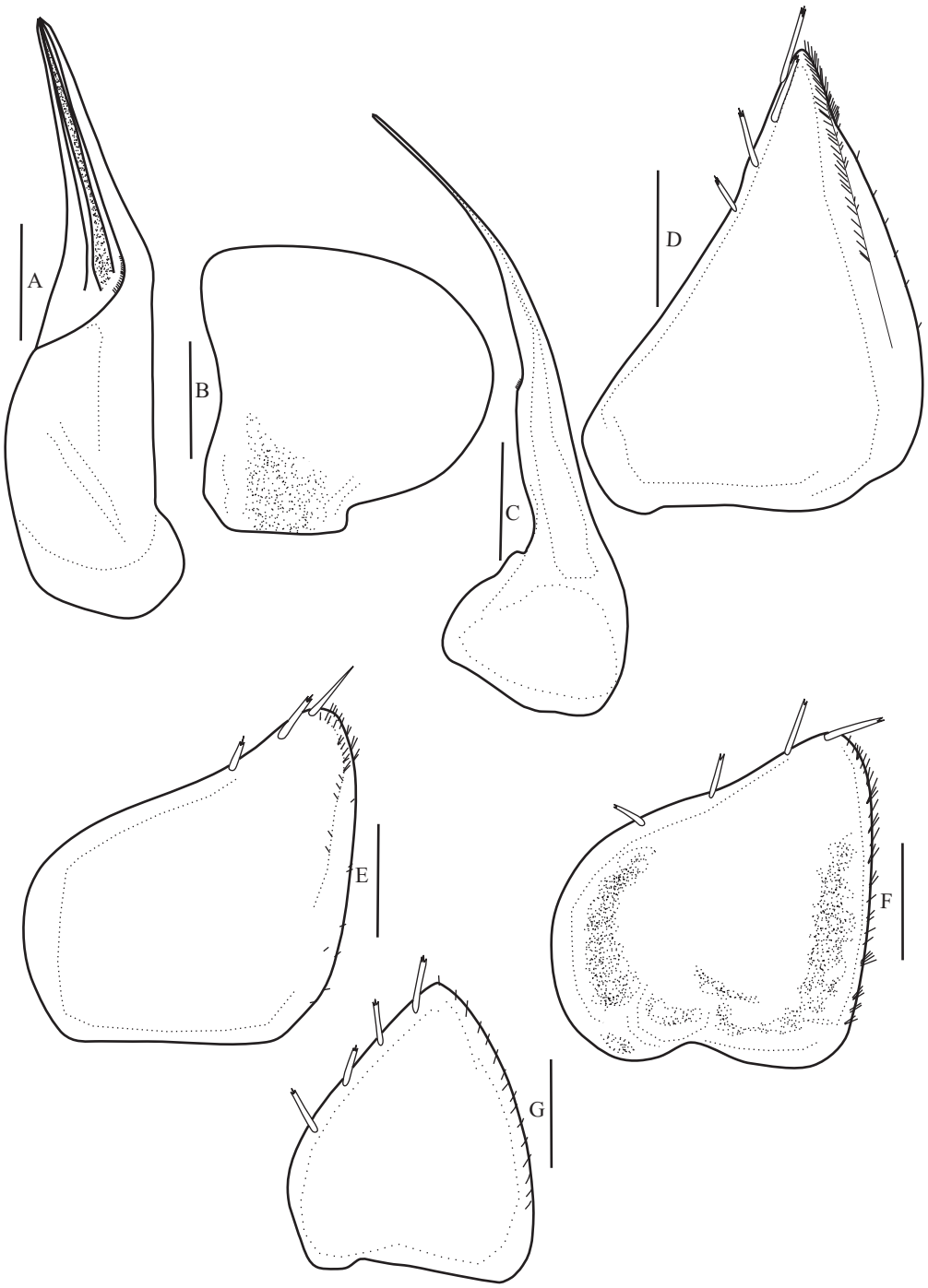

Fig. 11. *Paraplatyarthrus subterraneaus* Javidkar & King, sp. nov., holotype ♂: (*A*) pleopod 1 endopodite; (*B*) pleopod 1 exopodite; (*C*) pleopod 2 endopodite; (*D*) pleopod 2 exopodite; (*E*) pleopod 3 exopodite; (*F*) pleopod 4 exopodite; (*G*) pleopod 5 exopodite. Scale bars = 0.1 mm.

bilobate, lobes delimited by a fine suture, inner lobe smaller than outer lobe (Fig. 9*D*). Maxilliped endite with one large seta close to subapical inner corner; distal articles with one large proximal seta, a medial tuft of three large slender setae and an apical tuft of a few long setae; the outer margin of palp with one medial fine seta and two distal fine setae (Fig. 9*E*).

