Records of the Australian Museum (2019)
vol. 71, issue no. 1, pp. 1–32
https://doi.org/10.3853/j.2201-4349.71.2019.1677

Records of the Australian Museum
a peer-reviewed open-access journal
published by the Australian Museum, Sydney
communicating knowledge derived from our collections
ISSN 0067-1975 (print), 2201-4349 (online)

DNA Barcoding and Integrative Taxonomy of the *Heterolepisma sclerophylla* species complex (Zygentoma: Lepismatidae: Heterolepismatinae) and the Description of Two New Species

Graeme B. Smith[1, 2], Andrew Mitchell[1], Timothy R. C. Lee[1] and Luis Espinasa[3]

[1] Australian Museum Research Institute,
Australian Museum, 1 William Street, Sydney New South Wales 2010, Australia

[2] Federation University Australia, PO Box 663, Ballarat Victoria 3353, Australia

[3] School of Science, Marist College, 3399 North Rd, Poughkeepsie, New York 12601, United States of America

Abstract. We present one of the first studies of DNA barcodes (COI sequences) in the basal insect order Zygentoma, and compare the data with nuclear (28S) and mitochondrial (16S) rDNA sequences and morphology for an integrative taxonomic study of the *Heterolepisma sclerophylla* Smith species group. DNA sequence analyses identified deep divisions between Queensland and New South Wales populations, and among populations in each state. Detailed morphological and morphometric evaluation of the specimens failed, in most cases, to identify unambiguous morphological characters of diagnostic value for each population, possibly due to the interaction of morphological conservatism with high levels of variability resulting from their continued moulting after reaching sexual maturity. Several strong consistent characters were identified to support the description of a southern Queensland population as a new species (*Heterolepisma cooloola* sp. nov.). The combined molecular and morphological data support the view that the presence of lanceolate scales and the absence of macrochaetae from the anterior margin of the frons are more significant to phylogeny than the arrangement of styli and the shape of the thoracic sternites in *Heterolepisma*. Specimens from Glen Davis, NSW, while indistinguishable from *H. sclerophylla* in all other characters examined, were found to possess one fewer pair of abdominal styli in both sexes and are also described as a new species (*Heterolepisma coorongooba* sp. nov.). Five lineages are recognized within the remaining NSW material but as reliable (non-overlapping) morphological and morphometric differences could not be identified, they are not described here as new species. *Heterolepisma sclerophylla* sensu stricto is considered to be a complex of morphologically ill-defined species or perhaps subspecies.

The silverfish subfamily Heterolepismatinae Mendes, 1991 is poorly understood but quite diverse in spite of a certain superficial uniformity. Twenty-four species have been described with a mainly Gondwanan distribution extending to coastal southern Japan, Vietnam, Angola, Mozambique, Somalia, Zanzibar as well as to many Pacific and Indian Ocean Islands, but Smith (2017) suggested that there may be more than 100 morphospecies in Australia alone. In Australia the genus can be found in habitats ranging from the fringes of tropical rainforest to the dry deserts and even rarely in subalpine regions. Specimens are collected from dry leaf litter, under or within cavities in the bark of dead or living trees, sometimes under stones and occasionally within abandoned termite galleries. Several authors (Wygodzinsky,

Keywords: Thysanura; taxonomy; new species; DNA barcodes; 28S ribosomal DNA
Taxonomic registration: (LSID publication) http://zoobank.org/124BD25A-7712-4EC6-9A1E-48FC9C23B513
Corresponding author: Graeme B. Smith Ie_gbsmith@optusnet.com.au
Received: 2 May 2017 **Accepted**: 8 December 2018 **Published**: 13 March 2019 (in print and online simultaneously)
Publisher: The Australian Museum, Sydney, Australia (a statutory authority of, and principally funded by, the NSW State Government)
Citation: Graeme B. Smith, Andrew Mitchell, Timothy R. C. Lee, and Luis Espinasa. 2019. DNA barcoding and integrative taxonomy of the *Heterolepisma sclerophylla* species complex (Zygentoma: Lepismatidae: Heterolepismatinae) and the description of two new species. *Records of the Australian Museum* 71(1): 1–32. https://doi.org/10.3853/j.2201-4349.71.2019.1677

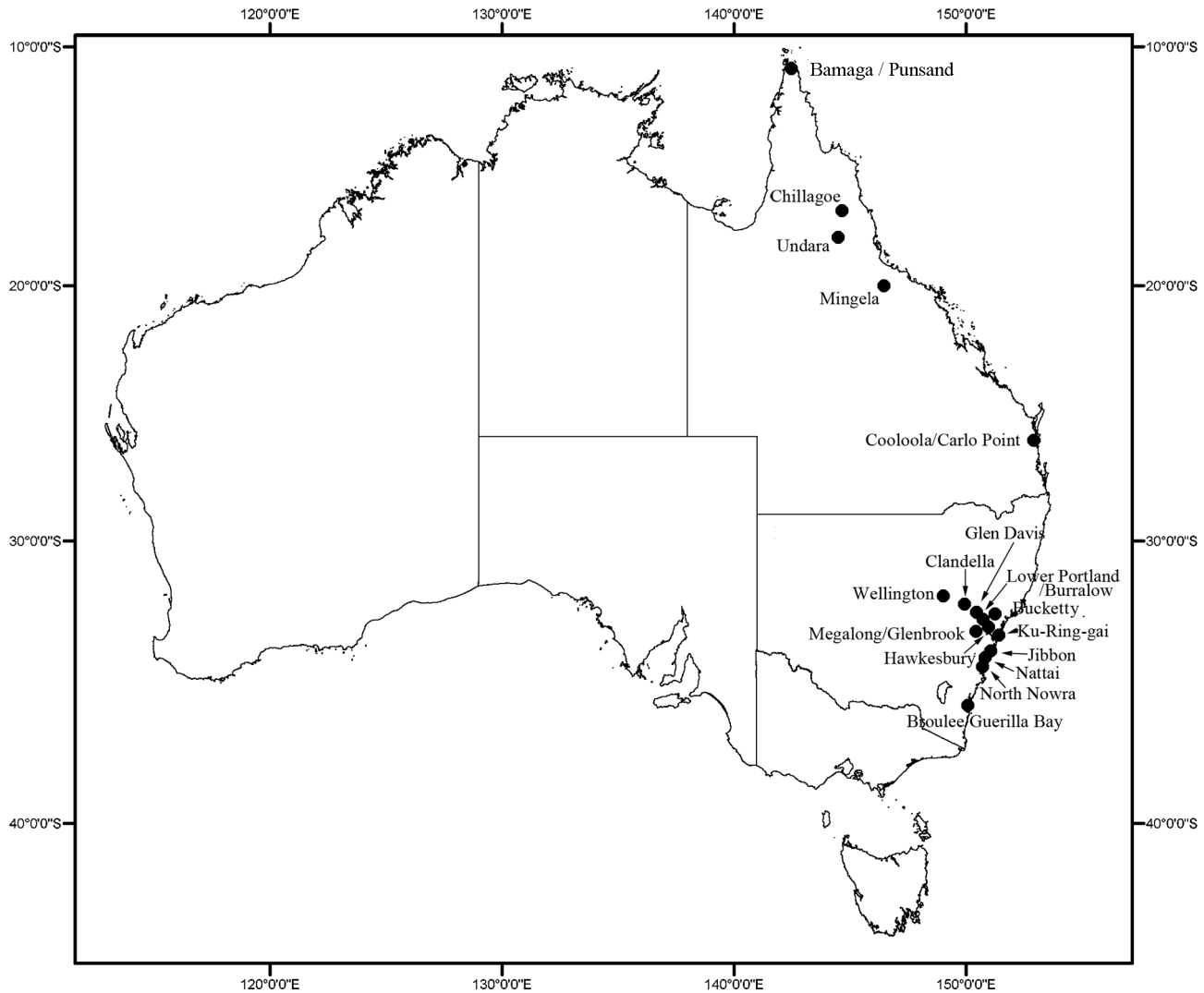


Figure 1. Collection localities.

1967, Irish, 1990 and Mendes, 1991) consider the genus to exhibit many plesiomorphies although Smith (2016b) discusses alternative interpretations.

The species *Heterolepisma sclerophylla* Smith was described from specimens collected at Broulee on the NSW south coast (Smith, 2014). Other localities listed included specimens from Glenbrook, Guerilla Bay, Lower Portland, Megalong Valley, Burralow (near Bilpin), Clandella State Forest, Hawkesbury Heights, Ku-ring-ai, Nattai and Wellington, all in NSW (Fig. 1). The species was distinguished from other *Heterolepisma* by a combination of features including a glabrous urosternite I, 1+1 single macrochaetae on urosternites II–VIII, styli on urosternites VII–IX in the ♀ and VIII–IX in the ♂, 2+2 combs on urotergite I, posterior combs on all nota, urotergal submedial combs each of two macrochaetae, a small apically-round, triangular prothoracic sternum which is longitudinally somewhat furrowed, and a rounded (rather than truncate) urotergite X. Variations in morphology between individuals (and even from left to right on a single individual) were mentioned in the original description but considered as "normal" given the continuous moulting of silverfish throughout their life, even after attaining sexual maturity. The large and "unlimited" number of moults during the life of silverfish results in considerable variation between specimens and makes morphological comparisons difficult.

Material with the same characters listed above has since been collected from a wide range of locations in Queensland, including Carlo Point and Cooloola in South East Queensland, Undara, Chillagoe and near Bamaga at the extreme northern tip of eastern Australia. Material was also obtained from further localities in New South Wales (Fig. 1). The material from Glen Davis was found to resemble *H. sclerophylla* in most aspects but had one fewer pairs of styli in both sexes. The number of pairs of styli has been considered variable in this genus and some species, notably *Heterolepisma stilivarians* Silvestri, from Western Australia, have been described as having variable numbers of styli (Silvestri, 1908). All the NSW and QLD material had been identified by the first author as belonging to, or being close to, *H. sclerophylla*, however preliminary molecular studies by the second author found distances of around 8% in DNA barcode sequences (the 5'-half of the COI gene) between populations from the type locality and the Blue Mountains (Megalong and Lower Portland) and even greater divergence from the Cooloola, southern Queensland, material (circa 14%). These large distances are similar to observed distances between the described species *H. highlandi* Smith, 2014

and *H. buntonorum* Smith, 2016, which raised the question of whether *H. sclerophylla* contains cryptic species, and prompted further study. The fourth author also performed preliminary analyses of the mitochondrial 16S rDNA gene for the population of "*sclerophylla*" from Cooloola and individual specimens from North Nowra and Bamaga, with similar results.

Further sequencing of both DNA barcodes and a nuclear gene, the D9-D10 region of the 28S ribosomal DNA, was conducted on several specimens each from Broulee, Megalong, Wellington and Cooloola/Carlo Point and a few specimens from other named localities. Detailed morphological and morphometric comparisons of several specimens from each locality were conducted. Some characters, such as the presence of lanceolate scales and the number of divisions in the ovipositor cannot be easily determined in alcohol material, so much of the material was dissected and mounted before examination.

Materials and methods

Abbreviations and definitions

Roman numerals are used to indicate abdominal segment number. In addition, the following abbreviations are used: AM: Australian Museum, 1 William St, Sydney 2010 Australia; asl: above sea level (in metres); HW: head width (in millimetres); H+B: head and body length (in millimetres); L/W: length to width (ratio); NSW: New South Wales; PI, PII, PIII: legs of prothorax, mesothorax and metathorax respectively; PCA: principal component analysis; penult: penultimate, referring to second last article of maxillary palp; QLD: Queensland; QM: Queensland Museum, Grey St & Melbourne St, South Brisbane QLD 4101; ult: ultimate (referring to last article). The prefixes pro, meso and meta are affixed to thoracic characters such as sterna, tibia and tarsus.

Specimens are currently stored in 80% ethanol/water unless specifically mentioned as being in 100% ethanol or else mounted on slides using Tendeiro medium. The term macrochaetae refers to the larger stronger bristles (always apically bifurcated), setae to smaller thinner bristles (bifurcated or simple), setulae to the very small, usually straight, setae associated with the combs and cilia to the curly thin hairs, often associated with the combs, setal collar or notal margins.

Collection of material, locality data and preparation

Details of the material examined are included with the description of each species. Locality co-ordinates were obtained using a hand-held Garmin eTrex®10 GPS with a claimed accuracy usually under 5 m. Dissected specimens have been mounted on two slides using Tendeiro medium (one slide with head and thorax, the other with the abdomen). Drawings were made with the aid of an Olympus CX31 binocular microscope fitted with a U-DA drawing attachment.

The holotype and a paratype of *Heterolepisma cooloola* are deposited with the Queensland Museum in Brisbane. Most of the remaining material is deposited with the Australian Museum in Sydney and each slide or specimen has an accession number starting with K (e.g., K.377269). Some material is still held by the first author, refrigerated in 100% ethanol. This material carries the author specimen data base number (e.g., gbs004932) and will eventually also be deposited with the Australian Museum.

Sampling, DNA extraction, PCR and DNA sequencing

Table 1 lists the 68 specimens subjected to DNA analysis, their collection localities, species identification, and BOLD and GenBank accession numbers for their DNA sequences. Species from three genera of Ctenolepismatinae were used as outgroups: *Ctenolepisma longicaudata* Escherich, *Qantelsella louisae* Smith, *Acrotelsella erniei* Smith and *Acrotelsella parvelar* Smith.

DNA extractions performed at the AM (for DNA barcode/COI and 28S rDNA sequences) used either the Bio Basic EZ-10 96 well plate Genomic DNA Isolation Kit (Astral Scientific, Taren Point, NSW) or the Bioline Isolate II Genomic DNA Kit (Bioline, Eveleigh, NSW) following the manufacturers' protocols, with exceptions noted below. DNA extractions performed at Marist College, New York (for 16S rDNA sequences) used a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD).

About five whole specimens from each key locality (Broulee, Megalong, Wellington and Cooloola/Carlo Point), collected directly into 100% ethanol, were soaked in DNA extraction buffer containing proteinase-K at room temperature for three hours. The remaining cuticle was returned to 100% ethanol and later dissected in 80% ethanol and mounted on to slides using Tendeiro medium (usually head and thorax on one slide, abdomen on the other).

