CSIRO PUBLISHING

Invertebrate Systematics, 2015, **29**, 223–238
http://dx.doi.org/10.1071/IS14043

Mitochondrial DNA allows the association of life stages to facilitate species recognition and delimitation in Australian stoneflies (Plecoptera : Gripopterygidae : *Newmanoperla*)

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Abstract. The larvae of stoneflies (Plecoptera) are important indicators for monitoring aquatic ecosystems, but the immature stages of some relevant species have not been described. Here, mitochondrial gene sequences are used to associate the adult and larval life stages for species of *Newmanoperla* McLellan. This study finds molecular and morphological support for five species, which include the four previously described species (*N. exigua*, *N. hackeri*, *N. prona* and *N. thoreyi*) and a newly recognised species, *N. theischingeri*, sp. nov., which is described herein. Molecular divergences between species for the COI fragment had minimum values of 15–18% while the maximum intraspecific divergence was 6–9%, and there was no overlap between species. Morphological characters for distinguishing the larvae of the five species were observed on the femora and included variations in the type of setation present and the area of occurrence. The combination of molecular and morphological methods enabled the larval morphology to be reassessed and has led to the following outcomes: the first formal generic larval description, a newly recognised species, updated descriptions for larvae of all species of *Newmanoperla* and a dichotomous key to larvae.

Additional keywords: Australia, DNA barcoding, cytochrome *c* oxidase subunit 1, taxonomy.

Received 29 July 2014, accepted 16 April 2015, published online 30 June 2015

Introduction

Plecoptera (stoneflies) are regarded as key indicator species for aquatic monitoring programs worldwide due to their ecological requirements, such as well-oxygenated water, and are used in combination with other benthic macroinvertebrates to assess water quality (Helešic 2001). Traditionally, many of these programs rely on sampling that targets the water column and benthos, which for stoneflies means identification of the larval form and not the terrestrial adult life stage. Reliance on larvae has posed issues in Australia as the current taxonomy of many stonefly species is restricted to the adult life stage. This disparity in taxonomy has left many species with larval forms either undescribed or with relatively poor descriptions, and therefore of lesser value in monitoring programs.

Stoneflies are a small insect order with ~3500 species worldwide (Fochetti and Tierno de Figueroa 2008). The majority of species (3058) occur in the northern hemisphere and 11 of the 16 stonefly families are considered as being of northern origin (Fochetti and Tierno de Figueroa 2008). The Australian fauna comprises ~190 species in four families. Of these, Gripopterygidae is by far the most species-rich and currently includes 12 genera and 134 species (Australian Biological Resources Study 2009).

The genus *Newmanoperla* (Gripopterygidae) was erected by McLellan in 1971 with two species – *N. thoreyi* (previously *Paranotoperla thoreyi* Banks, 1920; designated as the type species) and a newly recognised species, *N. hackeri* – described from southern Queensland and north-eastern New South Wales. Both species were described only from adult material. The larva of *Newmanoperla thoreyi* was first figured and briefly described by Hynes (1978), with a more detailed description provided by Suter and Bishop (1990). Since McLellan's (1971) work, a species described from adult material by Kimmin (1951) as *Leptoperla exigua* from Western Australian was synonymised with *N. thoreyi* but reinstated as *N. exigua* by Hynes (1982). The larval description of *N. exigua* was provided by Hynes and Bunn (1984). A further species, *N. prona*, was described from Tasmania by Hynes (1982) for both adult and larval life stages. A review of museum specimens by Theischinger and Cardale (1987) suggested an additional species, listed as *N.* cf. *hackeri*, based on adult material examined from north-eastern Queensland. Theischinger and Cardale (1987) considered this species to be tropical but did not formally name the taxon. Thus, there are currently five putative species of *Newmanoperla*: four described species (*N. exigua*, *N. hackeri*, *N. prona* and *N. thoreyi*) and *N.* cf. *hackeri*, as suggested by Theischinger and Cardale (1987).

A generic description of adults, or method for discriminating among species, has been stated in McLellan (1971), Hynes (1982) and Theischinger and Cardale (1987), but no formal generic

description of the larvae has been made. The larval characters currently used to distinguish the genus are those of the type *N. thoreyi*, as described in Hynes (1978).

This study aimed to associate the adult and larval life stages and increase our knowledge of larval taxonomy for the genus *Newmanoperla*, following similar studies of other stoneflies (e.g. Mynott *et al*. 2011; Boumans and Baumann 2012; Avelino-Capistrano *et al*. 2014). Descriptions provided in this paper are for larvae only, as detailed adult descriptions are contained in the literature and condensed in Theischinger and Cardale (1987). A formal generic description of the larvae of *Newmanoperla* is provided, as well as a larva-based taxonomic key to species.

Materials and methods

Specimen sampling

Specimens were collected in eastern Australia and south-western Western Australia (WA) with a focus on published locations recorded for the genus and museum records (site specific information listed in 'Material examined'; specimens used in genetic analyses shown in Table 1). A map of sampled locations is shown in Fig. 1. Aquatic sampling was conducted using dip nets and by hand picking of substrates such as waterfalls, fast flow rocky edge areas and submerged logs. Terrestrial sampling used a sweep net and black-light trapping with hand picking of specimens to reduce by-catch. All specimens were preserved in the field in 100% ethanol. Some specimens of *N. exigua* and two specimens considered to have slightly different morphology from *N. exigua* (referred to as *N.* sp. 1), from Western Australia were provided by the WA Department of Parks and Wildlife (DPaW, WA).

Adult males were identified to species using Theischinger and Cardale (1987). Larvae were identified using the original descriptions or the keys of Hynes (1978, 1989) and Yule (1997). Specimen imaging was performed using a Nikon SMZ1500 microscope and Nikon DS-Fi1 camera (Kawasaki, Kanagawa, Japan) running NIS-Element F ver. 3.2. Helicon Focus ver. 5.3.7 (Helicon Soft, Ukraine, www.heliconsoft.com) was used to create montaged photographs.

Molecular methods

Genetic techniques were used to confirm life stage associations where possible and were accepted only when sequences from identified adults nested with larval sequences or where a shared haplotype was shown (e.g. method of Zhou *et al*. 2007). Using shared genetic regions across life stages as the basis for associations applies the history-based phylogenetic species concept (as described by Baum and Donoghue 1995). Following similar life stage association studies on stoneflies (Gray 2009; Mynott *et al*. 2011; Avelino-Capistrano *et al*. 2014), the molecular data generated in this study were from mitochondrial DNA. DNA was extracted from a leg of selected specimens using a Qiagen DNeasy blood and tissue kit (Qiagen, Hildren, Germany) following standard protocols (Qiagen Handbook 2006). Two regions of the cytochrome *c* oxidase subunit 1 gene (COI) were amplified: the 5′ 'DNA barcode region' (COI5′; primers HCO2198 and LCO1490; Folmer *et al*. 1994) and the 3′ region (COI3′; primers Pat and Jerry; Simon *et al*. 1994). A full fragment of COI5′ for one specimen (JMH182) was not able to be obtained and an internal primer (COI-214Fm: 5′-GGDGCHCCWGAYATRGCWTTY CC-3′) was used with HCO2198. All primers were M13-tailed to facilitate sequencing. Initial reactions were conducted with the COI5′ fragment and analysed, after which a subset was chosen for reactions with the COI3′ fragment. Polymerase chain reaction (PCR) conditions for both COI fragments followed those of Webb and Suter (2010): 60 s at 94°C; five cycles of 60 s at 94°C, 90 s at 45°C, 90 s at 72°C; 35 cycles of 60 s at 94°C, 60 s at 50°C, 60 s at 72°C; and a final cycle of 4 min at 72°C. Polymerase chain reaction preparations consisted of 4 µL buffer reagent, 20 µL 50 mM $MgCl_2$, 0.8 µL of each primer, 0.1 µL Platinum taq polymerase (Invitrogen, Melbourne), 0.5–2 µL of DNA template, and ddH_2O to 40 µL. Polymerase chain reaction products were sent to Macrogen (Seoul, Republic of Korea) for purification and sequencing.

Genetic analysis

Sequence data were assembled in DNA Baser version 2.91.5 (Heracle BioSoft SRL, Romania, www.DnaBaser.com) with mismatches, if present, assessed visually. Alignments were generated in ClustalX in MEGA ver. 5.2 (Tamura *et al*. 2011) and translated to protein sequences to check for stop codons. Base frequency composition was assessed in MEGA and showed an unequal base frequency within the gene (average COI5′: A 24.9%, C 22%, G 19.9%, T 33.3%; average COI3′: A 26.5%, C 21.9%, G 17.5%, T 34.1%). Base composition was also assessed by codon position with COI5′ and COI3′ both showing a stronger AT bias at the more variable third codon position (COI5′: 1st codon 48.9%, 2nd codon 55.6%, 3rd codon 69.1%; COI3′: 1st codon 52.5%, 2nd codon 59.4%, 3rd codon 69.6%). Neighbour-joining (NJ) analysis was performed in MEGA using the Tamura-Nei substitution model (assumes unequal base frequencies), pairwise deletion option for missing data and 2000 bootstrap pseudoreplicates. Pairwise distances were calculated in MEGA using the p-distance model (uncorrected proportional difference) to assess divergence distances within and between the morphologically identified species. Lower pairwise distances are an indication of fewer changes in the nucleotide with lower p-distance values expected between sequences within a species than between the individual sequences of another species (Meyer and Paulay 2005). All new sequences from this study have been deposited on GenBank with accession numbers: COI5′ KP775643–KP775667 and COI3′ KP775668–KP775682.

