



## A NEW SPECIES OF *LEPTOPERLA* NEWMAN, 1839 (PLECOPTERA: GRIPOPTERYGIDAE)

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### ABSTRACT

*Leptoperla tsyrlini* sp. n. (Holotype male, Victoria, Casterton, Glenelg River at Sanford-Bahgallah Road, 37.61922, 141.42864, 15-Oct-2013) is described from the Glenelg catchment of southwestern Victoria, Australia. Mitochondrial DNA, using a 657 bp region of the cytochrome *c* oxidase I gene, was used to associate the adult male of this new species with the larval form. The new species is compared to its most similar congener, *L. neboissi* McLellan, 1971.

**Keywords:** taxonomy, genetics, life-stage association, Australia

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### INTRODUCTION

Australian stoneflies (Plecoptera) have long had a disparity in descriptive information between the life stages with most species being described from adult material (Theischinger and Cardale 1987). The genus *Leptoperla* is listed as having 30 species (Theischinger and Cardale 1987) and has a distribution along the east coast of Australia, south-east South Australia, south-west Western Australia and Tasmania (ABRS 2009, DeWalt et al. 2016). Tsyrlin (2001) published a larval key to the genus *Leptoperla* of Victoria, Australia. The study identified larvae that were suggested to belong to a new species, but the species was never formally described. The objective of this study is to describe the adult male and the larva.

### MATERIAL AND METHODS

Specimens were collected by the Environmental Protection Agency Victoria (EPA Vic) and sent to the senior author for genetic analyses. Adult illustrations were done with the aid of a camera lucida. Larval specimen imaging was performed using a Nikon SMZ1500 dissecting microscope and Nikon DS-Fi1 camera running NIS-Element F software version 3.2. Images focused at several depths on a specimen were stacked to produce a single image with greater depth of focus using Helicon Focus software version 5.3.7.

Mitochondrial DNA was used to confirm life stage associations using one adult male with several larvae of the new species. The association would be supported by the presence of a shared haplotype or

haplotypes (Zhou *et al.* 2007, Mynott 2015). A selection of larval specimens for the species was processed by the Canadian Centre for DNA Barcoding (CCDB; Guelph, Canada) following their standard extraction and sequencing methods.

A single adult male had a leg removed and DNA extracted using a Qiagen DNeasy blood and tissue kit, following standard protocols. Polymerase chain reaction (PCR) conditions and preparations followed those of Mynott (2015). The PCR product was sent to Macrogen Inc. (Seoul, Republic of Korea) for purification and sequencing.

Primers used were HCO2198 and LCO1490 (Folmer *et al.* 1994) which gave a 657 bp region of the cytochrome *c* oxidase subunit 1 (COI) for analyses. Alignments were generated in ClustalX in MEGA ver. 5.2 (Tamura *et al.* 2011). Genetic analyses used *Newmanoperla* and Austroperlidae sequences from Mynott (2015) as well as unpublished *Leptoperla* spp. sequences. 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. All new sequences from this study have been deposited on GenBank with accession numbers KX687986- KX687989.

The holotype and all larvae are deposited in the Australian Museum: Australia, New South Wales, Sydney (AM). Holotype and paratype catalogue numbers are provided for each specimen. Coordinates are provided as decimal degrees, negative for southern latitude and positive for eastern longitude.

## RESULTS AND DISCUSSION

### *Leptoperla tsyrkini* sp. n.

(Figs. 1-5)

<http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:492727>

**Material examined.** Holotype male, **Australia**, Victoria, Glenelg River at Sanford-Bahgallah Road, -37.61922, 141.42864, (EPA Victoria Site Code: JKN), 15 October 2013, EPA Victoria; Specimen accession number: JMH1829 (LTU); KX687986

(GenBank); K.462971 (AM).

**Male** (Holotype), juvenile.

**Head.** Grey with ill-defined irregular yellow patches along eye margin; antennae greyish.

**Thorax.** Largely dark brownish grey. Pronotum wider than long. Legs greyish yellow; in all legs the distal third to half of femur and basal fifth to sixth of tibiae and second tarsal segment somewhat darker than remainder; claws black; basal tarsal segment of hindleg as long as apical segment. Both wings basically hyaline, slightly suffused with greyish yellow, forewing extensively and irregularly mottled/clouded with yellowish grey.