DNA for specimens from most other localities was extracted from legs which had been removed from the specimen shortly after collection and stored in 100% ethanol at 4°C. The rest of the specimen was kept in 80% ethanol at room temperature.

Polymerase Chain Reaction (PCR) amplification of the DNA barcode region of the mitochondrial COI gene used the primers and followed the method of Mitchell (2015). For the 28S rDNA D9-D10 region, we used one forward (28S_8fm) and two reverse PCR primers (28S_10rm and 28S_11rm), which were simply 5'-M13-tailed versions of Machida and Knowlton's (2012) primers [28S] #8, [28S] #10_RC and [28S] #11_RC, respectively. PCR conditions for both genes followed those reported in Mitchell (2015) for COI. PCR products were purified using ExoSAP and sequenced in both directions using ABI Big Dye Terminator v.3.1 chemistry by Macrogen Inc. (Seoul, South Korea).

The 16S rDNA gene was amplified using primers 16Sar and 16Sb (Edgecombe *et al.* 2002). Amplification, PCR purification, sequencing, and chromatogram analyses were carried out as in Espinasa and Cappuccio (2008). One 16S sequence was downloaded from GenBank for *Tricholepidion gertschi* to serve as an outgroup.

DNA sequence assembly and phylogenetic analysis

Forward and reverse direction sequence trace files were assembled using Geneious v. 9.1.7 (Kearse *et al.*, 2012) and consensus sequences were aligned using Muscle (Edgar, 2004) and adjusted by eye. DNA sequences, sequence trace

files, and specimen collection data were uploaded to BOLD (Ratnasingham and Hebert, 2007) and GenBank. Two 16S sequences (samples K.377738 and K.377751) were uploaded to BOLD, but were not accepted by GenBank as they are less than 200 bp in length.

Four data sets were constructed: one for each of the three genes alone, COI, 28S, and 16S, and a concatenated gene data set. The COI alignment was trimmed from the 3'-end to 559 nucleotides to minimize missing data before phylogenetic analysis. The concatenated gene data set comprised only samples for which both 28S and COI sequences has been obtained. For outgroups *C. longicaudata* and *Acrotelsella* spp. the 28S and COI sequences were generated from different specimens (and for *Acrotelsella* from different species) thus the sequences are labelled with "Chimera" rather than with a Museum accession number in Fig. 4, although Table 1 lists the accession numbers of the specimens used for each gene. We did not include 16S sequences in the concatenated data set because there was only one specimen for which we had both 16S and other sequence data.

FABOX v. 1.4.1 (Villesen, 2007) was used to edit sequence names. MEGA v.7.0.26 (Kumar *et al.*, 2016) was used to calculate genetic distances. For phylogenetic analysis, nucleotide substitution models, and data partitions (codon positions) for COI, were tested using PartitionFinder2 (Lanfear *et al.*, 2012) on the CIPRES computing platform (Miller *et al.*, 2010), with the most appropriate model selected using the Bayesian Information Criterion. For 16S the best model was GTR+G, and for 28S the best model was K80+G. For COI the data had two partitions, codon positions 1 and 2 (best model TRNEF+I+G) and codon position 3 (best model HKY+G). For the concatenated data set PartitionFinder selected the same two partitions and models as COI, with 28S included in the partition with COI codon positions 1 and 2.

Exploratory phylogenetic analyses were performed in Fasttree 2 (Price *et al.*, 2010) while final analyses were performed using Maximum Likelihood (ML) as implemented in RAxML 7.2.8 (Stamatakis, 2006) and Bayesian Inference (BI) in MrBayes v.3.2.6 (Ronquist *et al.*, 2012) using the plugins available in Geneious. RAxML analyses used the ML search convergence criterion and performed 1,000 fast bootstrap replicates. MrBayes analyses used four heated chains with chain temperature = 0.2, a chain length of 2,000,000 generations, subsampling every 1,000 generations, and a burnin of 500 samples (25%). Convergence was assessed by examining the average standard deviation of splits frequencies, with values below 0.01 indicating convergence. Convergence was reached in two million generations for the single gene analyses and in one million generations for the concatenated genes data set.

Morphological and morphometric evaluation

Specimens were measured in alcohol according to the methodology in Smith (2013). Due to the continuous moulting, most measurements were compared as ratios, either length to width, or compared to head width. While some immature specimens were measured, especially when fewer "adult" specimens were available, the measurement data is intentionally biased towards the largest available sexually mature individuals. Consistent measurement data is difficult to generate, not only due to the large number of instars but it is also difficult to align the part to be measured completely horizontal. Some specimens are bent over and cannot be flattened for accurate measurement. Being soft-bodied the specimen can be distorted due to damage or preservation, with the various segments either contracted into each other or distended hence the unreliability of H+B, thorax and abdomen lengths. In addition, the nota can sometimes have lost their curvature and hence appear wider than expected probably because the specimen was partially squashed during collection. Ranges of measurement data are often quite wide and it is therefore difficult to find parameters where there is no overlap in the range. Some measurements, e.g., L/W of inner processes of coxites IX, L/W of thoracic sterna and L/W of urotergite X, can only be made using dissected material. The number of divisions in each of the ovipositor valves must also be made with dissected material.

The following measurement ratios were compared: thorax length/HW, thorax length/H+B, thorax length/width (widest part of mesonotum), abdomen length/H+B, width/HW of all nota, thorax length/abdomen length, H+B/HW, L/W of pedicel, L/W of the scape, the length of the ultimate article of the maxillary palp relative to its width, to head width and the length of the penultimate article, the length of both pedicel and scape relative to each other and to head width, length of tarsi of all legs/HW, length of tarsus /tibia of all legs, L/W of ultimate article of maxillary palp, length of ultimate/penultimate articles of maxillary palp, L/W ultimate article of labial palp, length of pedicel/scape, L/W of thoracic sterna, their width relative to head width, their length relative to each other, the width of the gap between the combs on the metathoracic sternite relative to the average length of the combs, the length of all tarsi and tibiae relative to both their width and HW, the length of the tibia and tarsi of PI relative to PIII, the L/W of urotergite X and the inner and outer processes of coxites IX (the latter independently for males and females), length stylus VII/IX, length of stylus VIII/IX and the length of the ovipositor relative to head width.

Morphological characters compared include the presence and distribution of lanceolate scales, the chaetotaxy of the frons, clypeus and labrum, the relative size and chaetotaxy of the pedicel and scape, the chaetotaxy of the outer face of the mandibles, the length of molar region and the chaetotaxy at the end of molar region, the number of teeth on lacinia and its length relative to galea, the number of lamellae and setae on the lacinia, the chaetotaxy of the articles of the maxillary palp, the relative length of the most distal articles and the presence, type and location of the branched papillae on the ultimate article of both the males and females, the chaetotaxy of the submentum and mentum, the chaetotaxy of the labial palp and the shape of the last article and the arrangement of its papillae, the density of anterior collar of the pronotum, location of trichobothrial areas and their association with macrochaetae, the lateral and posterior chaetotaxy of all nota, the chaetotaxy of the presternum, the shape, size and chaetotaxy of all thoracic sterna, the scale covering and chaetotaxy of PI and PIII, the chaetotaxy of the urotergites and urosternites (position of combs as well as the number of macrochaetae, marginal setae, setulae and cilia), the shape and chaetotaxy of urotergite X, the number and size of the styli, the number of divisions of the ovipositor, the size, position and chaetotaxy of the paramera and the distribution of pigment.

Table 1. Museum, BOLD and GenBank accession numbers for all sequences obtained.

					BOLD Numbers		GenBank Accession Numbers		
Museum ID	Species	Extra Info	State	Site	Sample ID	Process ID	COI	28S	16S
K.261126	*H. cooloola* sp. nov.	paratype	QLD	Cooloola	gbs004890	ZYI042-17	MF040956	—	—
K.261128	*H. cooloola* sp. nov.	paratype	QLD	Cooloola	gbs004891	ZYI043-17	—	KY951403	—
K.261130	*H. cooloola* sp. nov.	paratype	QLD	Cooloola	gbs004892	ZYI044-17	MF040955	KY951402	—
K.261132	*H. cooloola* sp. nov.	paratype	QLD	Cooloola	gbs004894	ZYI045-17	MF040954	—	—
K.261134	*H. cooloola* sp. nov.	paratype	QLD	Cooloola	gbs004895	ZYI046-17	MF040953	—	—
K.261136	*H. cooloola* sp. nov.	—	QLD	Carlo Point	gbs004868	ZYI038-17	MF040951	KY951399	—
K.261138	*H. cooloola* sp. nov.	—	QLD	Carlo Point	gbs004873	ZYI039-17	—	KY951406	—
K.261140	*H. cooloola* sp. nov.	—	QLD	Carlo Point	gbs004877	ZYI040-17	—	KY951405	—
K.261180	*H. cooloola* sp. nov.	—	QLD	Carlo Point	gbs004867	ZYI037-17	—	N/A [<200 nt]	—
K.261182	*H. cooloola* sp. nov.	—	QLD	Carlo Point	gbs004879	ZYI041-17	MK185704	KY951404	KY951372
K.261186	*H. cooloola* sp. nov.	paratype	QLD	Cooloola	gbs003331	ZYI059-17	—	—	KY951367
K.377746	*H. cooloola* sp. nov.	—	QLD	Cooloola	gbs003324	ZYI060-17	—	—	KY951368
K.377748	*H. cooloola* sp. nov.	—	QLD	Cooloola	gbs003353	ZYI016-17	MF040952	KY951400	—
K.377752	*H. cooloola* sp. nov.	—	QLD	Carlo Point	gbs004871	ZYI061-17	—	—	KY951371
K.377753	*H. cooloola* sp. nov.	—	QLD	Carlo Point	gbs004883	ZYI062-17	—	—	KY951369
K.377754	*H. cooloola* sp. nov.	—	QLD	Cooloola	gbs004893	ZYI063-17	—	—	KY951370
QM207012	*H. cooloola* sp. nov.	paratype	QLD	Cooloola	gbs003330	ZYI015-17	—	KY951401	—
K.261204	*H. coorongooba* sp. nov.	paratype	NSW	Glen Davis	gbs004629	ZYI024-17	MF040960	KY951410	—
K.261208	*H. coorongooba* sp. nov.	holotype	NSW	Glen Davis	gbs004626	ZYI021-17	MF040957	KY951407	—
K.261210	*H. coorongooba* sp. nov.	paratype	NSW	Glen Davis	gbs004627	ZYI022-17	MF040958	KY951408	—
K.261212	*H. coorongooba* sp. nov.	paratype	NSW	Glen Davis	gbs004628	ZYI023-17	MF040959	KY951409	—
K.260992	*H. sclerophylla*	paratype	NSW	Broulee	gbs001024	ZYI001-17	MF040943	KY951392	—
K.260994	*H. sclerophylla*	—	NSW	Guerilla Bay	gbs001032	ZYI002-17	MF040942	—	—
K.261084	*H. sclerophylla*	—	NSW	Nattai	gbs003094	ZYI014-17	—	KY951386	—
K.261142	*H. sclerophylla*	—	NSW	Broulee	gbs004969	ZYI048-17	MF040946	KY951396	—
K.261143	*H. sclerophylla*	—	NSW	Broulee	gbs004985	ZYI049-17	—	KY951375	—
K.261145	*H. sclerophylla*	—	NSW	Broulee	gbs004986	ZYI050-17	MF040945	KY951395	—
K.261150	*H. sclerophylla*	—	NSW	Broulee	gbs004987	ZYI051-17	MF040944	KY951394	—
K.261152	*H. sclerophylla*	—	NSW	Broulee	gbs004989	ZYI052-17	MK185703	KY951393	—
K.261158	*H. sclerophylla*	—	NSW	Wellington	gbs004856	ZYI036-17	MF040947	—	—
K.261160	*H. sclerophylla*	—	NSW	Wellington	gbs004853	ZYI034-17	MF040925	KY951377	—
K.261162	*H. sclerophylla*	—	NSW	Wellington	gbs004850	ZYI032-17	MF040926	KY951379	—
K.261164	*H. sclerophylla*	—	NSW	Wellington	gbs004851	ZYI033-17	—	KY951378	—
K.261166	*H. sclerophylla*	—	NSW	Megalong	gbs004820	ZYI027-17	MF040930	KY951382	—
K.261168	*H. sclerophylla*	—	NSW	Megalong	gbs004823	ZYI028-17	MF040929	—	—
K.261170	*H. sclerophylla*	—	NSW	Megalong	gbs004825	ZYI029-17	MF040928	—	—
K.261172	*H. sclerophylla*	—	NSW	Megalong	gbs004826	ZYI030-17	—	KY951381	—
K.261174	*H. sclerophylla*	—	NSW	Megalong	gbs004831	ZYI031-17	MF040927	KY951380	—
K.261184	*H. sclerophylla*	—	NSW	Jibbon	gbs004812	ZYI026-17	MF040931	—	—
K.261194	*H. sclerophylla*	—	NSW	Lower Portland	gbs001166	ZYI005-17	MF040940	—	—
K.261196	*H. sclerophylla*	—	NSW	North Nowra	gbs004241	ZYI054-17	—	—	KY951366
K.261200	*H. sclerophylla*	—	NSW	Bucketty	gbs004299	ZYI020-17	MF040932	KY951383	—
K.261234	*H. sclerophylla*	—	NSW	North Nowra	gbs004253	ZYI019-17	MF040933	KY951384	—
K.261240	*H. sclerophylla*	—	NSW	Nattai	gbs003090	ZYI013-17	—	KY951387	—
K.261246	*H. sclerophylla*	—	NSW	Wellington	gbs004855	ZYI035-17	—	KY951376	—
K.377577	*H. sclerophylla*	—	NSW	Lower Portland	gbs001178	ZYI006-17	MF040939	—	—
K.377580	*H. sclerophylla*	—	NSW	Glenbrook	gbs001125	ZYI004-17	MF040941	KY951391	—
K.377581	*H. sclerophylla*	—	NSW	Glenbrook	gbs003060	ZYI012-17	MF040935	KY951388	—
K.377583	*H. sclerophylla*	—	NSW	Megalong	gbs001409	ZYI009-17	MF040938	—	—
K.377592	*H. sclerophylla*	—	NSW	Burralow	gbs001730	ZYI010-17	MF040937	KY951390	—
K.377593	*H. sclerophylla*	—	NSW	Burralow	gbs001733	ZYI011-17	MF040936	KY951389	—
K.377738	*H. sclerophylla*	—	NSW	Megalong	gbs004841	ZYI057-17	—	—	N/A [<200 nt]
K.377751	*H. sclerophylla*	—	NSW	Megalong	gbs004839	ZYI056-17	—	—	N/A [<200 nt]
K.377772	*H. sclerophylla*	—	NSW	Bents Basin	gbs004782	ZYI025-17	—	MK185708	—
K.377749	*H.* sp. nr. *cooloola*	—	QLD	Undara	gbs003828	ZYI018-17	MF040934	KY951385	—
K.377755	*H.* sp. nr. *cooloola*	—	QLD	Chillagoe	gbs001266	ZYI007-17	MF040950	—	—
K.377756	*H.* sp. nr. *cooloola*	—	QLD	Chillagoe	gbs001277	ZYI008-17	MF040949	—	—
K.377757	*H.* sp. nr. *cooloola*	—	QLD	Bamaga	gbs003755	ZYI058-17	—	—	KY951364
K.377759	*H.* sp. nr. *cooloola*	—	QLD	Punsand	gbs003750	ZYI017-17	MF040948	KY951397	—
N/A	*H.* sp. nr. *cooloola*	—	QLD	Mingella	gbs001322	ZYI053-17	—	KY951398	—
K.261244	*H. buntonorum*	—	TAS	Knocklofty	gbs004929	ZYI047-17	MF040923	KY951373	—
K.377604	*H. highlandi*	paratype	NSW	Wee Jasper	gbs001119	ZYI003-17	MF040924	KY951374	—
N/A	*H.* sp. nr. *highlandi*	—	NSW	Red Hill	gbs004492	ZYI055-17	—	—	KY951365
gbs001275	*Ctenolepisma longicaudata*	outgroup	QLD	Chillagoe	gbs001275	ZYI064-18	MK185702	—	—
K.377675	*Ctenolepisma longicaudata*	outgroup	TAS	Hobart	gbs001836	ZYI065-18	—	MK185707	—
K.377609	*Acrotelsella erniei*	outgroup, holotype	NT	Mt Sonder	gbs001438	ZYI066-18	MK185701	—	—
K.261103	*Acrotelsella parlevar*	outgroup, holotype	TAS	Travellers Rest	gbs004624	ZYI067-18	—	MK185706	—
QM228755	*Qantelsella louisae*	outgroup, holotype	QLD	Bladensburg	gbs003917	ZYI068-18	MK185705	MK185709	—
N/A	*Tricholepidion gertschi*	outgroup	—	—	—	—	—	—	AY191994