Bayesian analyses were run to further assess support for the monophyly of the species using a non-distance-based method. Bayes analyses were performed in MrBayes version 3.2.2 (Huelsenbeck and Ronquist 2001) through the CIPRES portal (Miller *et al*. 2010). Prior to Bayesian analyses being run, models of nucleotide evolution were assessed for both the COI5′ and a concatenated dataset (subsequently referred to as COI53′) fragments by codon position (due to the variation in base composition at the 1st, 2nd and 3rd codon positions) using MrModeltest ver. 2.3 (Nylander 2004) run through PAUP* ver. 4.0 (Swofford 2003). The models of best fit selected for both COI fragments (individual COI5′ and concatenated COI53′) using the Akaike Information Criterion (AIC) were 1st position GTR+I (generalised time reversible model plus invariant sites),

Table 1. Location, voucher and GenBank accession numbers for *Newmanoperla* specimens used in genetic analysis

Asterisk (*) in georeferenced field denotes coordinates that have been estimated from location details. Western Australian site codes are those of WA Department of Parks and Wildlife

Species	Life stage	Location	Georeference	Collection date	Voucher number	GenBank accession number (COI5′)	GenBank accession number (COI3′)
Austroperlidae	Larva	Tas.: Creek at Lake Dobson, bridge at car park, Lake Dobson Road	Lat. –42.685° Long. 146.5942°	10.i.2012	JMH484	KP775666	KP775681
Austroperlidae	Larva	NSW: Polblue Creek at Polblue picnic ground	Lat. –31.9258° Long. 151.3875°	24.ii.2011	JMH482	KP775665	KP775680
Austroperlidae	Larva	Tas.: Bird River at Bird Bridge. Bird Track, Franklin-Wild Rivers National Park	Lat. –42.3431° Long. 145.5906°	7.i.2012	JMH495	KP775667	KP775682
N. exigua	Larva	WA: Stirling Dam (site code HAR21)	Lat. –33.10829° Long. 116.04199° *	15.ix.2010	JMH223	KP775660	KP775677
N. exigua	Larva	WA: Stream near Hoffman's Mill (site code HAR01	Lat. –33.00183° Long. 116.084210° *	07.x.2009	JMH231	KP775662	KP775678
N. exigua	Adult ♀	WA: River below Serpentine Falls	Lat. –32.36806° Long. 116.01111°	18.ix.2008	JMH197	KP775654	KP775671
N. exigua	Larva	WA: Rosa Brook, Lawson Road (site code BLA54)	Lat. –33.94292° Long. 115.49375° *	20.x.2009	JMH233	KP775663	–
N. exigua	Larva	WA: Big Hill Brook at Wheatley Coast Road	Lat. –34.49333° Long. 116.18083°	20.ix.2008	JMH182	KP775651	–
N. exigua	Larva	WA: Finlay Brook (site code MRY33)	–	06.x.2009	JMH224	KP775661	–
N. hackeri	Larva	NSW: Polblue Creek at Polblue picnic ground	Lat. –31.9258 Long. 151.3875	24.ii.2011	JMH1333	KP775647	–
N. hackeri	Larva	NSW: Williams River at Rocky Crossing	Lat. –32.11666 Long. 151.48334	11.xi.2012	JMH1206	KP775646	KP775669
N. hackeri	Adult ♂	NSW: Allyn River at upper campground	Lat. –31.1292 Long. 151.4734	11.xi.2012	JMH1536	KP775648	–
N. hackeri	Adult ♂	NSW: Allyn River at upper campground	Lat. –31.1292 Long. 151.4734	11.xi.2012	JMH1537	KP775649	–
N. hackeri	Adult ♂	Qld: Morans Creek above Morans Falls, O'Reillys (Green Mountains) section	Lat. –28.2318 Long. 153.125	17.xi.2011	JMH1202	KP775644	KP775668
N. hackeri	Adult ♂	Qld: Morans Creek above Morans Falls, O'Reillys (Green Mountains) section	Lat. –28.2318 Long. 153.125	16.xi.2012	JMH1129	KP775643	–
N. prona	Larva	Tas.: Esperance River at bridge Esperance River Road (crossroads with Rutherfords Road)	Lat. –43.27697 Long. 146.87688	15.i.2012	JMH307	KP775664	KP775679
N. theischingeri, sp. nov.	Larva	Qld: Rainforest stream on Kirrama Range Road.	Lat. –18.21372 Long. 145.79813	16.v.2010	JMH200	KP775655	KP775672
N. theischingeri, sp. nov.	Adult ♀	Qld: Rainforest creek with waterfall on Kirrama Range Road	Lat. –18.2108 Long. 145.8075	16.v.2010	JMH201	KP775656	KP775673
N. thoreyi	Larva	NSW: Bullock Head Creek crossing Snowy Mountains Highway	Lat. –35.8405 Long. 148.4911	30.xi.2009	JMH191	KP775652	KP775670

(*continued next page*)

Table 1. (*continued*)

Species	Life stage	Location	Georeference	Collection date	Voucher number	GenBank accession number (COI5′)	GenBank accession number (COI3′)
N. thoreyi	Larva	NSW: Diggers Creek crossing Summit Road	Lat. –36.36037 Long. 148.4868	01.xii.2009	JMH196	KP775653	–
N. thoreyi	Larva	NSW: Sawpit Creek crossing Summit Road	Lat. –36.35168 Long. 148.56599	01.xii.2009	JMH1205	KP775645	–
N. thoreyi	Larvae	Vic.: Snowy Creek on Omeo Highway, near Mitta Mitta	Lat. –36.545 Long. 147.384	12.viii.2009	JMH206; JMH207	KP775657; KP775658	KP775674; KP775675
N. thoreyi	Adult ♂	Vic.: McMahons Creek crossing Warburton-Woods Point Road	Lat. –37.70146 Long. 145.8303	12.xi.2008	JMH1538	KP775650	–
N. thoreyi	Larva	Vic.: East branch Buffalo River	Lat. –37.0407 Long. 146.83785	06.x.2009	JMH211	KP775659	KP775676

2nd position F81 (Felsenstein) and 3rd position GTR+G (generalised time reversible model plus gamma distributed). Bayesian analyses used the following input parameters for all runs: sequence data, partition by codon position, individual nucleotide models, default Markov chain Monte Carlo (MCMC) command (four chains), default number of runs (two), random starting tree, sampling frequency 1000, generations 5 000 000, parameters unlinked and a set burn-in of 25%. The concatenated dataset was restricted by the sequences that were generated for the COI3′ fragment: specimens for which only a COI5′ sequence was obtained were excluded from the concatenated dataset. Trace plots of MrBayes log files were viewed in TRACER version 1.5 (Rambaut and Drummond 2007) to visually check whether the analysis had reached stationarity. All runs had potential scale reduction factor (PSRF) values of 1 and effective sample size (ESS) greater than 200. Bayesian consensus trees were viewed in FigTree ver. 1.4.

Results

Life stage associations

The genetic analyses for the COI5′ fragment contained 25 sequences (22 *Newmanoperla* spp. and three Austroperlidae). Twenty-one sequences were the full COI5′ fragment of 657 base pairs (bp) with one bp trimmed so that the fragment started at a first codon position. The remaining four sequences were smaller fragments due to poorer quality sequencing results or being obtained using an internal forward primer: JMH495 (608 bp), JMH1205 (602 bp), JMH1538 (594 bp) and JMH182 (376 bp).

Neighbour-joining and Bayesian analyses (Fig. 2) showed five genetically distinct groups corresponding with the four morphologically identified species: *N. exigua*, *N. hackeri*, *N. prona* and *N. thoreyi*, and a morphologically and geographically distinct grouping regarded as a new species (*N. theischingeri*, sp. nov.). Four of these clades contained sequences that either nested with, or shared haplotypes across, life stages. The exception was *N. prona*, for which there was only a single sequence. The shared morphology between identified species and the nesting of corresponding sequences satisfies the history-based phylogenetic species concept. Two clades, *N. thoreyi* and *N. hackeri*, included adult males and larvae and thus provide life stage associations. The larva of *N. hackeri* had not previously been associated with adults and described. A single larval specimen of *N. prona* was collected during this study and was genetically distinct from the other species of *Newmanoperla*. The other two clades each comprised an adult female and one (*N. theischingeri*, sp. nov.) or more (*N. exigua*) larvae. Two sister clades (clades C and D; Figs 2, 3) were supported for *N. exigua*, one clade (Clade D) contained an adult female and three larvae while the other clade (Clade C) contained two larvae that had been suggested as a novel morphotype, *N.* sp. 1, by the DPaW, WA. The remaining clade contained only two specimens (an adult female and a larva) of *Newmanoperla* collected from Kirrama National Park (north-eastern Queensland). The larva in this clade showed distinct morphological characters and the female and sample location are similar to that described in Theischinger and Cardale (1987) as *N.* cf. *hackeri*. The distinct genetic separation of the clade from the other *Newmanoperla* species (minimum pairwise distance between the other clades ranged from 15% to

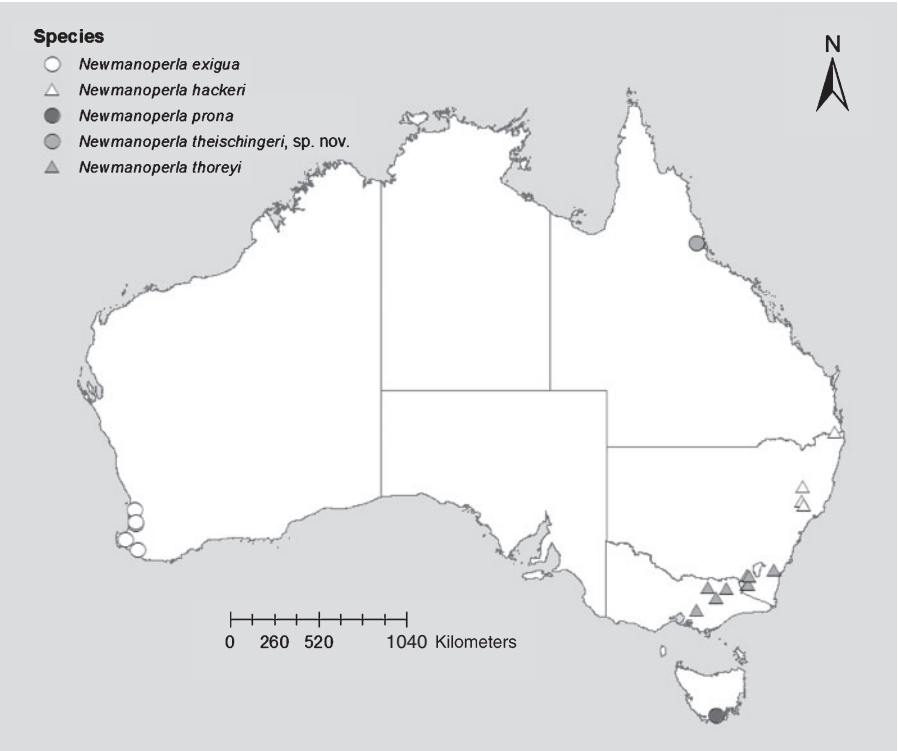

Fig. 1. Map of Australia with locations of specimens collected in this study labelled by species.