Epimeron 1 bluntly projecting anteriorly; in dorsal view, posterolateral corner of pereonites 1–3 rounded; posterolateral corner of pereonites 4–7 posteriorly directed. Noduli laterales present on pereonal tergites with sheath subtriangular (Fig. 10*C*).

Pereopod 1 carpus inner margin densely covered with large serrate setae, dense tuft of fine setae present medially near distal

margin; propodus with both simple setae and a few large serrate setae; dactylus with a long narrow seta exceeding claws, outer claw sickle-shaped (Fig. 10*A*); carpus inner side sexually dimorphic in density of setae (male with inner side more plumose). Pereopod 7 not showing any significant sexual dimorphism; carpus and merus inner side with serrate setae but less dense compared with those of pereopod 1; carpus, merus and ischium outer apical margin with large serrate setae; ischium inner side with few short simple setae; basis apical inner side with a single simple long seta (Fig. 10*B*).

Pleon outline continuous with pereon. Pleopod 1 endopodite with very fine small setae on medial and apical parts (Fig. 11*A*); exopodite with no marginal setae (Fig. 11*B*). Pleopod 2 endopodite long and slender (Fig. 11*C*); exopodite large, with four (inner side) long serrate marginal setae (Fig. 11*D*). Pleopod 3, 4, 5 exopodites with three, four and four marginal serrate setae, respectively (Fig. 11*E–G*). Pleotelson triangular and pointed. Uropodal exopodites well-surpassing pleotelson; endopodites slightly exceeding pleotelson.

Etymology

The species name is derived from the Latin word 'subterraneus' (meaning subterranean) due to its troglobitic lifestyle.

Distribution

The new species is confined to an individual calcrete aquifer, Laverton Downs, Eastern Murchison region, Western Australia.

Discussion

Evidence for a new family Paraplatyarthridae

Our study, based on a combination of molecular phylogenetic analyses and detailed morphological comparisons, revealed the presence of a divergent clade of oniscidean isopods comprising Australian and South American taxa, which we propose represents a morphologically distinct new family, Paraplatyarthridae. In both single and multiple gene phylogenetic analyses, members of the new family, which were previously but problematically assigned to Platyarthridae, were not only unrelated to platyarthrid taxa represented by *T. tomentosa*, *Platyarthrus* and *Niambia* species, but also formed a distinctive clade to 13 other included oniscidean families. Detailed morphological analyses on the second antenna, dorsal body ornamentation, cephalon structure and mouthparts uncovered diagnostic characters, which, in combination, distinguish species of Paraplatyarthridae from all known oniscidean families.

Relationships and geographic extent of Paraplatyarthridae

All our analyses recognised the Australian taxa (*Paraplatyarthrus* spp.) as being monophyletic and sister to taxon 10, the single South American species. However, relationships among *Paraplatyarthrus* spp. are poorly resolved in all analyses, and thus preclude any discussion of geographic patterns.

The world catalogue of terrestrial isopods (Schmalfuss 2003) lists 56 species of *Trichorhina*, mostly from the neotropical region. Following the recognition of a number of new species of *Trichorhina* from Brazil (Araujo and Almerão 2007; Souza *et al.* 2011) this number has increased to 64. Given the convergent occurrence of some characters, such as the presence of scale setae on the dorsal body in members of Paraplatyarthridae and *Trichorhina*, and the fact that the new family also occurs in Brazil, it is possible, even likely, that some species described as *Trichorhina* in fact belong to Paraplatyarthridae.