**Abdomen.** Largely greyish yellow, on each side with narrow greyish brown dorsal line close and parallel to imaginary mid-dorsal line and wider greyish brown lateral stripe.

**Genitalia** (Figs. 1, 2). Lateral sclerites of tergite X angled, the apices widely separated; posterior sclerite short and tapered, projecting posterodorsally from a rather deep base; epiproct in lateral view slightly arched dorsally, subtriangular in dorsal view, end-hook narrow, lateral teeth alternating in size from large subapical to large basal; paraprocts thin, slightly arched dorsally, inner margin with small subtriangular ventral lobe at about mid-length, apex narrowed to thin hook curving ventrolaterally.

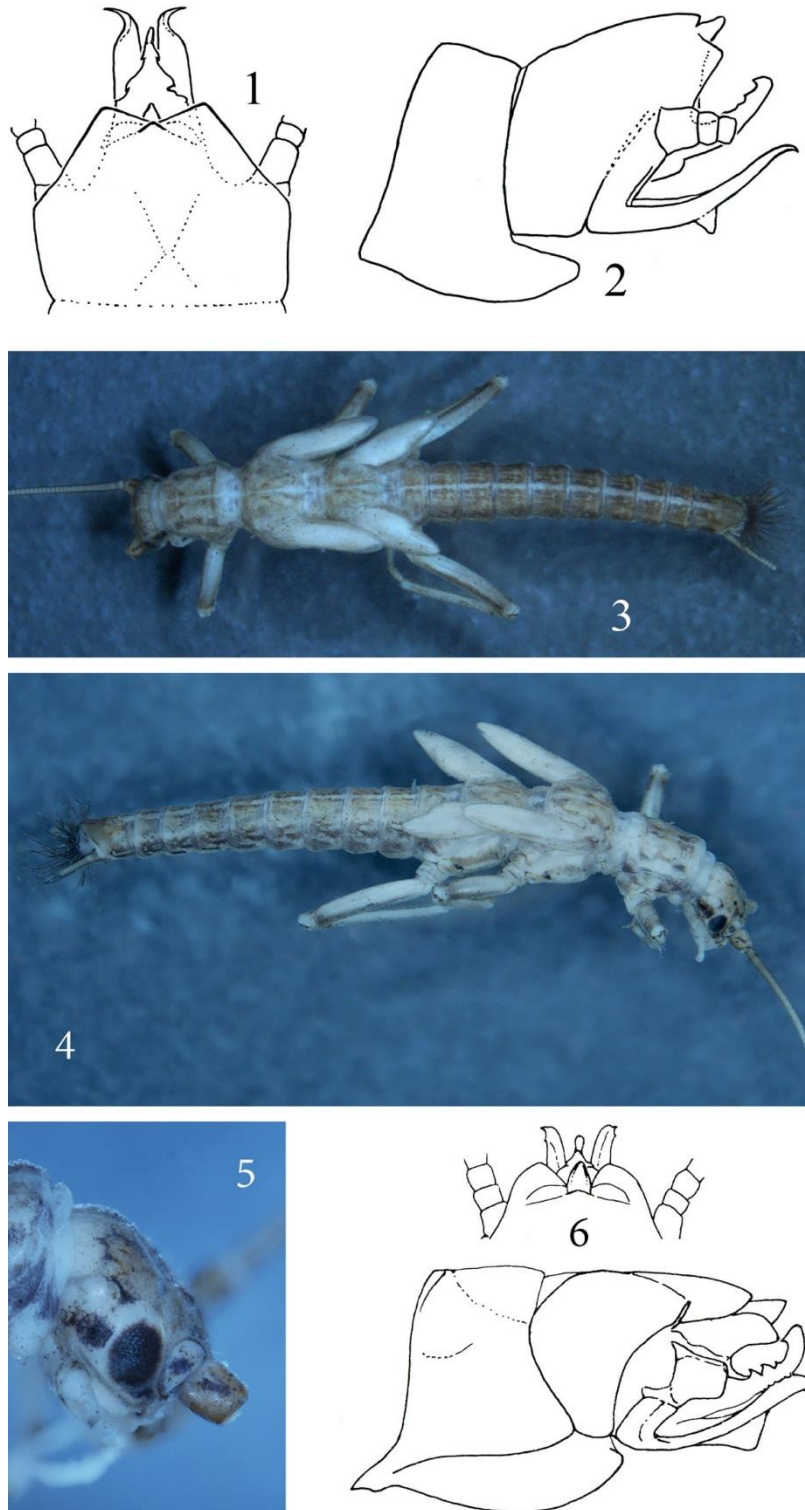
**Dimensions.** Body length 6.5 mm, forewing 7.5 mm.