20% for the COI5′ data) is further indicative of support for a tropical *Newmanoperla* species, described in this paper as *N. theischingeri*, sp. nov.

The COI3′ fragment length for eleven sequences was 764 bp, with the remaining four sequences being smaller fragments due to poorer quality sequencing results: JMH197 (718 bp), JMH231 (709 bp), JMH211 (658 bp) and JMH1202 (472 bp). The analysis with the concatenated COI3′ and COI5′ dataset (Fig. 3) included 15 specimens (12 *Newmanoperla* spp. and three Austroperlidae specimens), with results showing similar topology to the COI5′ derived tree and supporting the same clades. The COI5′ and COI53′ derived trees both show *N. thoreyi* as sister to the other *Newmanoperla* species. The placement of *N. prona* in the COI5′ NJ tree had low support and was highly variable, but in the COI53′ Bayesian tree the placement of *N. prona* was well supported as sister to a clade comprising *N. exigua*, *N. hackeri* and *N. theischingeri*, sp. nov.

Inter- and intraspecific divergences

Inter- and intraspecific divergences were based on the five clades that corresponded to the four previously described *Newmanoperla* species (*N. exigua*, *N. hackeri*, *N. prona* and *N. thoreyi*) and a fifth clade (*N. theischingeri*, sp. nov.) that is morphologically and genetically distinct from the four described *Newmanoperla* species. All five clades showed a large minimum interspecific genetic divergence for the COI5′ dataset that ranged from 15% to 20%, and all were distinctly higher than all maximum intraspecific divergences (Table 2). Three clades showed high maximum intraspecific divergence distances: *N. hackeri* (9%), *N. exigua* (7%) and *N. thoreyi* (8%). The *N. hackeri* clade comprised two sister clades (clades A and B; Figs 2, 3) that had intraspecific divergences between the two clades of 8–9%. The specimens in the two clades were from the opposite ends of the known geographic range for this species. One clade (Clade B) contained two specimens (JMH1202, JMH1129) from Lamington National Park (southern Queensland) whereas the other clade (Clade A) contained four specimens (JMH1206, JMH1333, JMH1536, JMH1537), sampled from Barrington Tops National Park (central-eastern New South Wales).

The pairwise p-distances from the COI53′ data (Table 3) were generally lower than those of the COI5′ data, although there were fewer specimens included. The minimum interspecific pairwise distances ranged from 15% to 18% and maximum intraspecific distances were: *N. exigua* 6%, *N. thoreyi* 6% and *N. hackeri* 9%.

Base composition frequency

The base frequencies recorded for *Newmanoperla* were compared with other datasets for previous stonefly studies (Table 4; Mynott *et al*. 2011; Weiss *et al.* 2012; Boumans and Baumann 2012) and showed similar composition to the genera from these studies. For COI5′, the average AT content recorded in this study (58.2%) was slightly lower but similar to the other genera (AT content 59.5–60.5%), with this pattern similarly across the individual codon positions (Table 4). The combined COI53′ dataset had average base frequencies for the genus *Newmanoperla* of: A 25.7%, C 22.1%, G 18.5% and T 33.6%. Total average AT frequency for the COI53′ fragment was 59.3% and when assessed by codon position the average values were: 1st position AT

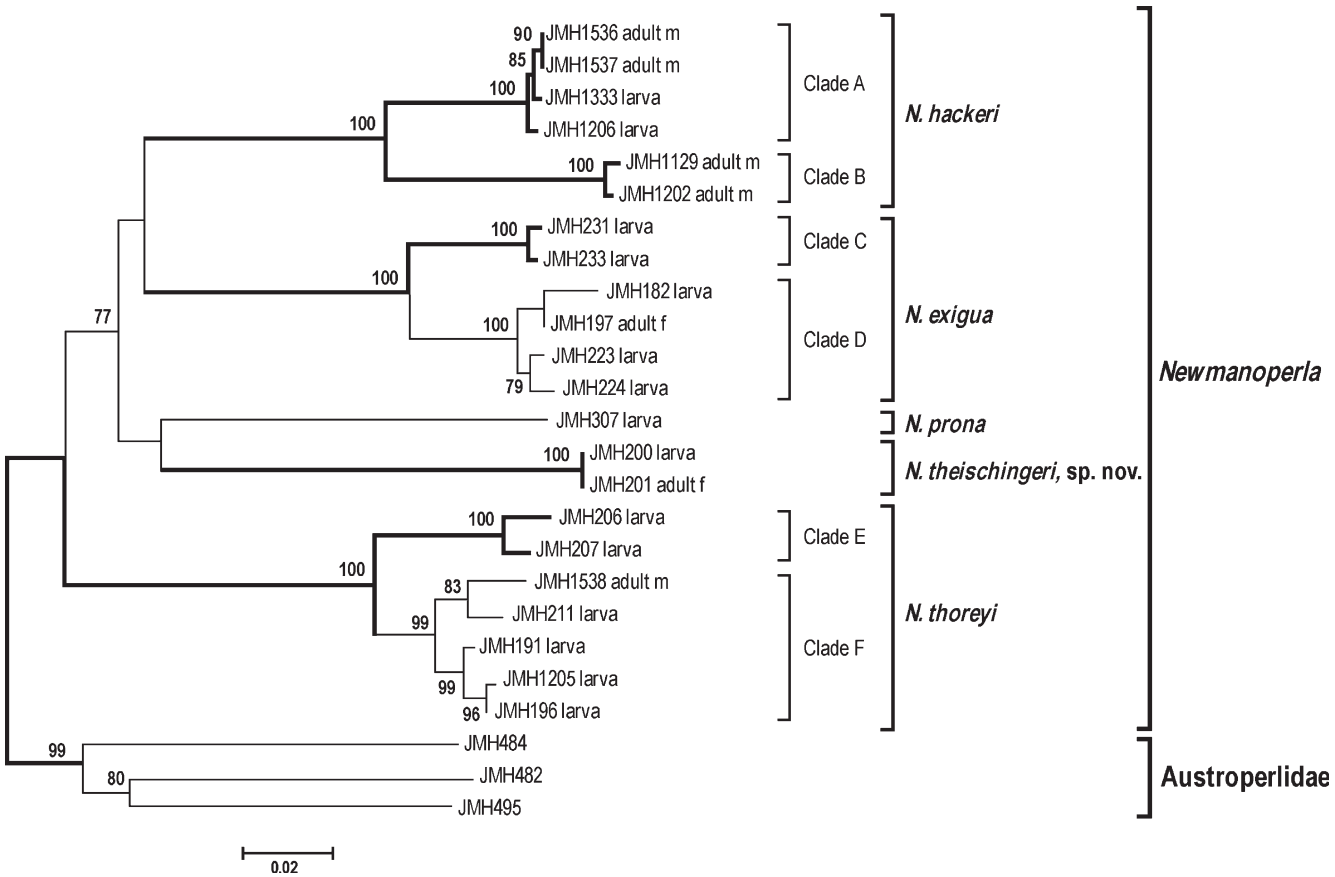

Fig. 2. Neighbour-joining (Tamura-Nei model of evolution) for COI5′ sequences tree using a K2P model of evolution with 2000 bootstrap replicates and pairwise deletion. Only bootstrap values ≥75% are shown. Bolded branches are those also supported in Bayesian analysis (posterior probability = 1). Scale bar indicates substitutions per site.

50.1%, 2nd position AT 57.7% and 3rd position AT 69.4%. This was comparable with the *Siphonoperla* dataset (Weiss *et al.* 2012), shown in Table 4, for which comparable sequence data were available. Insects in general are AT-biased especially within the mitochondrial genome (Cameron 2014). The AT content of the stonefly genera reported here appears to be comparable, although low, with previously shown COI data for Coleoptera (59–73%; Sheffield *et al*. 2009: 390, fig. 7).

Discussion

Combining morphological and molecular data can enable the rapid association of life stages and provide support for species delimitations (Zhou *et al*. 2007; Mynott *et al*. 2011). In this study, adult and larval morphology was initially assessed from collected material to determine species-level identifications for the *Newmanoperla* species, with additional morphotypes (*N.* sp. 1 and *N. theischingeri*, sp. nov.) also identified. Molecular techniques were then used to assess support for the morphological designations and to determine life stage associations, applying the history-based phylogenetic species concept. By using multiple lines of evidence (morphology, geography and molecular), support has been shown for five *Newmanoperla* species: *N. exigua*, *N. hackeri*, *N. prona*, *N. theischingeri*, sp. nov. and *N. thoreyi*.