While most species of *Trichorhina* have a tropical or subtropical distribution, as does taxon 10 from Brazil, *Paraplatyarthrus* spp. appear to be restricted to subtropical and arid to temperate habitats, with all recorded species to date being found in Western Australia from the west and south-west to arid regions in central WA, where the majority have a subterranean lifestyle. The systematic position of the two described Australian *Trichorhina* – *T. australiensis* Wahrberg, 1922 (WA; type in the Zoological Museum, University of Hamburg) and *T. tropicalis* (Queensland; type in the Australian Museum) – is still ambiguous as samples of these species were unavailable. Moreover, the published taxonomic descriptions of these taxa do not provide key diagnostic characters and one type is in poor condition, precluding their accurate identification. Furthermore, examination of several species in the WAM collection, which have been designated as *Trichorhina*, showed that none of them belong to this genus, while a number of undescribed taxa from Lord Howe Island and the Northern Territory, which have been tentatively assigned to *Trichorhina* (Lewis 1998; Moulds and Bannink 2012), are all in need of reassessment. A comprehensive molecular and morphological study of all Australian species that are morphologically similar to *Trichorhina* is required to determine whether all these taxa belong to Paraplatyarthridae or whether members of 'Platyarthridae' also occur on the continent.

Oniscidean relationships

Although far from definitive due to numerous missing critical taxa, our molecular phylogenetic analyses further showed that taxa currently considered members of the family Platyarthridae are polyphyletic. In all our analyses, the platyarthrid *P. hoffmannseggii* (type species of the genus) was never recovered in a monophyletic group with *T. tomentosa*. Rather, it was placed in a clade comprising *A. vulgare* (Armadillidiidae) (*28S*-only analysis), or with members of six other families (*18S*-only analysis), but with weak internal support, a result corroborated by the three-gene analysis, albeit with reduced taxon sampling. This finding is perhaps not surprising given that the *18S* analysis of Mattern (2003) also found 'Platyarthridae', represented by *T. tomentosa* and *P. schoeblii*, to be polyphyletic. The systematic position of *Niambia* ('Platyarthridae') is still uncertain and more oniscidean taxa are needed to further resolve its relationships. The occurrence of an undescribed species of *Niambia* in Australia (Adelaide, SA) may be due to its introduction from Africa, as it was found beside a river in an urban area. *Niambia capensis* is the only reported widespread species that is native to South Africa, but it has been introduced to different parts of the world including the USA (California, in a sandy intertidal zone) and New Zealand (Maloney *et al.* 2007; S. Taiti, pers. comm.).

In our study, representatives of Diplocheta, 'Philosciidae' and 'Trachelipodidae' also come out in different parts of the tree (*18S*-only), a finding for the latter family that is also supported by Mattern (2003). The status of *Haloniscus* is particularly

problematic and it has had a somewhat controversial taxonomic history. It was first considered a member of Oniscidae by Williams (1970) and then included in Philosciidae by Vandel (1973), but Poore (2002) regarded it as an unplaced taxon. In our *18S*-only analyses, *Haloniscus* was a sister lineage to Stenoniscidae, and well separated from *Philoscia muscorum* (Philosciidae), but data from additional oniscidean families are required to test this sister group relationship. Taiti *et al.* (1995) described a new aquatic *Haloniscus* species from New Caledonia, and discussed a possible relationship between *Alloniscus* Dana, 1854 (Alloniscidae) and *Haloniscus* on the basis of the absence of noduli laterales, second antennal flagellum with three articles, and the same structure of mouthparts. In our *18S*-only phylogeny, *Haloniscus* spp. 1–4, which are all subterranean taxa, were sister to taxon 11, a surface species from South Australia. Since taxon 11 was represented solely by female specimens its taxonomy remains uncertain.

These findings point to significant problems with the current higher-level classification of Oniscidea which have been articulated elsewhere, for example, in the very detailed morphological study of oniscidean relationships by Schmidt (2008). However, the molecular studies to date are obviously restricted in scope by their limited taxon sampling. Clearly there is a need for more detailed molecular analyses of oniscideans that incorporate more inclusive sampling from the 33 currently recognised families (Gruner *et al*. 1993; Schmidt 2008), preferably utilising type species and genera of families and additional markers. More comprehensive taxon sampling also needs to specifically target southern hemisphere continents, which contain a largely unstudied and undoubtedly rich fauna. Only then will a comprehensive picture of molecular-based relationships be forthcoming that can be rationalised with the available morphological data.