There is a large amount of variability in almost all these characters. Sometimes the variability is between individuals and other times from the left to the right side of the same specimen. The number and thickness of macrochaetae seems to be greater on larger specimens and the pigmentation seems to be stronger. Some populations were found to have more individuals with a certain character, e.g., a glabrous posterior medial region on the mesosternum but not in all cases and unless a character proved consistent for all individuals examined within one lineage versus another then it was concluded that the character did not qualify as diagnostic. Many or even most macrochaetae have been lost on dissected specimens and chaetotaxy comparisons are based largely on insertion points. It is possible that significant differences in the size and form of the bristles exist that could not be seen when comparing the slide material. For example, the apex of the more lateral macrochaeta of the posterior combs of the metanotum in a specimen from North Nowra (K.261196) was very thin and tapering, almost trichobothria-like, while that of a specimen from Lower Portland (K.261050) was delicately bifurcate and another from Broulee (K.261218) was thicker at the base than K.261196 but also simple apically. More specimens, in better conditions (perhaps collected live and held until after the following moult) may provide additional useful characters.

To explore the morphometric data, we carried out two Principal Components Analyses (PCA). In the first analysis (PCA1), we excluded juvenile specimens as well as specimens and morphological characters for which the data was incomplete. This resulted in a dataset consisting of 40 specimens (from seven lineages) and 36 morphological characters. The second analysis (PCA2) was performed on 21 adult female specimens, and included 15 female-only characters relating to the ovipositor morphology. A separate analysis was conducted on the gap between the combs versus the average length of the combs using only the available slide mounted material.

PCA was conducted in R v3.3.3, (R Core Team, 2017), and visualized using the R package "ggbiplot" (Vu, 2011). Data was log transformed before analysis, means were set to zero and variance was scaled between 0 and 1. As the dataset for the metathoracic combs was found to be non-normally distributed (Shapiro-Wilk test, W-statistic = 0.79283, $p = 4.061 \times 10^{-7}$), and without homogeneity of variance (Bartlett's test, Bartlett's $K^2 = 16.255$, df = 6, p = 0.01245), the Kruskal-Wallis test was used. The Kruskal-Wallis test returned a significant result ($\chi^2 = 35.609$, df = 6, p-value = 3.284×10^{-6}). The post-hoc Dunn Test without corrections (Dunn, 1964) was conducted in R using the package dunn.test v1.3.5 (Dinno, 2017).

Results

Molecular data

Table 1 lists the BOLD and GenBank accession numbers of all sequences obtained. There were 44 COI sequences, 34 of which were >485 bp in length, meeting the length requirement of the BARCODE standard (Hanner, 2009), with 10 partial sequences of 300–485 bp long. Mean base compositions in COI for A, C, G and T were 30.9%, 24.3%, 16.8% and 28.1%, respectively, or 41% GC.

Forty-two 28S sequences were obtained, with 32 being derived from primer pair 28S_8fm/28S_11rm and comprising 442 bp aligned, while the remaining 10 sequences were obtained with primer pair 28S_8fm/28S_10rm and comprised 230 bp aligned. For 28S the mean base compositions for A, C, G and T were 25.6%, 23.6%, 27.8% and 23.0%, respectively, or 51.4% GC.

Twelve 16S sequences were obtained and the alignment had a length of 503 bp.

Figures 2–4 show the ML trees obtained for the mitochondrial genes (COI and 16S rRNA), for the nuclear gene 28S rRNA, and for the concatenated 28S and COI genes, respectively. BI Posterior Probabilities (PP) are shown above branches if greater than 0.90 and ML bootstrap percentages (BP) are shown below branches if greater than 70%, while paratypes are indicated with an asterisk and holotypes with double asterisks.

All BI analyses converged. ML and BI analyses gave almost identical results, the only differences being that that placement of some taxa (e.g., sample K.377759 from Punsand, see below, and K.377604 *H. highlandi*) were strongly supported by BI but had no support (BP <50) under ML.

For COI the BI tree (Fig. 2) did not recover the ingroup (the *H. sclerophylla* group) as monophyletic. Instead, the *H. sclerophylla* group was split into Queensland and NSW lineages, although the single Punsand (Cape York) specimen was placed as sister-group to the NSW lineage. *Heterolepisma highlandi* is placed between the two *H. sclerophylla* group lineages while *H. buntonorum* is basal to them, and the two taxa are separated by an uncorrected distance of 10.8%.

Within the Queensland lineage, the eight samples from Cooloola and Carlo Point in South East Queensland formed a tightly clustered clade with 94% bootstrap support, although each sequence was unique. Seven of eight sequences showed 0.4–1.8% uncorrected distances among them, while the eighth sequence was somewhat separated at a distance of 1.8–4.0% from the others. Specimens from Chillagoe (n = 2), Undara (n = 1) and Punsand (n = 1) showed inter-population distances of 4.0–9.4%.

Within the NSW group six distinct clades were apparent, each with low intra-clade distances compared with inter-group distances, and with very strong bootstrap support (>90%) in all but a single case, where bootstrap support was still strong (74%). Clade 1 comprised a single sample from North Nowra, approximately 110 km north of the type locality, Broulee, on the NSW south coast. Clade 2 comprised five samples from Broulee, one of which is a paratype, one from the nearby locality of Guerilla Bay, as well as one specimen from Jibbon, another coastal locality 217 km north of Broulee. The maximum intra-clade DNA distance was 5.3%, which was the same as the nearest neighbour distance for a specimen from Lower Portland. Clade 3 comprised four specimens from Glen Davis with maximum intra-clade distance of 0.8% and a minimum nearest neighbour distance of 7.2%. Clade 4 comprised three samples from Wellington with a maximum intra-clade distance of 1.3% and a minimum nearest neighbour distance of 5.4%. Clade 5 comprised five samples from Burralow, Glenbrook and Bucketty, with a maximum intra-clade distance of 4.9% (for the specimen from Bucketty) and a minimum nearest neighbour distance of 4.0%. Clade 6 comprised seven samples, five from Megalong Valley and two from Lower Portland, with a maximum intra-clade distance of 4.0% and a minimum nearest neighbour distance of 3.6%.

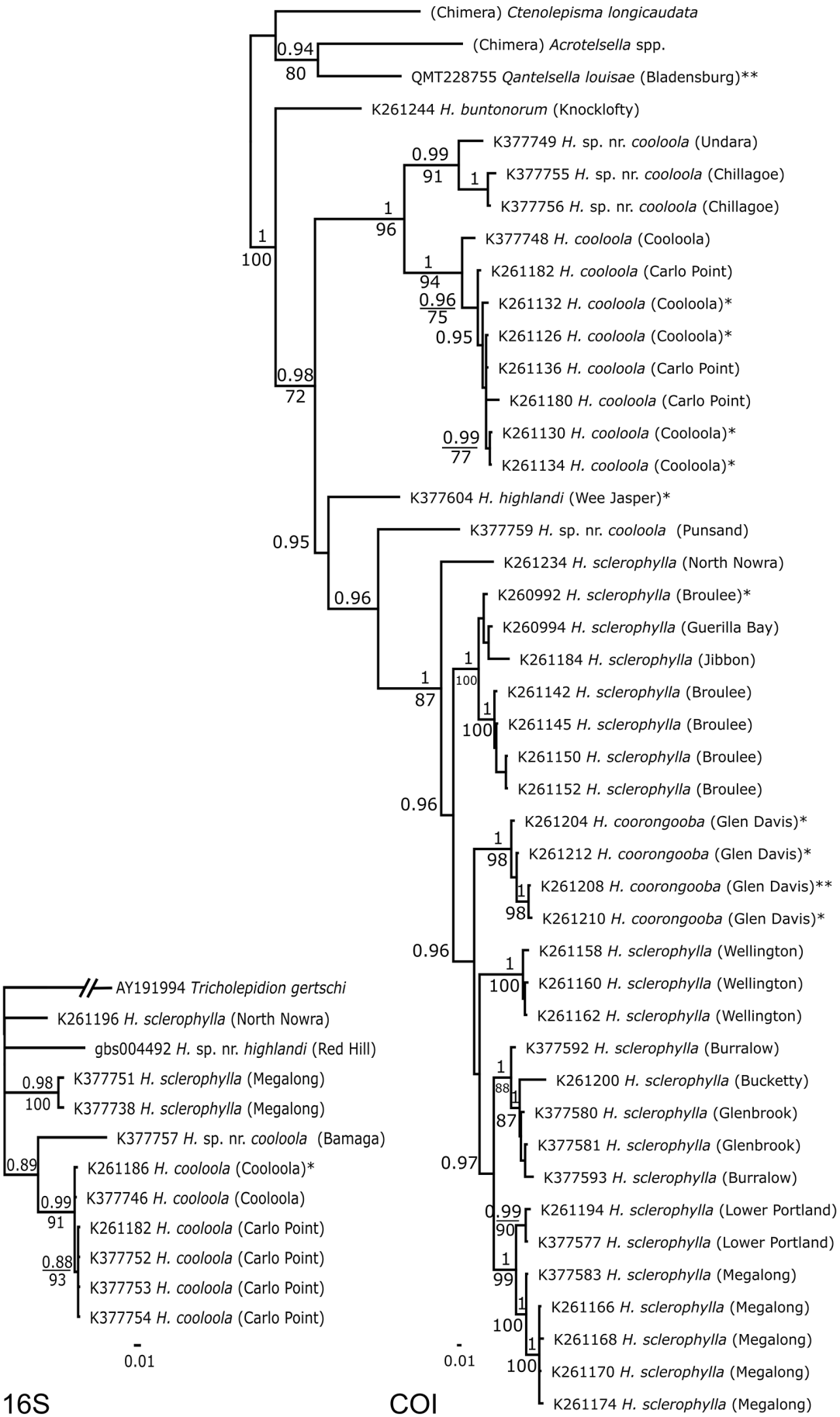


Figure 2. BI trees for mitochondrial genes 16S and COI. BI posterior probabilities and ML bootstrap values are shown above and below branches, if ≥0.9 or ≥70%, respectively. Asterisks indicate type specimens, with a single asterisk for paratypes and a double asterisk for holotypes.