The use of divergence distance-based data for species delimitations (in this study uncorrected pairwise p-distance) assumes that the intraspecific distances will be lower than the interspecific distances. For COI data this trend is referred to as the ‘barcode gap’ (Hebert *et al*. 2003). In this study, the molecular data showed no overlap between the morphologically grouped inter- and intraspecific divergence distances (COI5′: minimum 15–20% and maximum 7–9%, respectively; concatenated COI53′: minimum 15–18% and maximum 6–9%) and suggested a significant gap of 6% between inter- and intraspecific distances. However, if using a strict interpretation of the COI barcode species concept, using only molecular data, the intraspecific divergences would be arbitrarily placed at ≤3% (Hebert *et al*. 2003), and if applied to this dataset would suggest a further three ‘species’. The grouping into these eight ‘barcode species’ would show the presence of a small ‘barcode gap’ (1%), but would have no current morphological or ecological support. The identification of the five *Newmanoperla* species in this study is based on multiple lines of evidence: morphological, geographical and molecular support (using the history-based phylogenetic species concept).

The maximum intraspecific COI divergence for some species of *Newmanoperla* could be regarded as high (Hebert *et al*. 2003); however, similar results have been recorded in previous

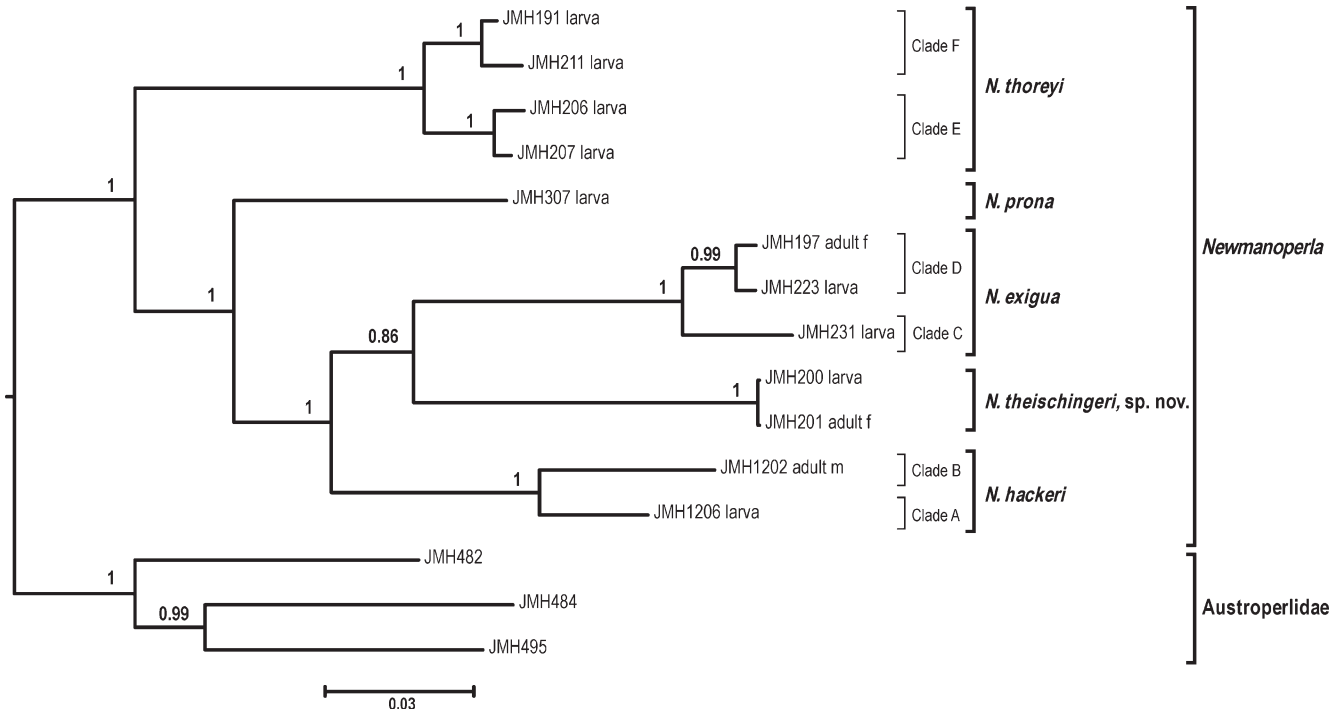

Fig. 3. Consensus Bayesian tree of concatenated COI5′ and COI3′ sequences. Analysis run with dataset partitioned by codon position (models of evolution were: 1st position GTR+I, 2nd position F81 and 3rd position GTR+G) and parameters unlinked for 5 000 000 generations. Scale bar indicates substitutions per site.

Table 2. Ranges of pairwise divergence (p-distance) among *Newmanoperla* taxa for the COI5′ dataset
Maximum intraspecific values in bold

Species	*N. hackeri*		*N. prona*		*N. exigua*		*N. thoreyi*		*N. theischingeri*, sp. nov.	
	min	max	min	max	min	max	min	max	min	max
N. hackeri	0.00	**0.09**	0.19	0.21	0.15	0.18	0.16	0.21	0.20	0.22
N. prona			–	–	0.19	0.20	0.20	0.21	0.16	0.16
N. exigua					0.00	**0.07**	0.16	0.19	0.17	0.19
N. thoreyi							0.00	**0.08**	0.20	0.21
N. theischingeri, sp. nov.									0.00	–

Table 3. Estimated evolutionary divergence distances for *Newmanoperla* COI53′ sequence dataset (p-distance)
Maximum intraspecific values in bold

Species	*N. hackeri*		*N. prona*		*N. exigua*		*N. thoreyi*		*N. theischingeri*, sp. nov.	
	min	max	min	max	min	max	min	max	min	max
N. hackeri	–	**0.09**	0.16	0.17	0.15	0.17	0.16	0.18	0.17	0.18
N. prona			–	–	0.16	0.17	0.17	0.17	0.17	0.17
N. exigua					0.01	**0.06**	0.16	0.18	0.16	0.16
N. thoreyi							0.02	**0.06**	0.18	0.18
N. theischingeri, sp. nov.									0.00	–

molecular studies on stoneflies, for example: *Riekoperla alpina* 5.8%, *R. karki* 5%, *R. compressa* 4.2% (Mynott *et al*. 2011); *Siphonoperla torrentium* 6.2%, *S. hajastanica* 5.1% (Weiss *et al*. 2012); *Kempnyia alterosarum* 6.4%, *K. colossica* 15.1%, *K. gracilenta* 11.2%, *K. jatim* 7.7%, *K. obtusa* 9.6%, *K. petersorum* 4.6%, *K. varipes* 9.6% (Avelino-Capistrano *et al*. 2014). These studies demonstrated large interspecific COI distances with no overlap shown with intraspecific

Table 4. Comparison of base compositional bias among stonefly genera
All values are percentage of AT content by fragment (all positions) and codon position (pos.)

	COI5′ fragment				COI3′ fragment			
	All pos.	1st pos.	2nd pos.	3rd pos.	All pos.	1st pos.	2nd pos.	3rd pos.
Amphinemura	60.5	48.8	56.4	75.8				
Newmanoperla	58.2	48.9	55.6	69.1	59.3	50.1	57.7	69.4
Riekoperla	60.0	49.9	55.7	74.3				
Siphonoperla	59.5	48.1	56.4	74.3	61.1	51.1	58.4	74.1

divergences; although, in another study (Fochetti *et al*. 2011), overlap was shown in the genus *Besodolus*. In these studies, authors did not observe morphological characters in groups with high intraspecific COI divergence that were suggestive of multiple species, even when using a reverse taxonomy approach (Kim *et al*. 2012). Mynott *et al*. (2011) suggested that cryptic species might be present and that further molecular study would need to be undertaken to test this idea. The use of arbitrary cut-offs (e.g. ≤3% intraspecific divergence; Hebert *et al*. 2003) for species delimitation might be misleading, with inter- and intraspecific divergence distances often varying between and within taxonomic groups (Meyer and Paulay 2005; Meier *et al*. 2006, 2008; Virgilio *et al*. 2010; Taylor and Harris 2012). Further investigation is required to assess species delimitation within stonefly groups and whether, perhaps, there are underlying molecular causes for the reported high intraspecific divergence distances (e.g. heteroplasmy; Magnacca and Brown 2010).

In this study, *N. hackeri* showed high intraspecific divergence (9%) between two distinct genetic clades (clades A and B). The specimens identified and sequenced for *N. hackeri* included four adult males: two were collected from the Lamington National Park (NP) and two from the Barrington Tops NP (southern Qld and the southern area of the NSW north coast, respectively). These divergence distances might be a consequence of the large geographical distance (~580 km) between the specimens that were sampled and sequenced. As stoneflies are regarded as poor dispersers, geographical separation of genetic populations within a species might be occurring, especially in mitochondrial genes if adult females are site-faithful. Avelino-Capistrano *et al*. (2014) collected specimens of *Kempnyia petersorum* from sites that were ~700 km apart. The sequences identified at those sites had intraspecific genetic pairwise distances of 2.6–4.6% and the authors stated that morphology was uniform over the range. In the current study, not having specimens or molecular data of *N. hackeri* from the area between the two collection sites is a limiting factor as the divergence distance range between the two clades (0–1% within each clade and 8–9% between the clades) may be due to incomplete sampling. A more complete sampling of *N. hackeri* across its distribution range may resolve whether *N. hackeri* is a single species with high divergences within the species or whether the high divergences indicate the presence of two species: *N. hackeri* and another with adult male morphology very similar to *N. hackeri*. Based on the current available morphological and molecular information, *N. hackeri* is here regarded as a single species as there are insufficient morphological data to support there being multiple species within the current circumscription. This will need to be tested with more data: more loci, specifically those from the nuclear genome, could provide insight into the genetic diversity, gene flow and species status of populations within *N. hackeri*.