Water conducting system

The unique furrow with modified hair-like setae on the ventral second antennae accompanied by leaf-like scale setae in Paraplatyarthridae distinguished the family from all members of the other oniscidian families examined, and is possibly linked to the development of an efficient water conducting system, required for the arid and semi-arid habitats where most species are found. In regard to the 'platyarthrid' genera studied, *Niambia*, which is widely distributed in southern Africa, has had its status questioned relative to *Trichorhina* (Vandel 1959; Schmalfuss and Ferrara 1978). According to our SEM study, *Niambia* can be separated from *Trichorhina* by the structure of its water conducting system which comprises a simple line of capillary setae (Fig. 4*E*, *F*), whereas this structure is absent in both *Trichorhina* and *Platyarthrus*. In addition, *Trichorhina* species do not have any supra-antennal line while this line is well-developed in *Niambia*. Further, in the *Niambia* species examined, a tracheal system is missing in all pleopod exopodites, which is in agreement with Vandel's (1959) study, while our SEM study also revealed that tracheae are absent in the pleopod exopodites of all paraplatyarthrids and *Trichorhina* spp. examined.

Our morphological examination of the second antenna of members of Armadillidae, Armadillidiidae, Ligiidae, Porcellionidae and Trachelipodidae revealed significant differences in structures associated with the water conducting system, suggesting it is likely to represent a robust suite of characters useful for taxonomic and phylogenetic evaluations at family and genus levels. In *Troglarmadillo* (Armadillidae), the furrow includes elongated setae which are similar to that of Paraplatyarthridae, but no leaf-like scale setae are present on the second antenna peduncle. Schmalfuss (1998) stated that when the second antennae are in contact with wet substrates, water (or fluid) can be either absorbed into the ventral water conducting system to regulate the water budget or emitted as excretion by capillary action. In the *Ligia* species examined there is just one simple furrow along the second antenna from the peduncle to the flagellum which does not include any capillary setae. It is still not clear whether this furrow is a more plesiomorphic form homologous to capillary furrows in other oniscidean groups, or whether it is a completely different structure. Our SEM results also show the cuticle of the second antennae in *Ligia* (found in the littoral zone) is relatively smooth and resembles more closely the condition found in aquatic isopods. In other examined oniscidean species, associated with terrestrial environments, the cuticle on the second antennae has a more complex architecture, for example, in Paraplatyarthridae. Schmalfuss (1978), who examined the structure of oniscidean cuticles using SEM, considered it to have an anti-adhesive function, preventing tiny wet particles from sticking to the body surface. Clearly a fruitful avenue for future research would be a detailed examination of the water conducting system of a wide sampling of oniscideans that assesses morphological differences both among and within genera.

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Appendix 1 Taxon list, geographic location and accession number of oniscidean species examined

Family names in inverted commas indicate they are likely to be non-monophyletic, as postulated in previous studies (see 'Introduction'); generic abbreviations (in brackets) are those used in the text and Figs 2 and3; species with an asterisk are those for which *18S* sequences were obtained from Genbank; voucher numbers are for the Western Australian Museum (WAM) except those indicated by 'Ω' which are deposited in the South Australian Museum

Section and family	Species	Locality	Voucher number	GenBank accession numbers		
				18S rDNA	*28S* rDNA	*COI*
Crinocheta Legrand, 1946						
Armadillidae Brandt & Ratzeburg, 1831	**Cubaris murina* Brandt, 1833	Thailand	–	AJ287064	–	–
	Troglarmadillo sp. 1	Sturt Meadows calcrete, E Murchison, WA	BES15550.1-2	KR424636, KR424635	KR424727	KR424597,KR424598
	Troglarmadillo sp. 2	Lake Miranda West calcrete, E Murchison, WA	BES15537.4/7, 9	KR424637, KR424638, KR424639	KR424726	KR424595,KR424596
	Troglarmadillo sp. 3	Laverton Downs, Shady Well, E Murchison, WA	BES15068.2/14603.1	KR424640, KR424641	–	KR424559,KR424560
Armadillidiidae Brandt, 1833	**Armadillidium nastum* Budde-Lund, 1885	Leipzig, Germany	–	AY048175	–	–
	**Armadillidium opacum* Koch, 1844	Bavaria, Piding near Berchtesgaden, Germany	–	AY048176	–	–
	Armadillidium vulgare Latreille, 1804	Adelaide, SA	ΩJA252-3-2012	KR424674, KR424675	KR424717, KR424718	KR424608,KR424609
Cylisticidae Verhoeff, 1949	**Cylisticus convexus* De Geer, 1778	Bavaria, Piding near Berchtesgaden, Germany	–	AJ287059	–	–
Detonidae Budde-Lund, 1906	*Deto marina* Chilton, 1884	Hallet Cove, SA	ΩJA258-9-2012	KR424676, KR424677	KR424728, KR424729	KR424585,KR424586
Oniscidae Latreille, 1802	**Oniscus asellus* Linnaeus, 1758	Bielefeld, Germany	–	AF255699.1	–	–
Paraplatyarthridae, fam. nov.	*Paraplatyarthrus*, gen. nov. (taxon 1)	Mt Morgans, E Murchison, WA	ΩJA100/103-2011	KR424624	–	KR424573, KR424572
	Paraplatyarthrus, gen. nov. (taxon 2)	Marradong, WA	ΩJA152/154/155-2011	KR424656, KR424657	KR424697, KR424696	KR424568, KR424569, KR424570
	Paraplatyarthrus, gen. nov. (taxon 3)	Sturt Meadows calcrete, E Murchison, WA	BES15548.1/15551.8-9	KR424627, KR424659, KR424658	KR424686, KR424687, KR424688	KR424563, KR424561, KR424562
	Paraplatyarthrus, gen. nov. (taxon 4)	Lake Miranda east calcrete, E Murchison, WA	BES15543.3	KR424665	KR424694	KR424567
	Paraplatyarthrus, gen. nov. (taxon 5)	Lake Miranda west calcrete, E Murchison, WA	BES15538.10	KR424664	–	KR424566