**Female.** Unknown.

**Larvae** (Paratypes): 3 specimens: Victoria, Heywood, Fitzroy River, 29 December 2012, Z. Billingham; Accession numbers: JMH1311 (LTU), KX687989 (GenBank), K.462972 (AM); JMH1312 (LTU), KX687988 (GenBank), K.462973 (AM); JMH1313 (LTU), KX687987 (GenBank), K.462974 (AM). (Non-paratypes): 8 specimens: same data.

**Head.** Pale brown with darker pigment on occiput; dark lateral stripe from scape to posterior head margin (Fig. 5). Antenna without long fine setae (Fig. 4).

**Thorax.** Pronotum longer than wide; pronotum pale with darker medial and lateral stripes; pleural area with dark patches (Fig. 3). Meso- and metanota with straight hind margin; all nota with



Figs. 1, 2. *Leptoperla tsyrlini* sp. nov., male, genitalia: (1) dorsal; (2) lateral.

Figs. 3-5. *Leptoperla tsyrlini* sp. nov., larva: (3) dorsal; (4) lateral; (5) head.

Fig. 6. *Leptoperla nevoissi*, male genitalia: (top) tip of tergite X, dorsal; (bottom) lateral.

on surfaces and margins. Legs: long and thin; femora and tibiae with short fine setae on all surfaces, tibiae with fringe of long fine setae on outer margin, femora with some scattered long fine setae on outer margin. Femora with dorsolateral dark longitudinal stripe, a broad dark transverse band distally. Tibiae with proximal, narrow dark band; inner margin of tibiae with long robust setae; hind tarsi with segment three greater than half as long as segment one.

**Abdomen.** Narrow, dorsal light stripe on abdominal segment one through nine, darker laterally (Fig. 3 & 4). All terga (surfaces and hind margins) with short fine setae; tergite X longer than wide, apex rounded. Cerci without long fine setae.

**Dimensions.** Body length 8.5 mm – 9.0 mm (n=11).

**Etymology.** This new species is dedicated to our colleague Edward Tsyrlin who recognised the distinctive larval morphology.

**Remarks.** A combination of widely angled lateral sclerites and short upright posterior sclerite of tergite X, an epiproct with a narrow end-hook, and paraprocts with a simple, narrow, laterally pointing end-hook and a small, triangular ventral lobe (Figs 1, 2) distinguishes the male of *Leptoperla tsyrlini* sp. n. from all other *Leptoperla* species. *Leptoperla tsyrlini* sp. n. is most similar to *L. nevoissi* McLellan, 1971, a species with more rounded lateral sclerites of tergite X and distinctly less hooked paraprocts with a much larger ventral lobe (Fig. 6). Tsyrlin (2001), in comparison to other *Leptoperla* larvae, indicates that the new species has a relatively long pronotum, a longer tarsomere one of the hind tarsus, and a complete absence of a fringe of setae on the pronotal edges. The new species appears endemic to the Glenelg and Fitzroy Rivers of southwestern Victoria.

#### ACKNOWLEDGEMENTS

This research was funded by an Australian Biological Resource Study Grant (CT211-32) with additional funding from La Trobe University and EPA Victoria. The authors would like to acknowledge the valuable input from Phil Suter, Ros St Clair, and Zac Billingham.

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Submitted 12 August 2016, Accepted 20 October 2016, Published 21 October 2016

Hosted and published at the University of Illinois, Illinois Natural History Survey, Champaign, Illinois, U.S.A.

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