The two COI clades of *N. exigua* (clades C and D; Figs 2, 3) were consistent with the two morphotypes (*N. exigua*: JMH182, JMH197, JMH223 and JMH224; *N.* sp. 1: JMH231 and JMH233) that had been suggested by the collectors (M. Pennifold, pers. comm.). Material available for the suggested *N.* sp. 1 (Clade C) was restricted to two early-instar larvae (wingbuds not developed) and these specimens were not morphologically sufficient to fully assess this possible morphotype. The specimens within *N. exigua* clades C and D overlapped geographically: Clade D ($n = 4$) was collected from Perth to south of Margaret River, and Clade C ($n = 2$) from north of Bunbury to Margaret River (specific site information listed in 'Material examined'). Additional specimens and molecular data are needed to assess whether Clade C (*N.* sp. 1) represents a distinct species that is morphologically similar to *N. exigua*.

Newmanoperla thoreyi also has considerable intraspecific COI divergence (COI53′ 6%), with sequences nesting into two clades (clades E and F; Figs 2, 3). This species is currently listed as having a widespread distribution, from south-west Queensland along the eastern ranges through to south-east South Australia. Considering the extent of this known distribution, the sampled area was relatively small: the alpine region of New South Wales and Victoria extending south to the Yarra Ranges (Clade F) and one site at Snowy Creek, Victoria (elevation 250 m) (Clade E). Given there is such COI divergence between collection localities that are relatively close together, there is likely to be considerably more divergence across the full geographic range of *N. thoreyi*, which needs to be investigated in respect to species status.

The designation of *N. theischingeri*, sp. nov. as a new species is a taxonomic hypothesis that can be summarised using the visualised approach of DeSalle *et al*. (2005) of a 'taxonomic circle', whereby geography (tropical Queensland), morphological characters (larval) and genetic support have been used to support the proposed taxonomic hypothesis. To complete the series of descriptions for the species *N. theischingeri*, sp. nov., the description of the adult male is required.

The primary focus of this study was on the life stage association and the description of the larvae for the genus *Newmanoperla*. While this study has provided an updated morphology for the larvae of the genus, the molecular results also generate questions about species delimitation, population dynamics and dispersal potential that have not yet been fully investigated. Questions about species delimitation within stonefly species will have broad implications for future biological monitoring programs that rely on molecular data and arbitrary cut-offs, such as DNA barcoding programs using

next generation sequencing techniques (e.g. Hajibabaei *et al*. 2011).

A strict application of DNA barcoding protocols (Hebert *et al*. 2003) is not likely to be highly successful with stoneflies. Stonefly literature in general reports high intraspecific COI divergences, which, if used in conjunction with species-level cut-offs of <3%, would result in recognising species for which there are no morphological or ecological bases. Also, without prior knowledge of the high COI divergence in stonefly species, identification using 'identity matching' is likely to fail to correctly assign samples. For example, a specimen that does not have an 'identity match' score of >97% to any known species will be returned as an unknown when, in fact, it is clearly assignable to a species morphologically. If *N. hackeri* is actually a single species with a large COI divergence (9%), a new specimen whose COI sequence is nested among the current samples but is 4% divergent from Clade A, and Clade B would not be identified as being *N. hackeri*, even though one of these clades might be its nearest neighbour. It is important that research continues to consider the morphology of species and continues to describe species, as this is still the base for many ecological and monitoring studies (Packer *et al*. 2009). Traditional taxonomy can be enhanced by using genetic techniques that have the additional advantage of generating a verified voucher sequence database that may be used in future molecular environmental research and monitoring programs.

Taxonomy

Family **GRIPOPTERYGIDAE** Enderlein, 1909

Genus ***Newmanoperla*** McLellan, 1971

Remarks

Hynes (1982) describes differences in the adults of the four species. Theischinger and Cardale (1987) provide illustrations and a descriptive overview of the adults. There is no previous generic description of the larvae. Hynes (1976) designated the characters of *N. thoreyi* as being distinct for the genus but the larva was not described until Hynes (1978) provided a brief description and illustrations.

Generic description

Adult. See Theischinger and Cardale (1987). Forewing CuA simple; hindwing stalk of Rs+MA shorter than crossvein between stalk and CuA; no fused anal veins. Male epiproct elongate with ventral spine. Female subgenital plate bilobed and posteriorly produced over sternite IX.

Larva. Light brown to grey dorsally with pale ventral surface. Antennae long, longer than abdomen and usually entire body, with long fine setae present on the first 15–20 segments (with the exception of *N. exigua*). Pronotum wider than long. Hind margin of mesonotum either straight or concave, usually with obvious notches at the base of wingpads (exception *N. prona* with small and not always obvious notches). Metanotum lacking notches at base of wingpads, hind margin often straight. Fine setae in lateral areas of sternites (at least on 6–10, with the exception of *N. exigua*). Paraprocts longer than basal width; narrow and tapering to a point, either dark or with darkened band near cercal base.

Newmanoperla exigua (Kimmins)

(Fig. 4*A*–*C*)

Material examined

Western Australia: 1 larva: Stirling Dam (Site Code HAR21), Lat. –33.10829° Long. 116.04199° (coordinates estimated), coll. Department of Parks and Wildlife (DPaW), M. Pennifold, 15.ix.2010, voucher number: JMH223. 1 larva: Stream near Hoffman's Mill (site code HAR01), Lat. –33.00183° Long. 116.084210° (coordinates estimated), coll. DPaW, M. Pennifold, 07.x.2009, voucher number: JMH231. 1 larva: Rosa Brook, Lawson Road (site code BLA54), Lat. –33.94292° Long. 115.49375° (coordinates estimated), coll. DPaW, M. Pennifold, 20.x.2009 by, voucher number: JMH233. 1 larva: Big Hill Brook at Wheatley Coast Road, Lat. –34.49333° Long. 116.18083°, coll. P. Suter, J. Webb, 20.ix.2008, voucher number: JMH182. 1 larva: Finlay Brook (site code MRY33), coll. DPaW, M. Pennifold, 06.x.2009 by, voucher number: JMH224. 1 adult ♀: River below Serpentine Falls, Lat. –32.36806° Long. 116.01111°, coll. P. Suter, J. Webb, 18.ix.2008 by, voucher number: JMH197. 1 adult ♀: Big Hill Brook at Wheatley Coast Road, Lat. –34.49333° Long. 116.18083°, coll. P. Suter, J. Webb, 20.ix.2008 by. 3 larvae: Rosa Brook, Lawson Road (site code BLA54), Lat. –33.94292° Long. 115.49375° (coordinates estimated), coll. DPaW, M. Pennifold, 20.x.2009 by.

Remarks

Larvae originally described and figured by Hynes and Bunn (1984), who associated the life stages by dissecting out the male genitalia from a mature larva. The following description is a modification of the description of Hynes and Bunn (1984) and includes additional distinguishing characters. This species is found only in Western Australia.

Description

Larva

Length. 4–6 mm.

Colour. Dorsal surface is dark with white spots clearly visible in each corner of pronotum and in anterior areas of meso- and metanota (Fig. 4*A*). Ventral surface is much paler by contrast.

Body. Not hairy. Dorsal cuticle when viewed in high magnification (×50) has a covering of minute setae.

Head. Antennae longer than abdomen; no long fine setae present.

Thorax. Hind margin of mesonotum concave; notches at base of wingpad (Fig. 4*A*).

Legs. Femora with dark band in distal and proximal areas (Fig. 4*B*); short robust setae present on outer surface in distal band. Tibiae with dark band in proximal area. Femora and tibiae with scattered hair fringe of long fine setae on outer margin. Tibiae with fringe of long robust setae on inner margin. Tarsal claws shorter than half of third tarsal segment in later instars; early instar tarsal claws longer than half third segment.

Abdomen. Dark markings on tergites; usually along hind margin and midline (Fig. 4*A*). Hind margins of tergites with robust setae present. Tenth tergite length equals width; mature male specimens with slightly raised knob on hind margin; females with tenth segment produced to a rounded point. Sternites pale.

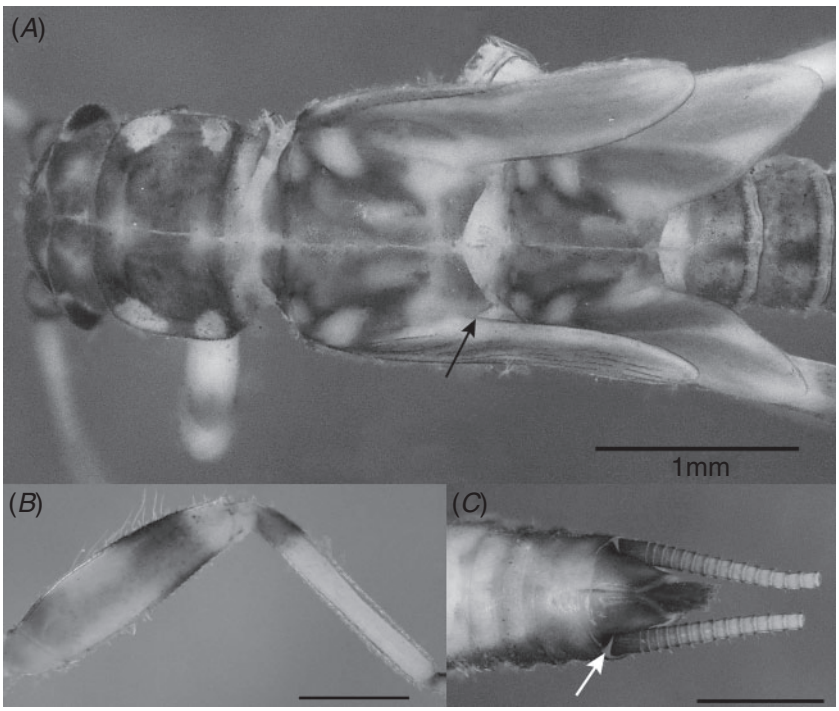

Fig. 4. *A–C*, *Newmanoperla exigua*: (*A*) dorsal view of thorax – arrow points to notch at base of hind margin and wingpad; distinct colouration on nota evident, especially white spots on corners of pronotum; (*B*) mid-leg – dark band in distal area on femora and proximal area of tibiae; scattered hair fringe on outer margin of femora; (*C*) paraprocts – dark band near base of cerci. Scale bars: *B* and *C* = 0.5 mm.