(*continued next page*)

Appendix 1. (*continued*)

Section and family	Species	Locality	Voucher number	GenBank accession numbers		
				18S rDNA	*28S* rDNA	*COI*
	Paraplatyarthrus subterraneus Javidkar & King, gen. & sp. nov. (taxon 6)	Laverton Down south calcrete, E Murchison, WA	WAM C53623-29/ BES15525.20/ BES15525.23/15524.6	KR424667, KR424625	KR424698	KR424564,KR424565
	Paraplatyarthrus, gen. nov. (taxon 7)	Cunyu calcrete, E Murchison, WA	BES15090.1/3	KR424660, KR424661	–	KR424580,KR424581
	Paraplatyarthrus, gen. nov. (taxon 8)	Uramurdah Lake calcrete, E Murchison, WA	BES15067.1/15088.1/ 15095.2	KR424673, KR424670, KR424671	–	KR424575, KR424577, KR424574
	Paraplatyarthrus, gen. nov. (taxon 9)	Lake Violet south, E Murchison, WA	BES16476.1/3	KR424668, KR424669	KR424690, KR424692	KR424579,KR424578
	New undescribed genus (taxon 10)	Porto Alegre, Belém Novo, RS, Brazil	ΩJa243-4-2011	KR424653, KR424654	KR424699, KR424700	KR424619,KR424621
Philosciidae Kinahan, 1857	**Philoscia muscorum* Scopoli, 1763	Leipzig, Germany	–	AJ287058.1	–	–
'Philosciidae'	*Haloniscus* sp. 1	Laverton Downs Windarra calcrete, E Murchison, WA	BES15094.1-2/14621.1	KR424645, KR424644, KR424646	KR424720	KR424611, KR424610, KR424612
	Haloniscus sp. 2	Lake Violet south calcrete, E Murchison, WA	BES15085	KR424649	–	KR424615
	Haloniscus sp. 3	Lake Miranda east calcrete, E Murchison, WA	BES15082/15082.1	KR424647, KR424648	KR424719	KR424613,KR424614
	Haloniscus sp. 4	Uramurdah calcrete, E Murchison, WA	BES15088.2-3/15089.3	KR424650, KR424651, KR424652	–	KR424617, KR424616, KR424618
	Genus indeterminate (taxon 11)	Douglas Scrub, SA	ΩJA249-2012	KR424678	–	KR424603
'Platyarthridae' Verhoeff, 1949	*Niambia* sp. 1	Bostwana, Africa	ΩJA245-2012	KR424626	KR424710	KR424591
	Niambia sp. 2	Adelaide, SA	ΩJA224-5-2012/JA237-9	KR424629, KR424628	KR424703, KR424709, KR424708, KR424707, KR424705	KR424588, KR424590, KR424589
	Platyarthrus aiasensis Legrand, 1954	Capo S Marco, Sinis Peninsula, Sardegna, Italy	ΩJA266-2011	–	–	–
	**Platyarthrus schoeblii* Budde-Lund, 1885	Mallorea, Perguera, Spain	–	AJ287060	–	–
	Platyarthrus costulatus Verhoeff, 1908	Giara di Gesturi, Sardegna, Italy	ΩJA267-2011	–	–	–