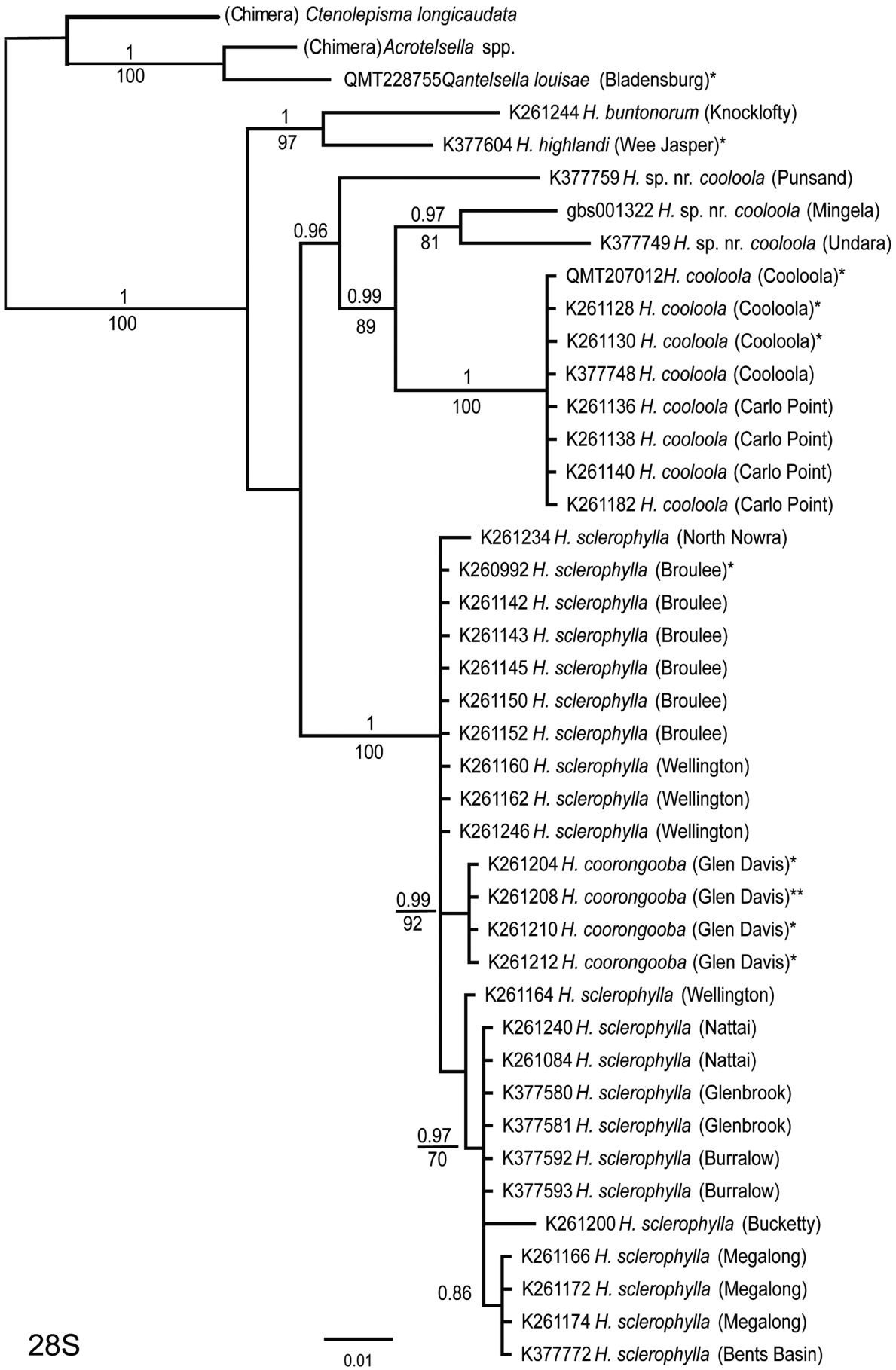


Figure 3. BI tree for nuclear gene 28S. BI posterior probabilities and ML bootstrap values are shown above and below branches, if ≥0.9 or ≥70%, respectively. Asterisks indicate type specimens, with a single asterisk for paratypes and a double asterisk for holotypes.

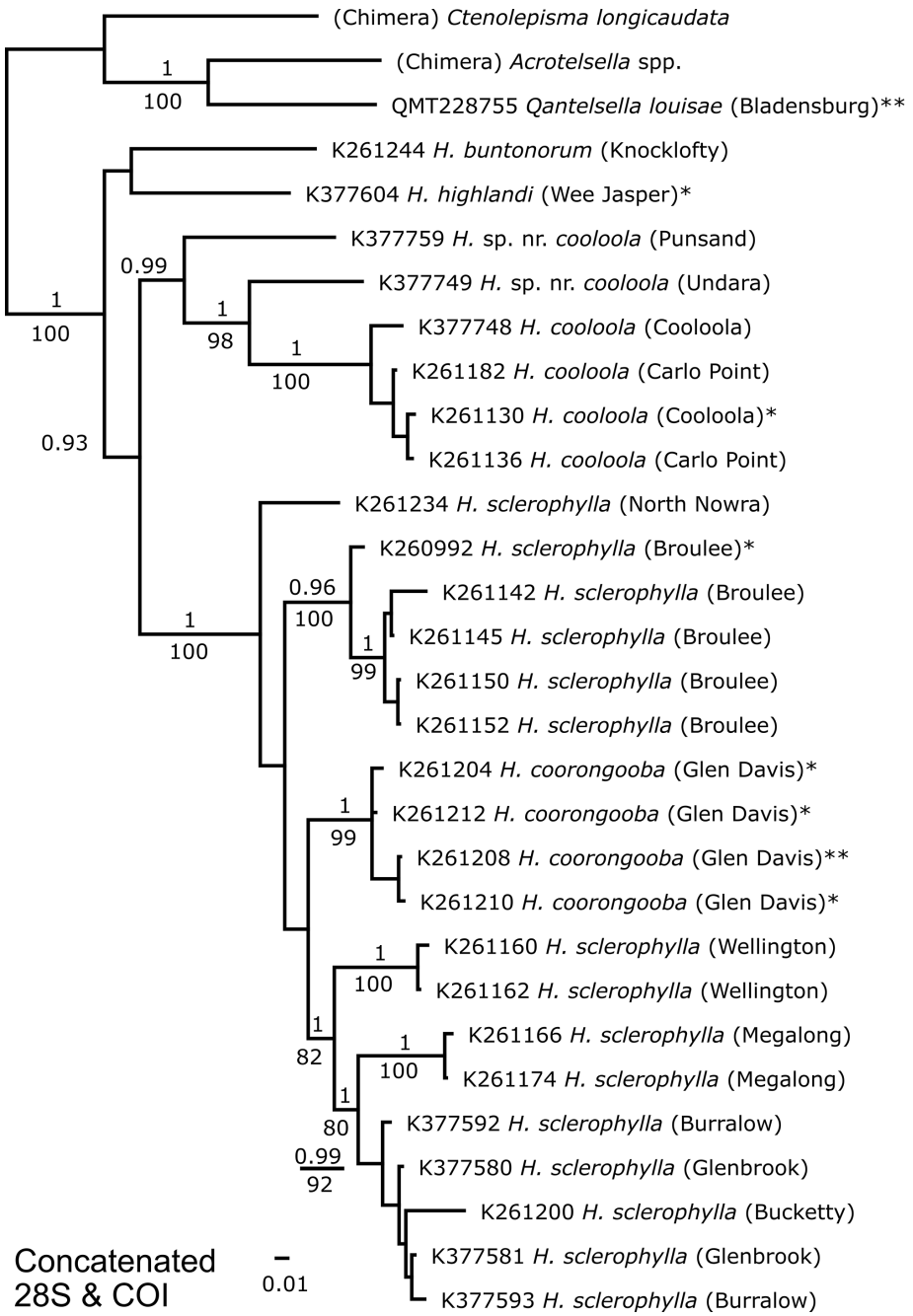


Figure 4. BI tree for concatenated COI and 28S genes. BI posterior probabilities and ML bootstrap values are shown above and below branches, if ≥0.9 or ≥70%, respectively. Asterisks indicate type specimens, with a single asterisk for paratypes and a double asterisk for holotypes.

The BI tree for the limited 16S data (Fig. 2) also showed the southern Queensland population to be distinct from the far North Queensland sample and separation of these taxa from the two NSW populations sampled, North Nowra and Megalong.

The 28S rDNA data showed a broadly similar BI tree topology (Fig. 3) with 6.4% distance between *H. buntonorum* and *H. highlandi*, a deep split between all Queensland (including Punsand) and NSW populations, deep splits among Queensland populations with the Cooloola/Carlo Point population distinct (nearest neighbour distance 10.8%). However, much less variation was observed among NSW populations. Considering the six lineages defined by the DNA barcode data, very little intra-lineage DNA variation was observed, except for the sample from Bucketty, which was 0.5% different from Glenbrook, Burralow and Nattai samples. This lineage could be distinguished from the Megalong population (0.5% distance). The North Nowra sample was 0.9–1.8% distant from other NSW *H. sclerophylla* group samples, however it was placed within a lineage containing both Broulee and Wellington samples. 28S sequences from the latter two localities are identical, except that specimen K.261164 from Wellington is longer at 442 nt versus 230 nt because the longer amplicon failed to amplify in most of these specimens.

The concatenated COI and 28S dataset yields a tree in which deeper relationships in the ingroup follow the 28S topology and shallower relationships, e.g., with the *H.*

Table 2. Morphometric comparison summary including all specimens (including juveniles) assigned to a lineage and not just those from the nominal locality.