Paraprocts with dark band near base of cerci; base broad, length of paraproct greater than width of base and tapering posterolaterally to a point (Fig. 4*C*). Cerci longer than abdomen.

Newmanoperla hackeri McLellan

(Fig. 5*A–D*)

Material examined

New South Wales: Barrington Tops National Park: 1 larva: Polblue Creek at Polblue picnic ground, Lat. –31.9258 Long. 151.3875, coll. J. Mynott and M. Shackleton, 24.ii.2011, voucher number: JMH1333. 1 larva: Williams River at Rocky Crossing, Lat. –32.11666 Long. 151.48334, coll. J. Mynott and D. Black, 11.xi.2012, voucher number: JMH1206. 6 larvae: Williams River at Rocky Crossing, Lat. –32.11666 Long. 151.48334, coll. J. Mynott and D. Black, 11.xi.2012, voucher number: JMH1207. 2 adult ♂: Allyn River at upper campground, Lat. –31.1292 Long. 151.4734, coll. J. Mynott and D. Black, 11.xi.2012, voucher number: JMH1536 and JMH1537. **Queensland:** Lamington National Park: 2 adult ♂: Morans Creek above Morans Falls, O'Reillys (Green Mountains) section, Lat. –28.2318 Long. 153.125, coll. J. Mynott and M. Shackleton, 17.xi.2011, voucher number: JMH1202; coll. J. Mynott and D. Black, 16.xi.2012, voucher number: JMH1129. 1 adult ♂: Morans Creek above Morans Falls, O'Reillys (Green Mountains) section, Lat. –28.2318 Long. 153.125, coll. J. Mynott and M. Shackleton, 17.xi.2011, voucher number: JMH1203.

Remarks

This species was described from adult material by McLellan (1971), but the larva has not previously been associated, described or figured. The following description is based on larvae associated with adult males by molecular methods in this study. The below description is based on late (not final) instar specimens.

Description

Larva

Length. 4–5 mm.

Colour. Dorsal surface light brown with the abdomen darker than head and thorax. Ventral surface pale.

Body. Dorsal surface covered with short robust blunt spines. Dorsoventrally flattened. General fuzzy appearance due to long fine setae.

Head. Antennae very long, longer than entire body. Long fine setae on dorsal surface of antennae, more pronounced on first 15 segments. Head slightly wider than pronotum. Variety of setae present posterior to eye and extending ventrally to gena.

Thorax. Meso- and metanota broad in appearance. Mesonotum with distinct flange in anterolateral area (Fig. 5*B*); long robust setae on outer margin of flange. Notches at the base of wingpads on mesonotum; hind margin relatively straight or slightly concave. Wingpads with short blunt spines following principal veins and long fine setae on posterolateral area.

Legs. Trochanter with long fine setae present in anterior area. Femora broad, about twice the width of tibia. Outer margin of femora and tibiae with fringe of long fine setae. Femora with short robust spines present on outer surface; long robust setae in proximal area of outer margin of femora (excluding the hind leg

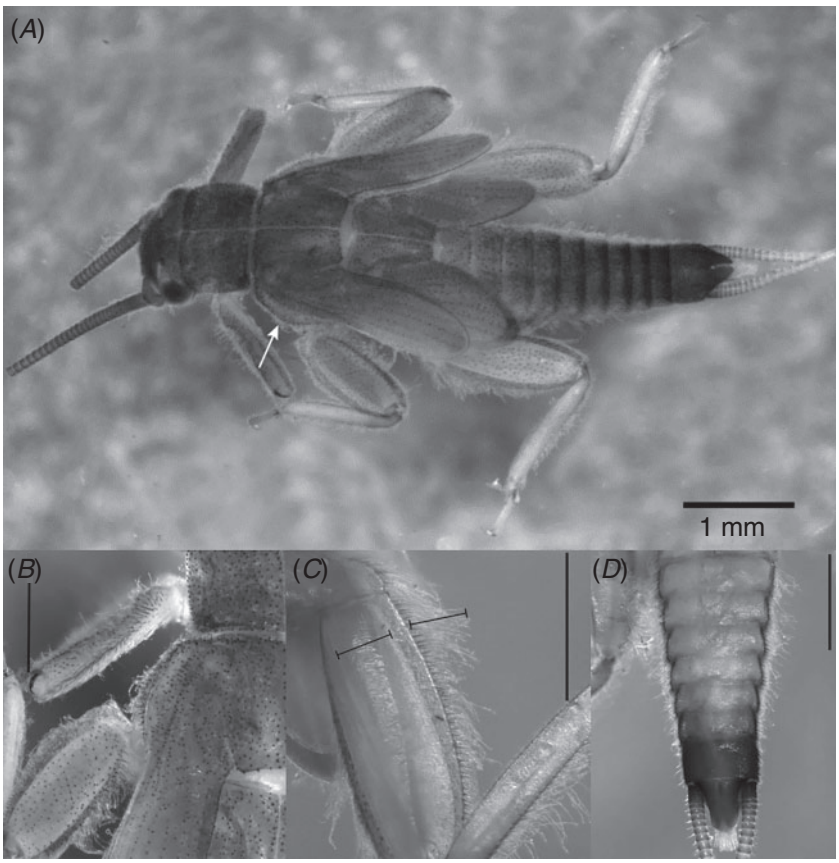

Fig. 5. *A–D*, *Newmanoperla hackeri*: (*A*) dorsal overview, dorsolaterally flattened and generally fuzzy appearance, arrow indicates mesonotal flange; (*B*) lateral area of thorax showing mesonotal flange and covering of short robust blunt setae; (*C*) ventral view of mid-leg showing fringe of long fine setae on inner margin of femora; (*D*) ventral view of abdomen showing lateral fringe of long fine setae. Scale bars: *B*, *C* (solid line only) and *D* = 0.5 mm.

that has some setae present). Inner margin of femora with fringe of long robust setae and long fine setae that do not exceed femora width (Fig. 5*C*). Inner margin of tibiae with robust setae. Tarsal claws shorter than half length of third segment.

Abdomen. Tapering posteriorly. Tergites with hind margin area darker, looking banded (Fig. 5*A*). Tenth tergite longer than wide; with slightly raised point in male specimens, females rounded. Lateral margins of sternites dark and with fringe of long fine setae; tenth sternite dark. Base of paraprocts wide with dark band near base of cerci; paraproct length greater than base width with straight margins, ends rounded medially (Fig. 5*D*). Long fine setae along dorsal surface of cerci, for first 15 segments.

Newmanoperla prona Hynes

(Fig. 6*A–E*)

Material examined

Tasmania: Southern Forests: 1 larva: Esperance River at bridge Esperance River Road (crossroads with Rutherfords Road), Lat. –43.27697 Long. 146.87688, coll. J. Mynott and M. Shackleton, 15.i.2012, voucher number: JMH307.

Remarks

Hynes' (1982) description has been modified here for consistency with some additional characters added. The life stages were associated by examination of a mature male larva in the type series that showed the adult male genitalia (Hynes 1982). This species is found only in Tasmania.

Description

Larva

Length. Final instar 4.5–5.5 mm.

Colour. Uniform brown on dorsal surface, pale ventral surface.

Body. Dorsoventrally flattened. Dorsal surface covered with short, blunt spines (Fig. 6*A*).

Head. Generally broad. Antennae longer than abdomen; lateral fringe of long fine setae on both sides of scape; long fine setae on both lateral sides of lower antennal segments (1–15) (Fig. 6*B*).

Thorax. Pronotum wider than long with long blunt spines on surface. Mesonotum flared in anterolateral area with long pointed setae on lateral margin (Fig. 6*C*); wingpads with short blunt spines