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Appendix 1. (*continued*)

Section and family	Species	Locality	Voucher number	GenBank accession numbers		
				18S rDNA	*28S* rDNA	*COI*
	Platyarthrus hoffmannseggii Brandt, 1833	Monte Moreilo, N of Florence, Italy	ΩJA204-5-2011	KR424630, KR424631	KR424711, KR424712	KR424592,KR424594
	Trichorhina anophthalma Arcangeli, 1936	De Santo António, Algar, Portugal	ΩJa268-2012	–	–	
	Trichorhina tomentosa Budde-Lund, 1893	Varginha Clube, MG, Brazil	ΩJA201-2/248-2012	KR424633, KR424634, KR424632	KR424715	KR424599,KR424600
Porcellionidae Brandt & Ratzeburg, 1831	**Porcellio scaber* Latreille, 1804	Leipzig, Germany	–	AJ287062	–	–
	**Porcellio spinicornis* Say, 1818	Drebkau, Germany	–	AY048183	–	–
	Porcellionides pruinosus Brandt, 1833	Laverton Downs,Mt Windarra, E Murchison, WA	BES16577.1-2	KR424622	KR424723, KR424724	KR424606,KR424607
	**Porcellionides sexfasciatus* Koch, 1847	Malorea, Es Trenc, Spain	–	AY048182	–	–
Stenoniscidae Verhoeff, 1908	Genus indeterminate	Laverton Downs,Mt Windarra calcrete, E Murchison, WA	BES16022-3	KR424643, KR424642	KR424721, KR424722	KR424604, KR424605
Trachelipodidae Strouhal, 1953	**Porcellium fiumanum* Verhoeff, 1918	Bavaria, Piding near Berchtesgaden, Germany	–	AY048180	–	–
	**Protracheoniscus politus* Koch, 1841	NeiBe valley near Hirschfelde, Germany	–	AY048184	–	–
	Orthometopon dalmatinum Verhoeff, 1901	Ionian Island, Greece	ΩJA264-2013	–	–	–
	Trachelipus cavaticus Schmalfuss, Paragamian & Sfenthourakis, 2004	Crete Island, Greece	ΩJA265-2013	–	–	–
	**Trachelipus ratzeburgii* Brandt, 1833	NeiBe valley near Hirschfelde, Germany	–	AJ287063	–	–
Diplocheta Vandel, 1957						
Ligiidae Leach, 1814	**Ligia oceanica* Linnaeus, 1767	Galicia, Spain	–	AF255698	–	–
	Ligia sp.	Rapid Bay, SA	ΩJA256-7-2012	KR424680, KR424681	KR424684, KR424685	KR424601, KR424602
	**Ligidium germanicum* Verhoeff, 1901	Bavaria, Piding near Berchtesgaden, Germany	–	AY048179	–	–

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Appendix 1. (*continued*)

Section and family	Species	Locality	Voucher number	GenBank accession numbers		
				18S rDNA	*28S* rDNA	*COI*
	**Ligidium hypnorum* Cuvier, 1792	Leipzig, Germany	–	AJ287056	–	–
Synocheta Legrand, 1946						
Styloniscidae Vandel, 1952	*Styloniscus* sp.	Douglas Scrub, SA	ΩJA250-2012	KR424679	KR424725	KR424587
Trichoniscidae Sars, 1899	**Haplophthalmus danicus* Budde-Lund, 1880	Leipzig, Germany	–	AJ287066	–	–
	**Hyloniscus riparius* Koch, 1838	Leipzig, Germany	–	AJ287065	–	–
	**Trichoniscus pusillus* Brandt, 1833	Leipzig, Germany	–	AJ287067	–	–
Outgroups						
Amphipoda	**Gammaracanthus lacustris* Sars, 1867	Savonranta, Finland	–	JF966191.1	–	–
	**Orchestia* sp.	Hallet Cove, SA	ΩJA261-2012	KR424682	–	–
Decapoda	**Heterocarpus* sp.	Taiwan	–	JF346257	–	–
Isopoda	*Sphaeroma serratum* Fabricius, 1787	Hallet Cove, SA	ΩJA263-2012	KR424683	–	KR424584
Syncarida	**Brevisomabatynella magna* Cho & Humphreys, 2009	Cunyu, WA	–	JQ446078.1	–	–