	Heterolepisma sclerophylla lineages										*H. coorongooba* sp. nov.		*H. cooloola* sp. nov.	
	Broulee		North Nowra		Glenbrook		Megalong		Wellington		Glen Davis		Cooloola	
Measurement parameter	mean (n)	range	mean (n)	range	mean (n)	range	mean (n)	range	mean (n)	range	mean (n)	range	mean (n)	range
Head width (HW)	1.19 (16)	0.84–1.40	1.19 (2)	1.13–1.25	1.09 (21)	0.79–1.50	1.15 (12)	0.88–1.38	0.99 (4)	0.98–1.01	1.16 (4)	1.08–1.30	1.08 (11)	0.98–1.20
H+B length	7.69 (15)	5.43–8.75	8.09 (2)	8.03–8.15	7.92 (16)	5.55–11.35	7.89 (9)	5.25–9.53	7.19 (4)	6.50–8.50	8.03 (3)	7.03–9.10	7.98 (11)	6.78–9.90
Thorax length/HW	2.01 (15)	1.85–2.35	2.11 (2)	2.06–2.16	2.21 (16)	2.05–2.34	2.07 (9)	1.83–2.30	2.15 (4)	2.00–2.33	2.15 (3)	1.92–2.35	2.14 (11)	1.98–2.38
Thorax length/H+B	0.31 (15)	0.29–0.34	0.31 (2)	0.30–0.32	0.30 (14)	0.28–0.33	0.30 (9)	0.29–0.33	0.30 (4)	027–0.31	0.31 (3)	0.27–0.36	0.29 (11)	0.26–0.32
Thorax length/width (meso)	1.23 (15)	1.10–1.42	1.35 (2)	1.24–1.45	1.48 (14)	1.29–1.48	1.28 (9)	1.07–1.39	1.40 (4)	1.23–1.58	1.33 (3)	1.22–1.44	1.34 (11)	1.19–1.52
Abdomen length/H+B	0.61 (15)	0.57–0.65	0.61 (2)	0.59–0.62	0.63 (15)	0.59–0.68	0.66 (9)	0.60–0.79	0.63 (4)	0.60–0.68	0.62 (3)	0.56–0.66	0.64 (11)	0.59–0.68
Prothorax width/HW	1.56 (15)	1.44–1.76	1.55 (2)	1.44–1.67	1.52 (15)	1.40–1.67	1.53 (9)	1.33–1.70	1.48 (4)	1.28–1.58	1.55 (4)	1.53–1.58	1.57 (11)	1.48–1.70
Mesothorax width/HW	1.64 (15)	1.53–1.84	1.58 (2)	1.42–1.73	1.59 (15)	1.45–1.67	1.62 (9)	1.47–1.72	1.54 (4)	1.36–1.67	1.61 (4)	1.58–1.64	1.60 (11)	1.54–1.72
Metathorax width/HW	1.54 (15)	1.39–1.61	1.48 (2)	1.34–1.62	1.53 (15)	1.38–1.63	1.54 (9)	1.41–1.68	1.46 (4)	1.23–1.62	1.51 (4)	1.46–1.55	1.51 (11)	1.40–1.57
Thorax length/abdomen length	0.50 (15)	0.45–0.59	0.51 (2)	0.48–0.54	0.48 (14)	0.41–0.54	0.46 (9)	0.40–0.50	0.47 (4)	0.40–0.52	0.51 (3)	0.41–0.64	0.45 (11)	0.39–0.54
H+B/HW	6.54 (15)	5.6–7.36	6.83 (2)	6.42–7.24	7.32 (15)	6.40–7.88	6.87 (9)	5.60–7.88	7.26 (4)	6.50–8.72	6.82 (3)	6.53–7.00	7.40 (11)	6.69–8.25
Ult max palp art/HW	0.30 (13)	0.24–0.33	0.34 (2)	0.33–0.35	0.32 (16)	0.29–0.35	0.31 (9)	0.30–0.37	0.32 (4)	0.29–0.37	0.32 (4)	0.29–0.33	0.22 (11)	0.20–0.25
Ult max palp art L/W	4.30 (13)	3.75–5.50	4.85 (2)	4.82–4.88	4.47 (16)	3.75–5.65	4.57 (9)	3.56–5.57	4.29 (4)	3.87–5.14	4.58 (4)	4.12–4.93	4.53 (11)	3.63–6.00
Penult max palp art L/W	2.72 (13)	1.41–3.87	3.25 (2)	3.16–3.33	3.13 (16)	2.22–3.60	3.23 (8)	2.63–3.88	2.66 (4)	2.42–3.16	3.14 (4)	2.95–3.53	2.67(11)	2.20–3.16
Ult/penult maxpalp art	1.38 (13)	1.09–2.00	1.33 (3)	1.30–1.37	1.29 (16)	1.16–1.75	1.22 (8)	1.11–1.36	1.32 (4)	1.20–1.43	1.23 (4)	1.16–1.27	1.30 (11)	1.16–1.57
Labial palp art L/W	1.04 (14)	0.90–1.21	1.01 (2)	0.86–1.16	1.09 (17)	0.86–1.35	1.09 (7)	0.89–1.33	1.05 (4)	0.92–1.12	1.12 (4)	0.77–1.30	1.05 (11)	0.90–1.19
Pedicel/scape	0.47 (14)	0.35–0.65	0.46 (2)	0.42–0.50	0.46 (16)	0.38–0.63	0.48 (6)	0.41–0.55	0.50 (4)	0.44–0.64	0.51 (4)	0.47–0.55	0.42 (9)	0.33–0.53
Pedicel/HW	0.08 (14)	0.07–0.10	0.07 (2)	0.06–0.08	0.08 (16)	0.06–0.10	0.09 (6)	0.07–0.10	0.08 (4)	0.07–0.09	0.08 (4)	0.08–0.08	0.07 (9)	0.05–0.09
Scape/HW	0.18 (14)	0.16–0.22	0.16 (2)	0.15–0.16	0.18 (16)	0.15–0.20	0.18 (6)	0.14–0.21	0.17 (4)	0.14–0.19	0.16 (4)	0.15–0.17	0.18 (10)	0.14–0.20
Prosternum L/W	0.97 (7)	0.86–1.14	1.07 (2)	1.05–1.09	0.98 (8)	0.88–1.06	0.99 (9)	0.96–1.06	0.99 (6)	0.92–1.07	0.95 (4)	0.91–1.00	1.08 (16)	1.00–1.15
Mesosternum L/W	1.08 (7)	1.00–1.13	1.17 (1)	1.17	1.14 (9)	1.09–1.24	1.08 (9)	1.01–1.15	1.12 (6)	1.03–1.18	1.08 (4)	1.01–1.12	1.09 (15)	0.98–1.18
Metasternum L/W	0.76 (6)	0.71–0.78	0.80 (2)	0.77–0.82	0.77 (8)	0.72–0.81	0.80 (7)	0.74–0.85	0.80 (4)	0.77–0.84	0.79 (4)	0.77–0.80	0.75 (11)	0.69–0.84
Prosternum width/HW	0.78 (7)	0.69–0.89	0.69 (2)	0.64–0.73	0.74 (8)	0.70–0.79	0.73 (7)	0.67–0.78	0.77 (6)	0.71–0.86	0.74 (4)	0.72–0.76	0.74 (16)	0.65–0.82
Mesosternum width/HW	0.88 (7)	0.77–0.98	0.80 (1)	0.8	0.80 (9)	0.75–0.85	0.81 (7)	0.77–0.89	0.86 (6)	0.75–0.97	0.80 (4)	0.75–0.84	0.83 (15)	0.71–0.92
Metasternum width/HW	1.10 (6)	1.00–1.23	1.03 (2)	1.00–1.06	1.05 (8)	1.00–1.11	1.03 (5)	0.96–1.12	1.08 (4)	0.97–1.26	1.03 (4)	1.02–1.06	1.10 (11)	1.00–1.19
Length mesosternum/prosternum	1.24 (6)	1.12–1.34	1.22 (1)	1.22	1.27 (8)	1.21–1.34	1.21 (8)	1.18–1.25	1.26 (6)	1.21–1.31	1.23 (4)	1.06–1.34	1.15 (15)	1.07–1.21
Length metasternum/prosternum	1.08 (5)	1.01–1.12	1.12 (2)	1.10–1.13	1.11 (7)	1.05–1.21	1.10 (6)	0.99–1.18	1.15 (4)	1.13–1.18	1.14 (4)	1.11–1.21	1.03 (11)	0.97–1.08
Metasternum L gap/L combs	2.37 (8)	1.66–2.67	2.55 (2)	2.18–2.92	2.98 (9)	2.38–3.39	2.61 (9)	1.85–3.72	2.00 (5)	1.57–2.59	3.06 (4)	1.83–3.89	7.31 (13)	5.60–8.90
PI tarsus/HW	0.47 (15)	0.43–0.51	0.48 (2)	0.45–0.51	0.50 (17)	0.45–0.54	0.47 (9)	0.40–0.54	0.50 (4)	0.42–0.58	0.5 (4)	0.47–0.54	0.50 (9)	0.48–0.55
PI tarsus/PI tibia	1.17 (14)	1.03–1.48	1.16 (2)	1.16–1.17	1.18 (17)	0.96–1.43	1.11 (9)	0.91–1.24	1.19 (4)	1.02–1.46	1.21 (4)	1.09–1.36	1.33 (9)	1.23–1.38
PI tibia/HW	0.41 (14)	0.30–0.45	0.41 (2)	0.38–0.44	0.43 (17)	0.37–0.53	0.43 (9)	0.37–0.49	0.43 (4)	0.36–0.56	0.42 (4)	0.36–0.43	0.38 (9)	0.35–0.40
PI tarsus L/W	5.87 (15)	4.84–7.00	5.70 (2)	5.18–6.22	6.34 (17)	5.76–7.78	6.02 (9)	5.20–7.00	6.24 (4)	5.25–7.00	6.99 (4)	6.25–8.00	6.70 (9)	5.44–7.60
PI tibia L/W	3.06 (14)	2.27–3.94	2.94 (2)	2.88–3.00	2.83 (17)	2.33–3.48	2.83 (8)	2.63–3.05	2.84 (4)	2.33–3.44	3.01 (4)	2.86–3.19	2.64 (9)	2.48–3.00
PII tarsus/HW	0.53 (14)	0.47–0.57	0.57 (2)	0.54–0.59	0.54 (15)	0.46–0.67	0.56 (9)	0.51–0.63	0.55 (4)	0.46–0.67	0.57 (4)	0.56–0.58	0.55 (7)	0.51–0.60
PII tarsus/PII tibia	1.09 (14)	0.96–1.17	1.12 (2)	1.10–1.14	1.08 (15)	0.93–1.29	1.10 (9)	0.98–1.26	1.08 (4)	1.02–1.16	1.12 (4)	1.07–1.20	1.22 (7)	1.00–1.37
PII tibia/HW	0.49 (14)	0.42–0.55	0.51 (2)	0.59–0.52	0.50 (16)	0.45–0.55	0.51 (9)	0.44–0.60	0.51 (4)	0.45–0.65	0.51 (4)	0.47–0.54	0.46 (7)	0.40–0.51
PII tarsus L/W	6.71 (14)	6.18–7.37	7.67 (2)	7.33–8.00	6.78 (15)	5.75–8.11	6.96 (9)	6.12–8.11	6.16 (4)	5.89–6.50	7.02 (4)	6.32–7.68	6.73 (7)	6.00–8.00
PII tibia L/W	3.28 (14)	2.89–3.94	3.37 (2)	2.97–3.76	3.21 (16)	2.86–3.69	3.34 (9)	2.83–3.93	3.45 (4)	3.14–3.71	3.42 (4)	2.78–4.00	2.98 (7)	2.39–3.47
PIII tarsus/HW	0.66 (13)	0.54–0.71	0.67 (2)	0.64–0.69	0.71 (17)	0.63–0.78	0.70 (8)	0.66–0.75	0.73 (4)	0.65–0.84	0.7 (4)	0.68–0.73	0.74 (7)	0.70–0.81
PIII tarsus/PIII tibia	1.06 (13)	0.99–1.17	1.04 (2)	1.00–1.08	1.06 (17)	0.93–1.16	1.05 (8)	0.98–1.15	1.04 (4)	0.96–1.23	1.04 (4)	1.00–1.07	1.14 (7)	1.01–1.20
PIII tibia/HW	0.63 (13)	0.53–0.69	0.64 (2)	0.64–0.64	0.67 (17)	0.50–0.74	0.68 (9)	0.59–0.77	0.71 (4)	0.63–0.85	0.68 (4)	0.64–0.73	0.66 (8)	0.59–0.72
PIII tarsus L/W	8.04 (13)	6.57–9.80	8.32 (2)	8.21–8.42	8.66 (17)	7.58–9.90	8.42 (8)	7.44–9.78	8.79 (4)	7.65–10.0	8.51 (4)	7.40–9.65	8.75 (7)	7.40–10.00
PIII tibia L/W	3.83 (13)	3.35–4.56	3.66 (2)	3.51–4.81	3.87 (17)	2.89–4.38	3.81 (9)	3.15–4.09	4.19 (4)	4.06–4.37	3.93 (4)	3.54–4.24	3.62 (7)	3.14–4.06
Tibia PI/PIII	0.65 (12)	0.51–0.75	0.64 (2)	0.60–0.68	0.64)17)	0.50–0.75	0.63 (9)	0.59–0.75	0.60 (4)	0.57–0.66	0.62 (4)	0.54–0.67	0.58 (10)	0.53–0.65
Tarsus PI/PIII	0.71 (13)	0.63–0.90	0.72 (2)	0.70–0.73	0.70 (17)	0.64–0.75	0.66 (8)	0.58–0.72	0.69 (4)	0.65–0.72	0.72 (4)	0.68–0.76	0.69 (7)	0.63–0.73
Urotergite X (L/W)	0.53 (7)	0.49–0.59	0.65 (2)	0.57–0.73	0.57 (8)	0.48–0.66	0.55 (8)	0.52–0.63	0.49 (5)	0.43–0.62	0.52 (2)	0.51–0.55	0.61 (5)	0.54–0.70
Coxite IX inner process (L/W) ♀	1.52 (8)	1.38–1.68	1.65 (2)	1.65–1.66	1.59 (12)	1.31–1.94	1.59 (16)	1.38–1.83	1.54 (10)	1.33–1.73	1.52 (5)	1.43–1.58	1.85 (8)	1.63–2.07
Coxite IX inner process (L/W) ♂	1.33 (4)	1.26–1.44	1.00 (2)	0.86–1.14	1.24 (6)	1.10–1.47	1.21 (4)	1.11–1.31	—	—	1.30 (2)	1.26–1.35	1.81 (2)	1.70–1.93
Ovipositor/HW	1.68 (6)	1.49–1.95	1.91 (1)	1.91	1.99 (8)	1.58–2.39	2.30 (4)	1.96–2.45	2.65 (1)	2.65	2.27 (3)	2.17–2.34	2.09 (7)	1.79–2.30
No. divisions of ovipositor	35.4 (5)	32–37	34 (1)	33–35	38.2 (5)	35–40	39.1 (3)	38–41	38.1 (3)	37–39	36.2 (3)	34–39	37.1 (8)	34–40
Stylus VII/IX	0.42 (6)	0.34–0.51	0.39 (1)	0.39	0.43 (7)	0.38–0.50	0.36 (3)	0.36–0.37	0.35 (1)	0.35	—	—	0.34 (4)	0.27–0.44
Stylus VIII/IX	0.47 (9)	0.43–0.52	0.42 (2)	0.38–0.46	0.42 (12)	0.31–0.63	0.41 (5)	0.27–0.47	0.34 (4)	0.27–0.45	0.43 (3)	0.36–0.46	0.42 (10)	0.35–0.55

sclerophylla group, follow the COI topology. The following relationships are strongly supported: monophyly of *Heterolepisma*, monophyly *H. cooloola* and of the *H. cooloola* species group (i.e. all Queensland samples of *Heterolepisma*), monophyly of the *H. sclerophylla* species group, within which six groups are each strongly supported: North Nowra (a single specimen, sister-group to the remainder), Broulee, Glen Davis, Wellington, Megalong, and finally Burralow, Glenbrook and Bucketty forming the sixth group.

Morphology

Morphological examination identified several strong characters differentiating the southern Queensland population from those in NSW including the absence of medial anterior chaetotaxy of the frons, the presence of lanceolate scales on the clypeus and legs, a macrochaeta mediad of the anterior trichobothrial area on the pronotum, a reduction in size of the medioposterior macrochaeta of the submedial dorsal combs and an elongated tapered urotergite X and it is described below as new. While the available molecular data is limited, what is available for the three

Table 3. Percentage of variation explained by all PCs in PCA1 accounting for at least 5% of the variation.

principal component	percentage of variation explained
PC1	17.44
PC2	15.62
PC3	10.54
PC4	8.15
PC5	6.81
PC6	6.32
PC7	5.81
PC8	5.31

Table 4. Loading matrix for principal components 1–8, based on 36 morphological characters in PCA1. Only principal components accounting for more than 5% of the variation are included. Strongest correlations are highlighted in the darkest grey.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Head width	0.145	-0.057	0.026	-0.317	0.173	-0.297	0.143	-0.265
H+B length	0.030	-0.252	0.000	-0.277	0.205	-0.187	0.140	-0.166
Thorax length/HW	-0.130	-0.257	0.101	0.267	0.120	-0.024	0.105	-0.025
Thorax length/H+B	0.053	0.176	0.162	0.350	0.003	-0.174	0.057	-0.188
Thorax length/width (meso)	-0.237	-0.158	0.216	0.164	-0.152	-0.052	0.082	0.095
Abdomen length/H+B	-0.052	-0.111	-0.069	-0.324	0.082	0.263	0.072	0.292
Prothorax width/HW	0.319	-0.048	-0.119	0.121	0.096	-0.145	-0.052	-0.068
Mesothorax width/HW	0.184	-0.120	-0.190	0.118	0.400	0.048	0.012	-0.186
Metathorax width/HW	0.178	-0.189	-0.141	0.086	0.315	0.044	0.050	-0.109
Thorax length/abdomen length	0.068	0.159	0.130	0.385	-0.043	-0.259	-0.014	-0.257
Thorax length/thorax width	-0.327	-0.126	0.144	0.079	-0.004	0.063	0.079	0.002
PI tarsus/HW	-0.124	-0.223	0.085	0.107	0.113	-0.094	-0.452	0.074
PI tarsus/PI tibia	-0.281	0.112	-0.153	-0.018	0.106	-0.141	-0.298	0.011
PI tibia/HW	0.190	-0.278	0.221	0.098	-0.020	0.092	-0.031	0.044
PI tarsus L/W	-0.212	-0.143	0.083	-0.146	-0.010	-0.186	-0.208	-0.060
PI tibia L/W	0.211	-0.121	0.218	-0.064	-0.005	0.050	0.080	0.032
PII tarsus/HW	-0.021	-0.202	-0.275	0.147	0.210	-0.049	-0.063	0.236
PII tarsus/PII tibia	-0.205	0.088	-0.261	0.041	0.212	-0.118	0.070	0.152
PII tibia/HW	0.199	-0.286	0.003	0.096	-0.037	0.068	-0.135	0.079
PII tarsus L/W	0.071	0.067	-0.117	-0.048	0.049	-0.369	-0.165	0.125
PII tibia L/W	0.202	-0.141	0.154	0.011	-0.168	0.081	-0.047	0.067
PII tarsus/HW	-0.188	-0.282	-0.090	0.249	0.046	0.041	0.046	0.026
PIII tarsus/PIII tibia	-0.200	0.060	0.179	0.205	0.282	-0.024	0.150	0.139
PIII tibia/HW	-0.010	-0.322	-0.233	0.064	-0.189	0.060	-0.073	-0.093
PIII tarsus L/W	-0.150	-0.209	0.027	-0.027	-0.139	-0.065	0.032	-0.292
PIII tibia L/W	0.060	-0.179	-0.245	0.011	-0.320	-0.077	-0.143	-0.245
Tibia PI/PIII	0.203	-0.015	0.395	0.033	0.138	0.032	0.021	0.108
Tarsus PI/PIII	0.078	0.069	0.195	-0.151	0.065	-0.111	-0.528	0.048
Ult max palp art/HW	0.245	-0.036	0.018	0.034	-0.179	-0.247	-0.035	0.311
Ult max palp art L/W	0.008	-0.132	0.097	-0.150	0.139	-0.161	0.248	0.057
Penult max palp art L/W	-0.018	-0.205	0.075	-0.064	-0.144	-0.330	0.170	0.277
Ult/penult max palp art	0.067	0.158	0.051	0.022	0.216	0.231	-0.082	-0.117
Labial palp art L/W	-0.019	0.041	-0.184	-0.017	-0.232	-0.185	0.275	-0.094
Pedicel/scape	0.189	0.052	-0.228	0.196	0.003	-0.035	0.020	0.262
Pedicel/HW	0.148	0.071	-0.189	0.150	-0.178	0.211	0.049	0.108
Scape/HW	-0.037	-0.119	0.019	-0.064	-0.038	0.285	-0.120	-0.267

genes found specimens from several localities in far northern Queensland (Bamaga, Punsand Bay, Chillagoe, Undara and Mingela) to be related to but distinct from the southern Queensland group. Preliminary morphological examination of some of this northern material confirms it shares most of the traits that distinguished the southern Queensland material from the NSW specimens, but from the degree of genetic difference it is likely that these northern Queensland specimens could represent new species. The investigation of this lies outside the scope of the current work.