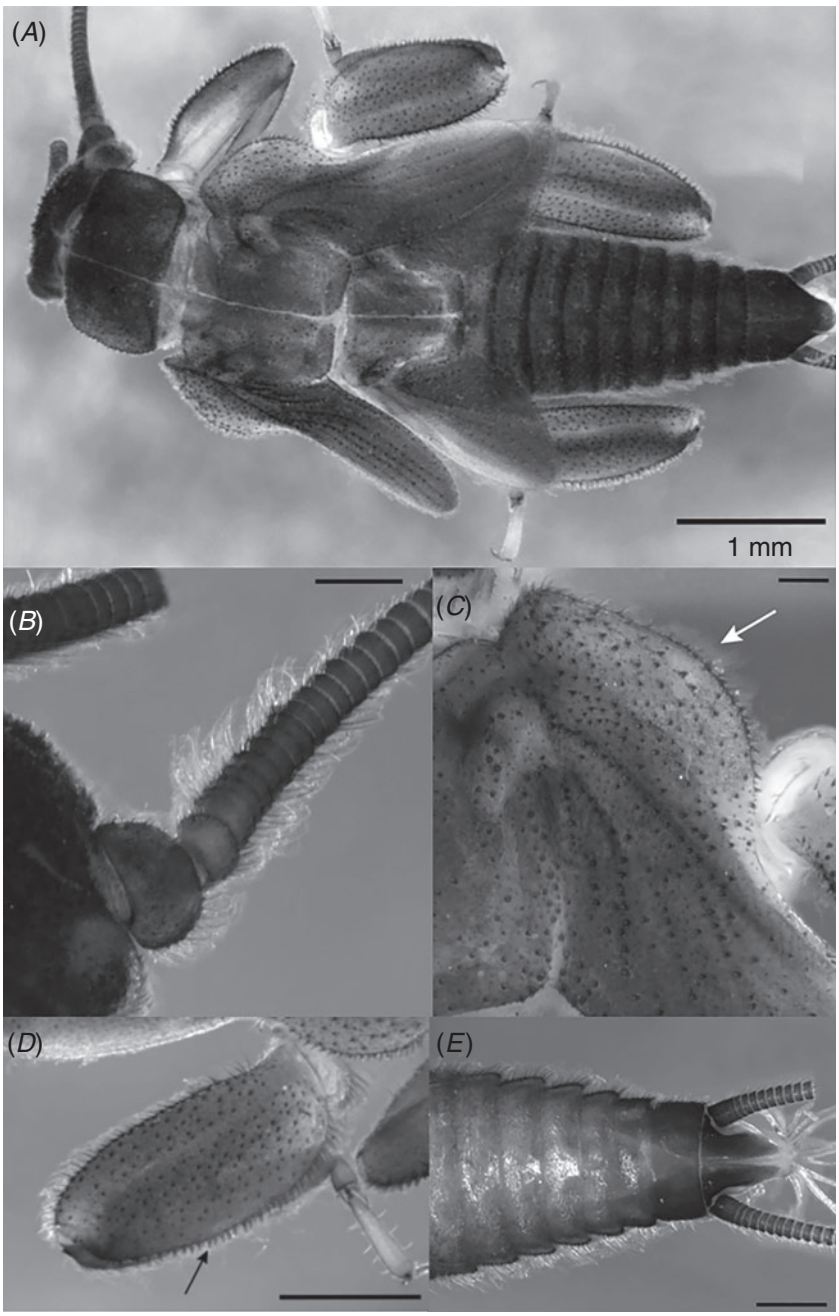

Fig. 6. *A–E*, *Newmanoperla prona*: (*A*) dorsal overview, dorsolaterally flattened appearance; (*B*) antennae with lateral fringes of long fine setae; (*C*) mesonotal flange indicated by arrow; (*D*) dorsal view mid-leg with flattened femora and short stout pointed setae on inner margin (arrow); (*E*) ventral view abdomen showing tapering posteriorly of abdomen and lateral fringe on margin of tergites. Scale bars: *B* and *C* = 0.1 mm; *D* and *E* = 0.5 mm.

following principal veins. Mesonotum with small notches at base of wingpads (not always obvious), hind margin straight (Fig. 6*A*).

Legs. Flattened with a wide femur that completely covers the tibia when leg is folded. Tarsal claws less than half as long as third segment. Outer surface of femora covered with short stout spines; femora with hair fringe on outer margin composed of long fine setae and long robust pointed setae (Fig. 6*D*); femora with short stout pointed setae on inner margin; tibiae with a fringe of long robust setae on outer margin. Trochanters with anterior hair fringe of long fine setae.

Abdomen. Flattened ventrally and tapering posteriorly. Tergites and hind margins covered with short blunt setae; median row slightly longer than others on tergite. Lateral margins of segments 1–9 fringed with long fine setae and long

robust setae (Fig. 6*E*). Paraprocts long and narrow, tapering to a point. Gill filaments number ~20–25. Cerci shorter than abdomen; basal third with long setae present, dorsal ones longer and more robust than the others.

Newmanoperla theischingeri, sp. nov.

(Fig. 7*A–D*)

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Material examined

Holotype. Australia: Queensland: Kirrama National Park: 1 larva: Rainforest stream on Kirrama Range Road. Lat. –18.21372 Long. 145.79813, coll. J. Webb and M. Shackleton, 16.v.2010, voucher number: JMH200. Deposited Queensland Museum, registration number: QMT199586.

Paratypes. Australia: Queensland: Kirrama National Park: 1 adult ♀: Rainforest creek with waterfall on Kirrama Range Road. Lat. –18.2108 Long. 145.8075, coll. J. Webb and M. Shackleton, 16.v.2010, voucher number: JMH201. Deposited Queensland Museum, registration number: QMT199587. Same data as for paratype, 2 adult ♀. Deposited Queensland Museum, registration numbers: QMT199588 and QMT199589.

Remarks

A species, *N.* cf. *hackeri*, from north-east Queensland was suggested by Theischinger and Cardale (1987) after examination of adult material held at the Australian National Insect Collection (Canberra). The description they provided states that the male epiproct and paraprocts, as well as the female subgenital plate, were much the same as *N. hackeri*. The adult female genital plate was figured (Theischinger and Cardale 1987: 53, fig. 101). The only specific distinction mentioned by Theischinger and Cardale (1987) is the number of distal crossveins between R and CuA: three or more in *N. hackeri* and 2,2,1,1 for *N.* cf. *hackeri*.

Here, *N. theischingeri*, sp. nov. is suggested to be the same species mentioned by Theischinger and Cardale (1987) based on the similarities of the adult female and the collection of the specimens in the same geographical area. The morphology of the larva is distinct from other species of *Newmanoperla* and the genetic data support these specimens as forming a distinct species of *Newmanoperla* (COI53′ minimum interspecific pairwise p-distances of 16–18%). The description of the adult male is required to complete the species description – none were available for this study.

Description

Adult

Female. General unicolourous appearance with the exception of darker bands on lateral areas of pronotum and hind margins of tergites. Body length 4.3–4.6 mm; forewing length 6.5–7.2 mm. Legs with long fine setae present on inner and outer margin of femora. Wings unicolourous; wingveins number 2,2,1,1. Abdomen with hair fringe on lateral margins. Hind margin of tenth tergite produced and rounded. Subgenital plate long (as long as segment), produced over ninth sternite and bilobed.

Distinguishing characters. Female *N. theischingeri* are distinguishable from *N. hackeri* by the number of crossveins in the distal area of the forewing being 2,2,1,1 (*N. hackeri* with three or more in each area). The long subgenital plate distinguishes *N. theischingeri* from *N. prona*, which has a short subgenital plate (length not greater than segment). The distinction between the two other *Newmanoperla* species is by geographic distribution (*N. exigua*, Western Australia; *N. theischingeri*, Northern Queensland) and size (body length

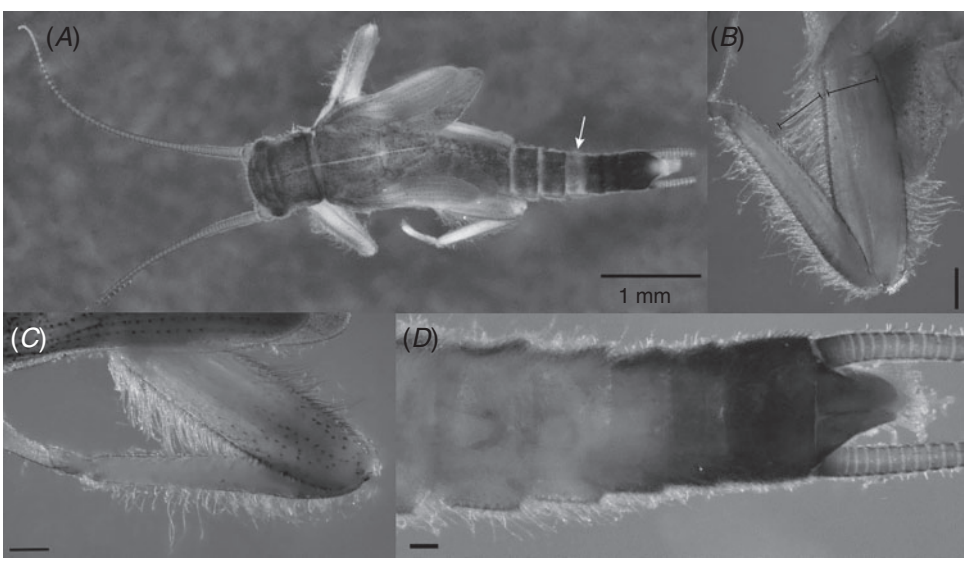

Fig. 7. *A–D*, *Newmanoperla theischingeri*, sp. nov.: (*A*) dorsal overview, arrow points to pale seventh tergite; (*B*) ventral view mid-leg showing long fine setae on inner margin subequal to femora width; (*C*) dorsal view mid-leg, showing long fine setae on inner and outer margin and short robust setae on outer surface; (*D*) ventral view abdomen showing lateral fringe of long fine setae. Scale bars: *B* (solid line only), *C* and *D* = 0.1 mm.

of *N. theischingeri* 4.3–4.6 mm; *N. thoreyi* 7.5 mm; McLellan 1971).

Larva

Length. 4 mm (not final instar).

Colour. Dorsally brown with abdominal segments 8–10 darker brown. Legs and wingpads pale.

Body. Dorsal surface covered with short robust blunt spines. Not dorsoventrally flattened.

Head. Antennae long, as long as entire body (Fig. 7*A*). Long fine setae on dorsal surface of antennae, on first 10–15 segments. Lateral margins of scape with long fine setae. Head slightly wider than pronotum.

Thorax. Pronotum with short robust setae on entire margin. Meso- and metanota broad in appearance. Mesonotum without distinct flange in anterolateral area but with short robust setae and long fine setae. Notches at the base of wingpads on mesonotum; hind margin relatively straight or slightly concave. Wingpads with short blunt spines following principal veins and long fine setae on posterolateral area. Pleural area of mesonotum with patch of long fine setae.

Legs. Trochanter with long fine setae present in anterior area. Outer margin of femora and tibiae with fringe of long fine setae (Fig. 7*B*, *C*), some very long. Femora with short robust setae present on outer surface; inner margin of femora with fringe of long robust setae and long fine setae, as long as femoral width. Inner margin of tibiae with robust setae. Tarsal claws shorter than half length of third segment.