The group from Glen Davis was easily separated from all other groups as both males and females had one fewer pair of styli. No other consistent morphological differences could be found. The Glen Davis material is described below as a new species.

Consistent morphological differences between the remaining five New South Wales groups were not found although some over-lapping differences were noted. Suspected morphological differences and difficulties in interpretation are discussed under each lineage. In the absence of consistent, quantifiable differences, we have chosen to keep them within the *H. sclerophylla* species group at present, recognizing them as distinct lineages. They may indeed represent separate species, however this would require a lot more molecular and morphological work including further samples from additional localities.

Morphometrics

A summary of all measurement data is found in Table 2.

PCA1. Our PCA reduced the 36 variables to eight principal components (PCs) that each accounted for at least 5% of the variation (Tables 3 and 4). Together they explained 76.00% of the variation.

Discrimination between lineages is most clear when plotting PC1 against PC2 (Fig. 5). Here, the Cooloola group was the only lineage that could be distinguished from the others along the PC1 axis, which is most strongly correlated with prothorax width/head width, thorax length/thorax width, and PI tarsus/PI tibia. Lineages other than the Cooloola group were not clearly identified.

Table 5. Loading matrix for principal components 1–2, based on 15 morphological characters in PCA2. Only principal components accounting for more than 5% of the variation are included.

	PC1	PC2
Head width	-0.281	0.187
H+B length	-0.260	-0.384
Thorax length	-0.280	-0.049
Mesothorax width	-0.274	0.197
Abdomen length	-0.226	-0.485
Ovipositor	-0.208	-0.290
Stylus IX	-0.257	-0.170
PI tarsus length	-0.246	0.126
PI tibia length	-0.260	0.348
PII tarsus length	-0.267	-0.218
PII tibia length	-0.275	0.181
PIII tarsus length	-0.278	-0.031
PIII tibia length	-0.271	-0.060
Ult max palp art length	-0.226	0.433
Penult max palp art length	-0.250	0.137

PCA2. Our PCA reduced the 15 variables to two PCs that each accounted for at least 5% of the variation: PC1, (73.51%) and PC2, (7.81%). Together they explained 81.32% of the variation.

Discrimination between lineages was not very clear, but the Broulee specimens were shifted relative to the other specimens along the PC2 axis, which is most strongly correlated with ultimate maxillary palp article length, abdomen length, PI tibia length and ovipositor length. This may indicate that these characters are most useful in delineating this lineage from others.

Significant differences were found for the gap between the metathoracic combs between the Cooloola group and all other groups, with the only other pair showing significant differences being Wellington and Glenbrook (Table 6).

Table 6. Results of Dunn's post-hoc test on the length to gap width ratio of the combs of the metasternum. Each cell contains the pairwise *z*-test statistic, followed by the *p*-value. Significant differences between groups ($p < 0.05$) are indicated by asterisks.

	Broulee	Cooloola	Glen Davis	Glenbrook	Megalong	North Nowra
Cooloola	-4.63, 0.00*	—	—	—	—	—
Glen Davis	-1.12, 0.26	2.34, 0.02*	—	—	—	—
Glenbrook	-1.85, 0.06	2.62, 0.01*	-0.33, 0.74	—	—	—
Megalong	-0.60, 0.55	3.98, 0.00 *	0.65, 0.52	1.25, 0.21	—	—
North Nowra	-0.31, 0.76	2.32, 0.02*	0.50, 0.62	0.80, 0.42	0.05, 0.96	—
Wellington	0.67, 0.50	4.79, 0.00*	1.59, 0.11	2.33, 0.02*	1.21, 0.23	0.73, 0.46

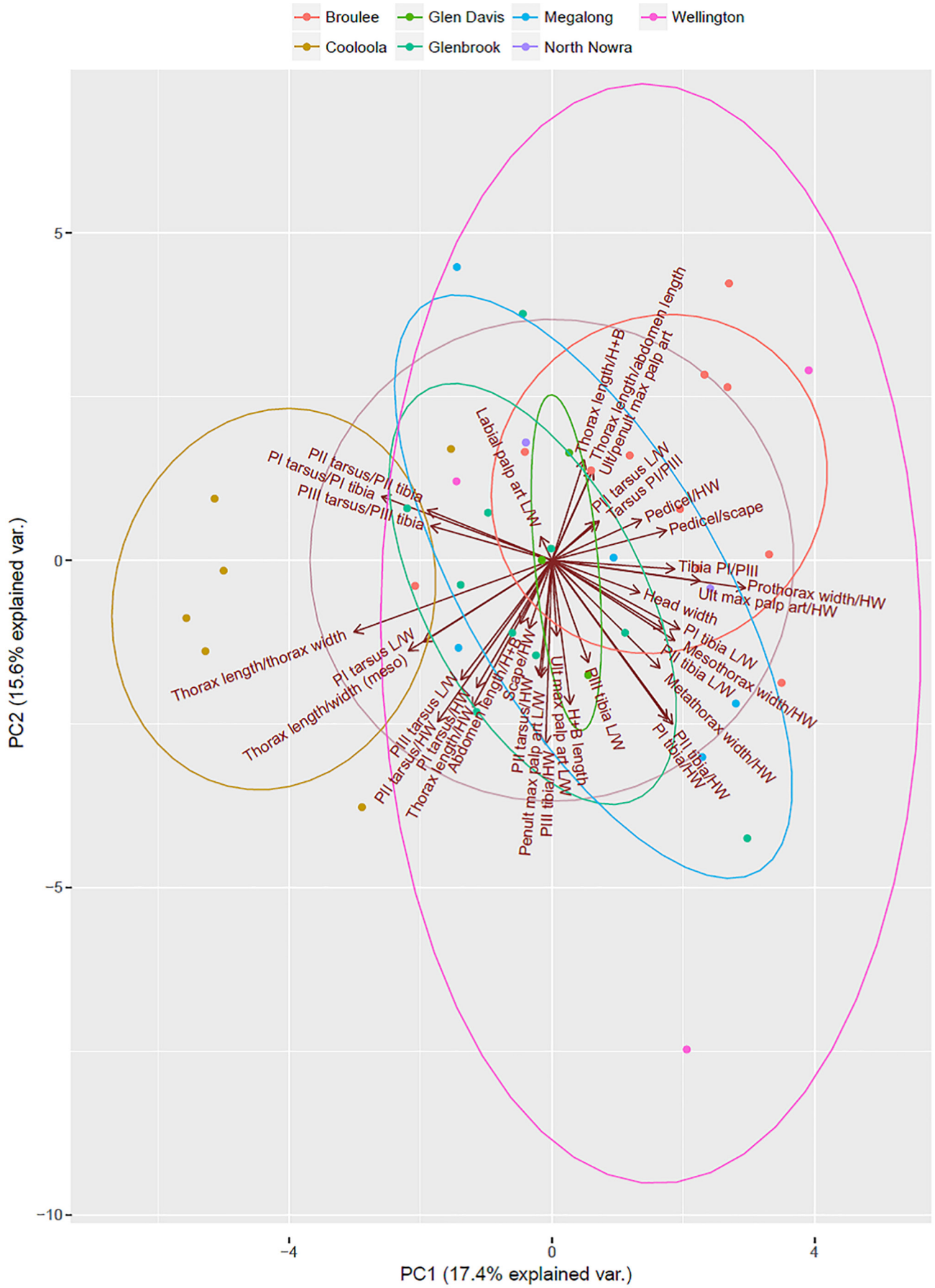


Figure 5. Scatterplot of principal component analysis 1. Members of lineages are indicated by a ⅔ confidence interval ellipse.

Systematics

Family Lepismatidae Latreille, 1802
Subfamily Heterolepismatinae Mendes, 1991

Heterolepisma Escherich, 1905

Heterolepisma Escherich, 1905: 63. Type species: *Lepisma pampeana* Silvestri, 1902 by subsequent designation (Paclt, 1967: 25).
Isolepisma Escherich, 1905: 61.
Notolepisma Tillyard, 1924: 241.

Heterolepisma buntonorum Smith 2016

Heterolepisma buntonorum Smith 2016a: 58.

Material examined. 1♀ (HW 1.43) (AMS K.261244 K.261245 on two slides) TAS: Knocklofty, 42.8752°S 147.2957°E 270 m asl, 13.ii.2016, Stephen Bunton.

Heterolepisma highlandi Smith 2014

Heterolepisma highlandi Smith, 2014: 16.

Type material (paratype). 1 juvenile ♀ (HW 0.88) (AMS K.377604 in ethanol) NSW: Wee Jasper, 35.0591°S 148.6489°E 552 m asl, 21.viii.2010, Graeme Smith and Phil Fleming.

Heterolepisma sclerophylla Smith, 2014

Fig. 7

Heterolepisma sclerophylla Smith, 2014: 9.

Type material examined. Holotype. 1♀ (HW 1.28) (AMS K.260990, K.260991 on two slides) NSW: Broulee, 35.8578°S 150.1621°E 15 m asl, 17.xi.2010, Graeme Smith. Paratypes. 1♀ (HW 1.23) (AMS K.377563 in ethanol) same data as holotype; 1♀ (HW 1.25) (AMS K.261218, K.261219 on two slides) same data as holotype; 1♂ (HW 1.25) (AMS K.260992, K.260993 on two slides) same data as previous; 1♂ (HW 1.23) (AMS K.377565 in ethanol) same data as holotype; 1♂ (HW 1.13) (AMS K.377566 in ethanol) same data as holotype; 1♀ (HW 1.15) (AMS K.377567 in ethanol) same data as holotype; 1 juvenile ♀ (HW 0.84) (AMS K.377568 in ethanol) same data as holotype; 1♂ (HW 1.15) (AMS K.377569 in ethanol) same data as holotype; 1♂ (HW 1.10) (AMS K.261074, K.261075 on two slides) same data as holotype; 1♀ (HW 1.08) (AMS K.377570 in ethanol) same data as holotype; 1♀ (HW 1.20) (AMS K.377561 in ethanol) same locality and collector as holotype 19.xi.2010; 1♀ (HW 1.40) (AMS K.377562 in ethanol) same data as previous.

Other topotypic material examined. 1♀ (HW 1.20) (AMS K.261216, K.261217 two slides) same locality as holotype, 18.ii.2016, Graeme Smith; 1♀ (HW 1.10) (AMS K.261142 head, thorax and abdominal segments I–IV on one slide); 1♀ (HW 1.10) (AMS K.261143, K.261144 on two slides) same data as previous; 1♂ (HW 1.13) (AMS K.261145, K.261149 on two slides) same data as previous; 1♀ (HW 1.13) (AMS K.261152, K.261153 on two slides) same data as previous; 1♀ (HW 1.18) (gbs004988 in 100% ethanol) same data as previous; 1♀ (HW 1.03) (AMS K.261150, K.261151 on two slides) same data as previous.

Material from other localities of the same lineage. 1♂ (HW 1.18) (AMS K.260994, K.260995 two slides) NSW: Guerilla Bay headland, 35.8321°S 150.2313°E 87 m asl, 17.xi.2010, Graeme Smith; 1♀ (HW 1.23) (AMS K.261202, K.261203 on two slides) NSW: Jibbon, 34.0781°S 151.1674°E 18 m asl, 1.i.2016, Graeme Smith; 1♀ (HW 1.00) (AMS K.261184, K.261185 on two slides) same data as previous.

Comments. The Broulee group has a comparatively short ovipositor both relative to head width (1.49–1.95 HW) and in the number of divisions (32–37). Most other lineages have ovipositors in mature specimens exceeding twice the head width and with 35–41 divisions. It also seems to have lower levels of pigment overall and the density and strength of the macrochaetae on the head and notal collar is less than for other lineages (Fig. 7). The Nowra lineage has a similarly short ovipositor but appears to have much darker pigment on the labial and maxillary palps and to a lesser extent on the legs. It has denser chaetotaxy along the sides of the head near the eyes (2–4 rows wide versus 2–3 rows wide).

The ovipositor length was reported in Smith (2014) as up to 2.09 times head width. This was based on the result from a specimen (originally recorded as K.377564 in ethanol but now replaced by K.261219 as slide) and was quite different to other specimens measured (range 1.49–1.79). This specimen was re-measured and the measurement is here corrected to 1.95 times HW. This is still much longer than other specimens from Broulee but the specimen only has 35–37 divisions which is within the normal range for the Broulee specimens (32–37). Each individual division is longer than seen with other specimens of this lineage.

The lineage has been collected from three localities along the central and southern coasts of New South Wales. All localities listed have sandy soils, but this may represent a sampling bias rather than be a true indication of habitat. In the type locality it is quite common among dry Eucalypt leaves caught between the fronds of cycads (*Macrozamia* sp. Zamiaceae). At Guerilla Bay it was collected within abandoned termite galleries on a standing tree and at Jibbon it was collected from dry leaf litter protected from rain beneath a fallen tree or in a large hollow at the base of the tree. It was occasionally found hiding within the bark of certain spongy-barked *Eucalyptus* or *Corymbia* trees using pyrethrum sprays, where it was usually collected together with a more abundant (but not yet described) species related to *Heterolepisma highlandi* Smith.

One Broulee specimen (K.261145) was unusual in that one of its maxillary palps was shorter than usual and had several plumose sensilla along its length rather than the usual three, as found on the other palp where they were more evenly spaced along the article rather than in the distal half. In all specimens examined the plumose sensilla on the palps of the males are much larger (2–3 times larger diameter) and more elaborate than those on the female.

North Nowra Lineage

Material examined. 1♀ (HW 1.25) (AMS K.261196, K.261197 on two slides) NSW: North Nowra, 34.8443°S 150.5742°E 60 m asl, 18.v.2014, Graeme Smith; 1♂ (HW 1.13) (AMS K.261234, K.261235 on two slides) NSW: North Nowra, 34.8447°S 150.5745°E 60 m asl, 18.v.2014, Graeme Smith.

Comments. The North Nowra lineage appears closest to the Broulee lineage in terms of ovipositor length. In the available slide material it stands out for having very darkly pigmented articles on the labial and maxillary palps (especially the penultimate and preceding articles). The lateral nota and more distal leg articles are also more heavily pigmented. Some caution needs to be exercised here because pigment seems to diminish in both alcohol and slide mounted material over time and there is quite a bit of difference in pigment between the two specimens examined, especially in PI.

Pigment is also generally weak in juvenile specimens making it a difficult character to use.

The Nowra lineage also appears to have denser macrochaetae around the sides of the head, towards the eyes and more on the face of the mandible (around 60–70 vs about 50 in the Broulee material) however the Glenbrook strain also appears to have a similar number of macrochaetae on the mandibles. Counting macrochaetae on the mandibles is also fairly subjective; when does one decide an insertion is that of a macrochaeta or a seta? The meso- and metathoracic sterna may also be more elongate (L/W 1.17 versus 1.00–1.13 and 0.77–0.82 versus 0.71–0.78 respectively).

On the tibia of PI most *H. sclerophylla* sensu lato have three dorsal carrot-shaped macrochaetae whose position along the margin can be quite variable and three carrot-shaped macrochaetae near the ventral margin with a stronger seta proximally that sometimes approaches carrot-shaped. Of the three PI legs available of the Nowra lineage, there only appears to be two macrochaetae on the dorsal surface and four quite solid macrochaetae on the ventral margin. Insertion points on the dorsal surface can be hard to see and the number of specimens insufficient. Urotergite IX may have more strongly developed and numerous setae in the infralateral corners. Urotergite X is also on average longer than the Broulee lineage (L/W 0.57–0.73 vs 0.49–0.59) and the inner processes of coxites IX in the male may be shorter (L/W 0.86–1.14 versus 1.26–1.44.

It may well be possible to describe this lineage as a morphologically distinct species using these characters. However, given the small number of North Nowra specimens examined, from just a single location and the limited molecular data, we have taken a conservative approach and will consider this as a lineage of *H. sclerophylla* until more data suggest otherwise.

Glenbrook Lineage

Material examined. 1♂ (HW 1.00) (AMS K.377580 in ethanol) NSW: Blue Mountains N.P., Glenbrook, 33.8019°S 150.6193°E 131 m asl, 19.vi.2010, Graeme Smith; 1♂ (HW 1.13) (AMS K.261048, K.261049 on two slides) NSW: Blue Mountains N.P., Glenbrook, 33.8013°S 150.6194°E 115 m asl, 19.vi.2010, Graeme Smith;1♀ (HW 1.30) ♂ (HW 0.95) 1♀ (HW 0.98) (K.377581 together in ethanol) same data as previous.

Material examined from other localities in the same lineage. 1♀ (HW 1.40) (AMS K.261076, K.261077 on two slides) NSW: Burralow campsite, 33.5554°S 150.6054°E 354 m asl, 20.xi.2011, Graeme Smith; 1♂ (HW 1.29) (AMS K.377592 in ethanol) same data as previous; 1♀ (HW 1.00) 1♀ (HW 1.15) 1♂ (HW 1.10) (AMS K.377593 together in ethanol) NSW: Burralow campsite, track to waterfall, 33.5524° S 150.5962°E 352 m asl, 20.xi.2011, Graeme Smith; 1♀ (HW 1.50) (AMS K.261240, K.261241 on two slides) NSW: Nattai, above Middle Flat, 34.2408°S 150.3541°E 201 m asl, 30.iii.2013, Graeme Smith; 1♀ (HW 1.21) (AMS K.261084, K.261085 on two slides) NSW: Nattai, plateau above Lady Amanda Creek 34.2691°S 150.3912°E 623 m asl, 31.iii.2013, Graeme Smith; 1♀ (HW 1.08) (AMS K.261200, K.261201 on two slides) NSW: Bucketty, 33.0808°S 151.1385°E 264 m asl, 15.viii.2014, Graeme Smith; 1♀ (HW 1.06) (AMS K.261220, K.261221 on two slides) same data as previous; 1♀ (HW 0.93) (AMS K.377727 in ethanol) same data as previous; 1♀ (HW 1.18) (AMS K.261224, K.261225 on two slides) NSW: Bucketty 33.0807°S 151.1372°E 266 m asl, 16.viii.2014, Graeme Smith; 1♂ (HW 1.08) (AMS K.261222, K.261223 on two slides) same data as previous; 1 juvenile ♀ (HW 0.79) (AMS K.377729 in ethanol) same data as previous; 1♂ (HW 0.93) (AMS K.261239, K.261240 on two slides) NSW: Bucketty 33.0822°S 151.1419°E 207 m asl, 17.viii.2014, Graeme Smith.

Comments. This lineage, as well as the next two lineages, have a longer ovipositor than that of the more coastal Broulee and Nowra lineages although there is some overlap both in length relative to HW (1.58–2.39 versus 1.49–1.95 HW) and also in the number of divisions (35–40 versus 32–37). No correlation could be found between number of divisions and HW in clearly adult specimens including one specimen, from Nattai which was particularly large (HW 1.50 H+B over 11mm) but had only 36 divisions in the ovipositor. This suggests that divisions are not added with increasing size.

Apart from the ovipositor length, no reliable diagnostic character has been identified. The Glenbrook, Nattai and Burrawang specimens also seemed to have more macrochaetae on the external face of the mandibles (around 60 versus around 50) as well as more setae in the lateral group near the molar area (12–16 versus around 11) but this was not the case with the Bucketty specimens, which also differed in generally having more pigment in the palps as well as at least the tarsi and tibia of PII and PIII.

All of the limited number of specimens examined from Glenbrook, Nattai and Burrawang differed from most specimens from other populations in having just three macrochaetae located on the posterior bulge of the femur of PI, instead of the usual four. Specimens from Bucketty however had the more usual four macrochaetae and two of the Broulee specimens also had only three macrochaetae so this character does not appear to be sufficiently reliable. The chaetotaxy of the legs, while having a basic pattern, differed quite considerably between specimens, in terms of the number and size of stout macrochaetae and their position.

Specimens from all four localities sometimes had five macrochaetae in the lateral combs of one or two urotergites whereas other lineages had a maximum of four, but most urotergites had the usual 3–4 macrochaetae.

The posterior margin of urotergite X in all dissected Bucketty specimens also has a wider glabrous area medially than seen in all other dissected and mounted *H. sclerophylla* sensu lato specimens.

All specimens of this lineage were collected in leaf litter which had gathered in places protected by rain e.g., beneath the trunk of a fallen tree that was not in contact with the ground.

Megalong Lineage

Material examined. 1♂ (HW 1.03) (AMS K.377583 in ethanol) NSW: Megalong Valley above Dunphy's campsite, 33.7906°S 150.2314°E 640 m asl, 19.vi.2011, Graeme Smith; 1 juvenile ♀ (HW 0.91) (AMS K.261046, K.261047 two slides) NSW: Megalong Valley, track out to Bellbird ridge lookout, 33.7931°S 150.2354°E 736 m asl, 19.vi.2011, Graeme Smith; 1 juvenile ♂ (HW 0.91) 1 juvenile ♂ (HW 0.85) (AMS K.377585 together in ethanol) same data as previous; 1♀ (HW 1.20) (AMS K.261166, K.261167 on two slides) NSW: Megalong Valley, Bellbird Ridge 33.7931°S 150.2353°E 736 m asl, 20.i.2016, Graeme Smith; 1♀ (HW 1.15) (AMS K.261168, K.261169 on two slides) same data as previous; 1♀ (HW 1.30) (AMS K.261170, K.261171 on two slides) same data as previous; 1♂ (HW 1.10) (AMS K.261172, K.261173 on two slides) same data as previous; 1♀ (HW 1.38) (AMS K.377735 in ethanol) same data as previous; 1♀ (HW 1.18) (AMS K.261174, K.261175 on two slides) same data as previous; 1♂ (HW 1.33) (AMS K.377751 in 100% ethanol) same data as previous; 1♀ (HW 1.38) (AMS K.377738 in ethanol) same data as previous.

Material examined from other localities in the same lineage. 1♀ (HW 1.20) (AMS K.261194, K.261195 on two slides) NSW: Lower Portland, 33.4221°S 150.8945°E 39 m asl, 26.iii.2011, Graeme Smith; 1♀ (HW 1.25) (AMS K.261050, K.261051 on two slides) same data as previous; 1♂ (HW 1.21) (AMS K.261052, K.261053 on two slides) same data as previous; 1♂ (HW 1.08) (AMS K.377577 in ethanol) NSW: Lower Portland, 33.4220°S 150.8939°E 66 m asl, 26.iii.2011, Graeme Smith; 1♀ (HW 1.21) (AMS K.261192, K.261193 on two slides) same data as previous; 1♂ (HW 1.20) (AMS K.377772 in ethanol) NSW: Bents Basin, 33.9337°S 150.6309°E 111 m asl, 13.ix.2015, Graeme Smith.

Comments. No morphological characters could be found to separate this lineage from the previous. It also has an ovipositor which appears to be longer than the coastal lineages being 1.96–2.45 HW with 36–41 divisions vs 1.49–1.95 HW and with 32–37 divisions for the Broulee lineage. The macrochaetae on the margins of the head and the anterior margin on the pronotum are somewhat denser and thicker than on Broulee specimens, similar to that seen in the Glenbrook lineage and the species from Glen Davis (described below).

The lineage has been collected from the Blue Mountains west of Sydney and their foothills, where sandy soils and rocky sandstone outcrops predominate. It is found in the same types of microhabitat, i.e. *Eucalyptus* and *Casuarina* leaf litter lying in places protected from rain or where the leaf litter accumulates but dries out quickly e.g., in the forks of trees or on exposed rocks. One specimen was taken from bark using a pyrethrum spray.

Wellington Lineage

Material examined. 1♀ (HW 1.08) (AMS K.261086, K.261087 on two slides) NSW: Mt Arthur Reserve, Wellington 32.5479°S 148.9090°E 450 m asl, 10.iii.2012, Graeme Smith; 1♀ (HW 1.23) (AMS K.261214, K.261215 on two slides) NSW: Mt Arthur Reserve, Wellington 32.5477°S 148.9091°E 453m asl, 11.iii.2012, Graeme Smith; 1♂ (HW 0.96) (AMS K.377595 in ethanol) same data as previous; 1♀ (HW 1.18) (AMS K.261162, K.261163 on two slides) NSW: Mt Arthur Reserve, Wellington 32.5497°S 148.9122°E 420 m asl, 22.i.2016, Graeme Smith; 1 juvenile ♀ (HW 0.90) (AMS K.261164, K.261165 on two slides) same data as previous; 1 juvenile ♀ (HW 0.98) (AMS K.377737 in ethanol) same data as previous; 1 juvenile ♀ (HW 0.88) (AMS K.261160, K.261161 on two slides) same data as previous; 1♂ (HW 1.01) (gbs004854 in 100% ethanol) same data as previous; 1 juvenile ♀ (HW 0.93) (AMS K.261246 K.261247 on two slides) same data as previous; 1 juvenile ♀ (HW 0.85) (AMS K.261158, K.261159 on two slides) same data as previous.

Comments. This lineage is similar in appearance to the Megalong and Glenbrook lineages with perhaps an even longer ovipositor (2.65 times HW versus up to 2.45) however data only exist for a single large female so the range may overlap with more material. It has the same number of divisions, but each was more elongate. Elongation of the divisions is probably quite variable between individuals as observed when examining a range of specimens of the Cooloola species described below. The Wellington lineage appears to be narrower in the body but there are insufficient larger specimens available and this parameter needs to be verified as it may represent a sampling bias. The gap between the combs of the metathoracic sternum was also comparatively small relative to the combs (1.55–2.75) but only statistically significantly different from the Glenbrook population (2.16–3.47) with the degree of overlap being unhelpful.

The Wellington habitat is more inland and drier than that of the other two lineages but the specimens were collected in the same typical microhabitat, i.e. dry *Callitris*, *Eucalyptus* and *Casuarina* leaf litter protected from rain, in rocky country with sandy soils.