Abdomen. Tergites 1–6 with hind margin area slightly darker, the seventh tergite conspicuously pale; tergites 8–10 darker than other tergites; the posterior area on tenth tergite pale; tenth tergite longer than wide; hind margin of female rounded. Hind margins of tergites 1–5 with robust blunt setae. Lateral margins of sternites with fringe of long fine setae (Fig. 7*D*); tenth sternite dark. Paraprocts with dark band near base of cerci; long with inner margins straight, ends rounding laterally; outer margin straight with hook-like curl anterodorsally beside cerci. Long fine setae along dorsal surface of cerci, for first 10 segments (all that remained on the examined specimen).

Etymology

This species is named after Gunther Theischinger, who has contributed greatly to the knowledge of the Australian Plecoptera.

Newmanoperla thoreyi (Banks)

(Fig. 8*A–C*)

Material examined

New South Wales: Kosciuszko National Park: 1 larva: Bullock Head Creek crossing Snowy Mountains Highway, Lat. –35.8405 Long. 148.4911, coll. J. Webb, J. Mynott, M. Shackleton and S. Moore, 30.xi.2009, voucher number: JMH191. 9 larvae: Bullock Head Creek crossing Snowy Mountains Highway, Lat. –35.8405 Long. 148.4911, coll. J. Webb, J. Mynott, M. Shackleton and S. Moore, 30.xi.2009, voucher numbers: JMH1331, JMH1332. 1 larva: Diggers Creek crossing Summit Road, Lat. –36.36037 Long. 148.4868, coll. J. Webb, J. Mynott, M. Shackleton and S. Moore, 01.xii.2009, voucher number: JMH196. 1 larva: Sawpit Creek crossing Summit Road, Lat. –36.35168 Long. 148.56599, coll. J. Webb, J. Mynott, M. Shackleton and S. Moore, 01.xii.2009, voucher number: JMH1205. 1 larva: Sawpit Creek crossing Summit Road, Lat. –36.35168 Long. 148.56599, coll. J. Webb, J. Mynott, M. Shackleton and S. Moore, 01.xii.2009, voucher number: JMH1204. 2 larvae: Alpine Creek crossing Snowy Mountains Highway, at chain bay, Lat. –35.91908 Long. 148.5918, coll. J. Webb, J. Mynott, M. Shackleton and S. Moore, 30.xi.2009, voucher numbers: JMH69, JMH71. Monga National Park: 2 larvae:

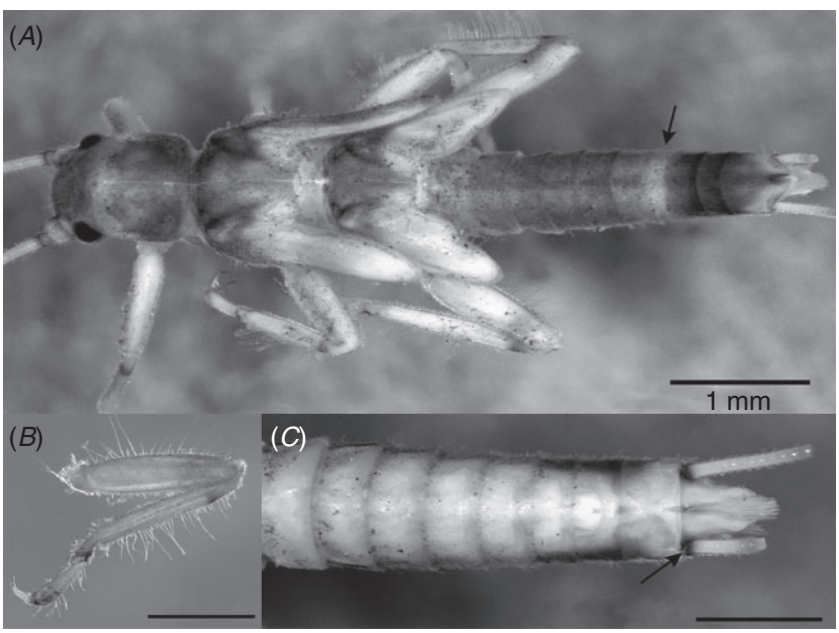

Fig. 8. *A–C*, *Newmanoperla thoreyi*: (*A*) dorsal overview, arrow points to pale seventh tergite; (*B*) dorsal view of mid-leg, scattered hair fringe on outer margin of femora and tibiae; (*C*) ventral view of abdomen arrow showing dark bands at base of cerci (arrow). Scale bars: *B* and *C* = 0.5 mm.

Mongalowe River at picnic spot (near Penance Grove), Lat. –35.56083 Long. 149.9222, coll. J. Mynott and M. Crump, 24.ix.2009, voucher numbers: JMH1329, JMH1330. **Victoria:** 2 larvae: Snowy Creek on Omeo Highway, near Mitta Mitta, Lat. –36.545 Long. 147.384, coll. J. Webb, J. Mynott and S. Moore, 12.viii.2009, voucher numbers: JMH206 and JMH207. 17 larvae: Mitta Mitta River at Mitta Mitta, coll. J. Webb, J. Mynott and S. Moore, 12. viii.2009 . 1 larva: Buffalo River at Blades picnic area, Lat. –36.4932 Long. 146.3968, coll. J. Mynott and S. Coates, 06.x.2009. 1 adult ♂: McMahons Creek crossing Warburton-Woods Point Road, Lat. –37.70146 Long. 145.8303, coll. J. Webb, 12.xi.2008, voucher number: JMH1538. Alpine National Park: 1 larva: East branch Buffalo River, Lat. –37.0407 Long. 146.83785, coll. J. Mynott and S. Coates, 06.x.2009, voucher number: JMH211.

Remarks

The larva was first figured by Hynes (1978) with a brief description. This was expanded by Suter and Bishop (1990) who added further descriptive characters. The association of life stages was by Hynes (1978), who used two approaches in his study, breeding out or the collection of freshly emerged adults with their exuvia. Hynes (1978) did not say which specific technique was used for *N. thoreyi*.

Description

Larva

Length. 4.2–7 mm (combining measurements by Hynes (1978) and Suter and Bishop (1990)).

Colour. Dorsally light brown with pale areas posteriorly on meso- and metanota (Fig. 8*A*). Tergites 7 and 10 usually noticeably paler than other tergites, especially in comparison with tergites 8 and 9 that commonly are a darker brown (Fig. 8*A*). Ventrally pale. Legs pale.

Body. Not dorsoventrally flattened. Dorsal surface covered with scattered long setae.

Head. Antennae longer than abdomen (4–5 mm) with long fine setae on dorsal margin (easily detached but most prominent on the first 20 segments); also with short setae around whorl. Setae also present on lateral margins of scape. Head not wider than pronotum.

Thorax. Pronotum wider than long. Mesonotum with concave hind margin and small notches at the base of wing pads. Long fine setae often noticeable along outer margin of wingpads. Pleural area of nota with long fine setae.

Legs. Tibiae with a weak dark band in proximal area (Fig. 8*B*), occasionally the femora in the distal area also has a very weak band. Femora and tibiae with a scattered fringe of very long fine setae (Fig. 8*B*); long setae on outer surface in distal area of femora. Tibiae with fringe of long robust setae on inner margin. Tarsi with scattered long fine setae on outer area of segments. Tarsal claws approximately half as long as third segment.

Abdomen. Lateral areas of sternites 6–10 with fine setae present. Tenth tergite length and width subequal. Mature male larvae with a slightly raised projection on posterior margin on tenth tergite. Cerci (often broken off) with long setae on dorsal and ventral margin of segments. Paraprocts pale with dark brown mark near base of cerci (Fig. 8*C*); base of cerci also with darkened band. Paraprocts longer than base, triangular with long straight margins that taper laterally to a point.

Key to larvae of *Newmanoperla*

1. Dense hair fringe on outer and inner margin of femora, may be fine or robust setae (Figs 5*B*, *C*, 6*D*) 2
   Inner margin of femora without hair fringe, outer margin with hairs present (Figs 4*B*, 8*B*) 4
2. Inner and outer margin of femora with robust setae, femora broad, distribution Tasmania (Fig. 6*D*) ..... *N. prona*
   Inner margin of femora with long fine setae, distribution Australian mainland 3
3. Hairs on inner margin of femora as long as width of segment (Fig. 7*B*); anterolateral area on mesonotum not with obvious flange; distribution north-eastern Queensland ..... *N. theischingeri*
   Hairs on inner margin of femora not as long as width of segment (Fig. 5*C*); anterolateral area on mesonotum with obvious flange; femora broad; distribution south-eastern Queensland, north-eastern New South Wales ..... *N. hackeri*
4. Long fine hairs on outer margin of femora and tibiae, length greater than segment width (Fig. 8*B*); distribution south-eastern states (excluding Tasmania) ..... *N. thoreyi*
   Some scattered hairs on femora, length not greater than segment width (Fig. 4*B*); pronotum with white spots in each corner (faint but noticeable in small specimens) (Fig. 4*A*) ..... *N. exigua*

Acknowledgements

This research was funded by an Australian Biological Resource Study Grant (CT211-32) with additional funding from La Trobe University. Specimens were collected under the following permits: Queensland Scientific Research Permits WITK06190909 and WITK10277111; New South Wales Scientific Research Permit P07/0095 and National Parks Service Scientific Licence numbers S11442, SL12404, S13223, SL100434; Victorian Scientific Research Permits: 10004636 and 10005961; Western Australia National Park Authority CE002143 and Western Australian Licences: Scientific SF006555 and Export ES002034; and Tasmanian Scientific Permit TFA11036. The author would like to acknowledge the valuable input from Phil Suter, Mark Carey and two anonymous reviewers during the writing of this work.

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