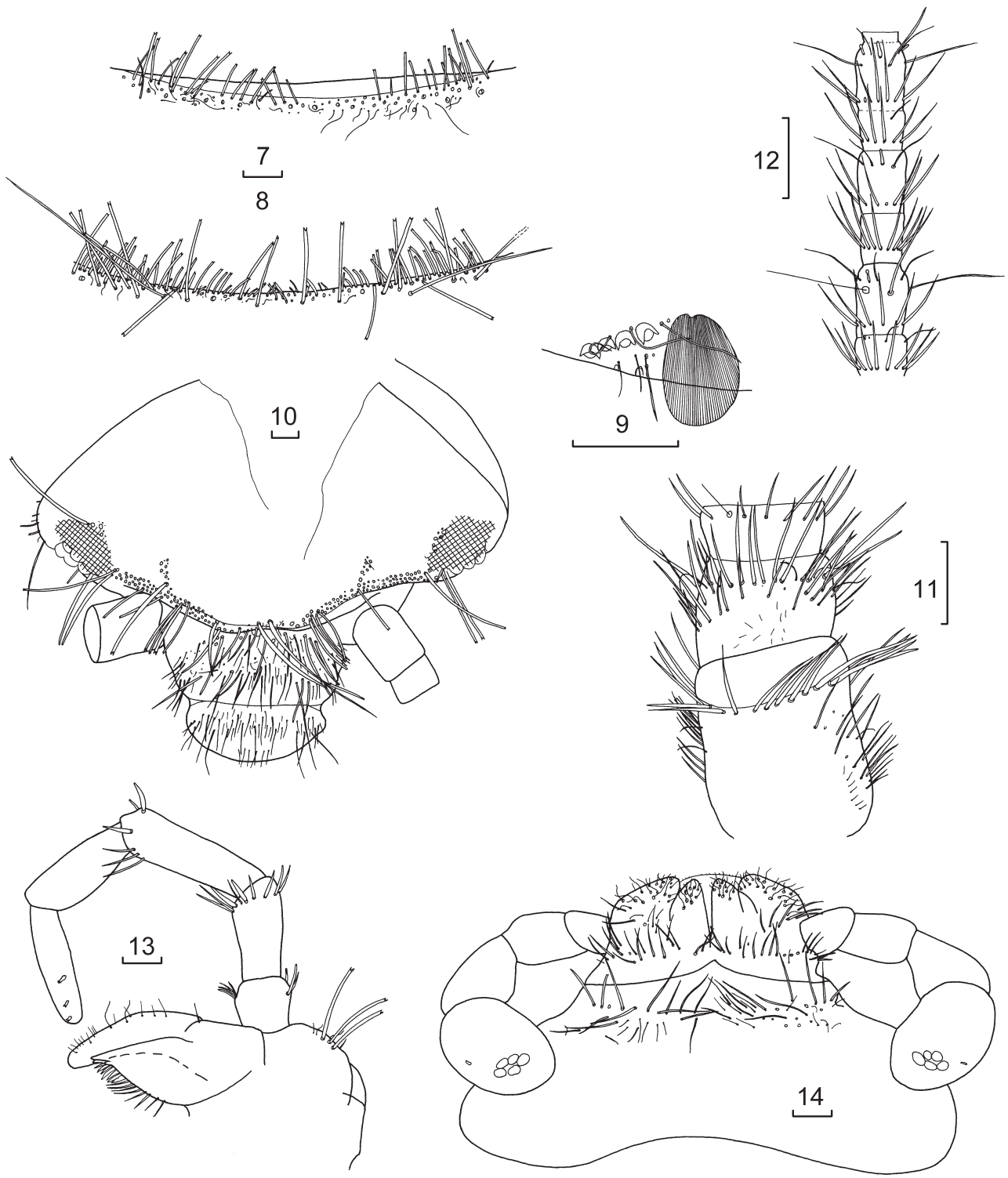
Heterolepisma coorongooba sp. nov.

Figs 6, 8–36

Holotype. ♀ (HW 1.30) (AMS K.261208, K.261209 on two slides) NSW: Glen Davis, above Coorongooba campground, 33.1271°S 150.3232°E 313m asl, 20.vi.2015, Graeme Smith.
Paratypes. 2♀♀, 1♂, 1 juvenile, all same data as holotype including 1♀ (HW 1.15) (AMS K.261210, K.261211 on two slides); 1♀ (HW 1.11) (AMS K.261212, K.261213 on two slides); 1♂ (HW 1.08) (AMS K.261204, K.261205 on two slides); 1 juvenile (HW 0 3) (AMS K.377726 in ethanol).

Diagnosis. This species is very similar to *Heterolepisma sclerophylla* differing in having one fewer pairs of styli in both the male and female (i.e. IX only in ♂, VIII and IX in the ♀). Compared to the lineage from the type locality it also has a longer ovipositor and thicker and perhaps more densely packed chaetotaxy along the pronotal collar and margins of the head.

Figure 6. *Heterolepisma coorongooba* sp. nov. Glen Davis, NSW.

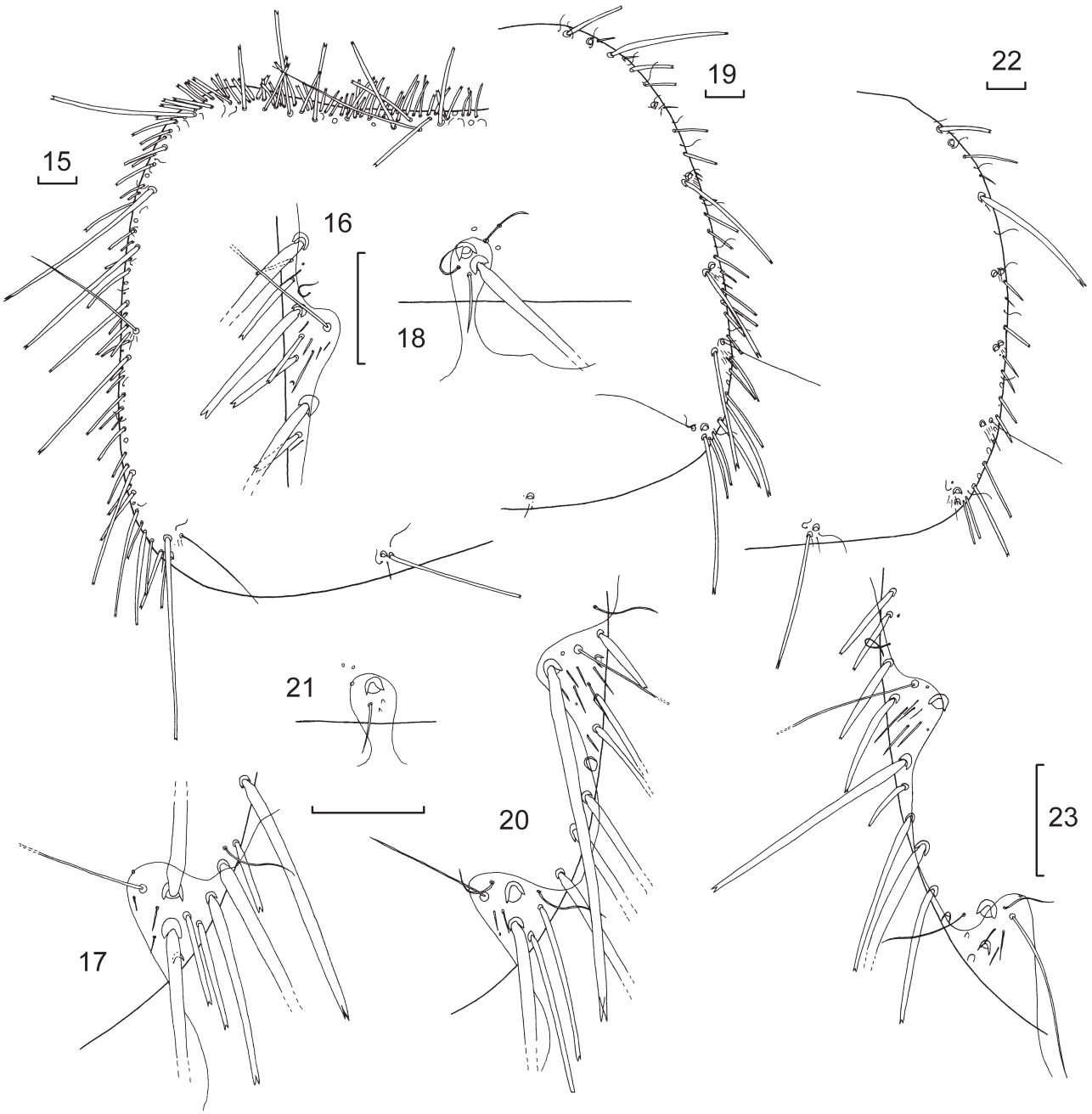


Figures 7–14. *Heterolepisma sclerophylla* Smith and *Heterolepisma coorongooba* sp. nov. *(7) Heterolepisma sclerophylla* Smith, holotype ♀, medial section of pronotal collar. *(8–14) Heterolepisma coorongooba* sp. nov. holotype ♀ *(8)* medial section of pronotal collar; *(9)* lateral bristlecomb of urotergite III and adjacent scale; *(10)* head; *(11)* scape, pedicel and basal interval of flagellum, from above; *(12)* antenna, most distal surviving annuli; *(13)* maxilla, only larger setae of palp illustrated; *(14)* labium, setae of palp not strong so not illustrated. Scale bars = 0.1 mm.

Description

Appearance: Medium to large silverfish, scale covering in life uniform or slightly mottled grey with brown antennae, terminal filaments brown with lighter annuli around larger macrochaetae resulting in distinctly banded appearance (Fig. 6).

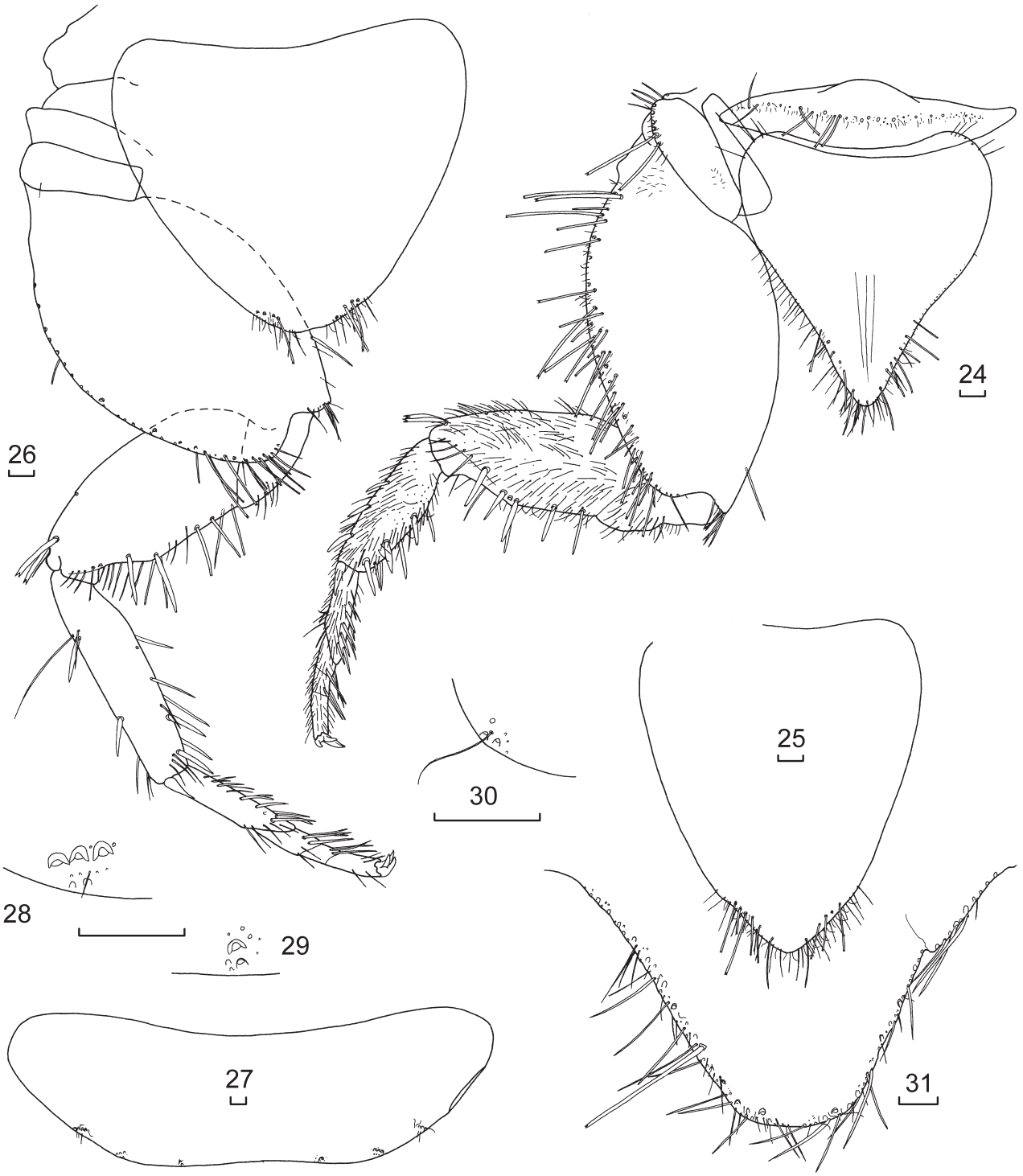
Body length: H+B up to 9.1 mm (♀) 7.0 mm (♂); maximum HW 1.30 mm; thorax: length up to 2.5 mm (or 0.27–0.36 H+B); width up to 2.05 mm, usually slightly widest at the mesonotum; antennae damaged in all specimens, maximum preserved length of antenna 4.4 mm (or 0.56 H+B); terminal filaments damaged in all specimens, maximum preserved



Figures 15–23. *Heterolepisma coorongooba* sp. nov. holotype ♀ *(15)* left lateral, anterior and posterior margins of pronotum; *(16)* idem, detail of left anterior trichobothrial area; *(17)* idem, detail of right posterior trichobothrial area; *(18)* idem, left posterior comb of pronotum; *(19)* lateral margin of mesonotum; *(20)* idem, trichobothrial areas of right side; *(21)* idem, left posterior comb of mesonotum; *(22)* lateral margin of metanotum; *(23)* idem, trichobothrial areas of left side. Scale bars = 0.1 mm.

length of cercus 2.8 mm (or 0.31 H+B); maximum preserved length of median dorsal appendage 3.4 mm (or 0.42 H+B). Body neither elongate nor broad with thorax slightly wider than abdominal segment I, the following abdominal segments about the same width until the fourth or fifth after which the abdomen tapers posteriorly.

Pigmentation: Pigment brown in alcohol preserved specimens. Present around eyes and to a lesser extent behind the peri-antennal group of macrochaetae; pedicel and scape very lightly pigmented distally, rest of flagellum uniformly lightly pigmented; all articles of maxillary palp with pigment especially in the four most distal articles although much less in the ultimate article; labium with pigment on the three most distal articles, being strongest on the penultimate, especially distally. Pigment present in anterior corner and along margins of all nota. Legs not heavily pigmented, with light pigment along outer margin of the coxa and the trochanter, apically on the outer femur and strongest on the tibia especially on the dorsal surface, present on basal article of tarsi, urotergite X and coxites IX with light pigmentation; styli IX pigmented in distal three quarters; other styli with less pigment. Ovipositor with yellowish hue. Terminal filaments with rings of pigment, with the pigment present in all annuli except the annuli bearing the large macrochaetae. Pigmentation is much reduced in juvenile specimens.

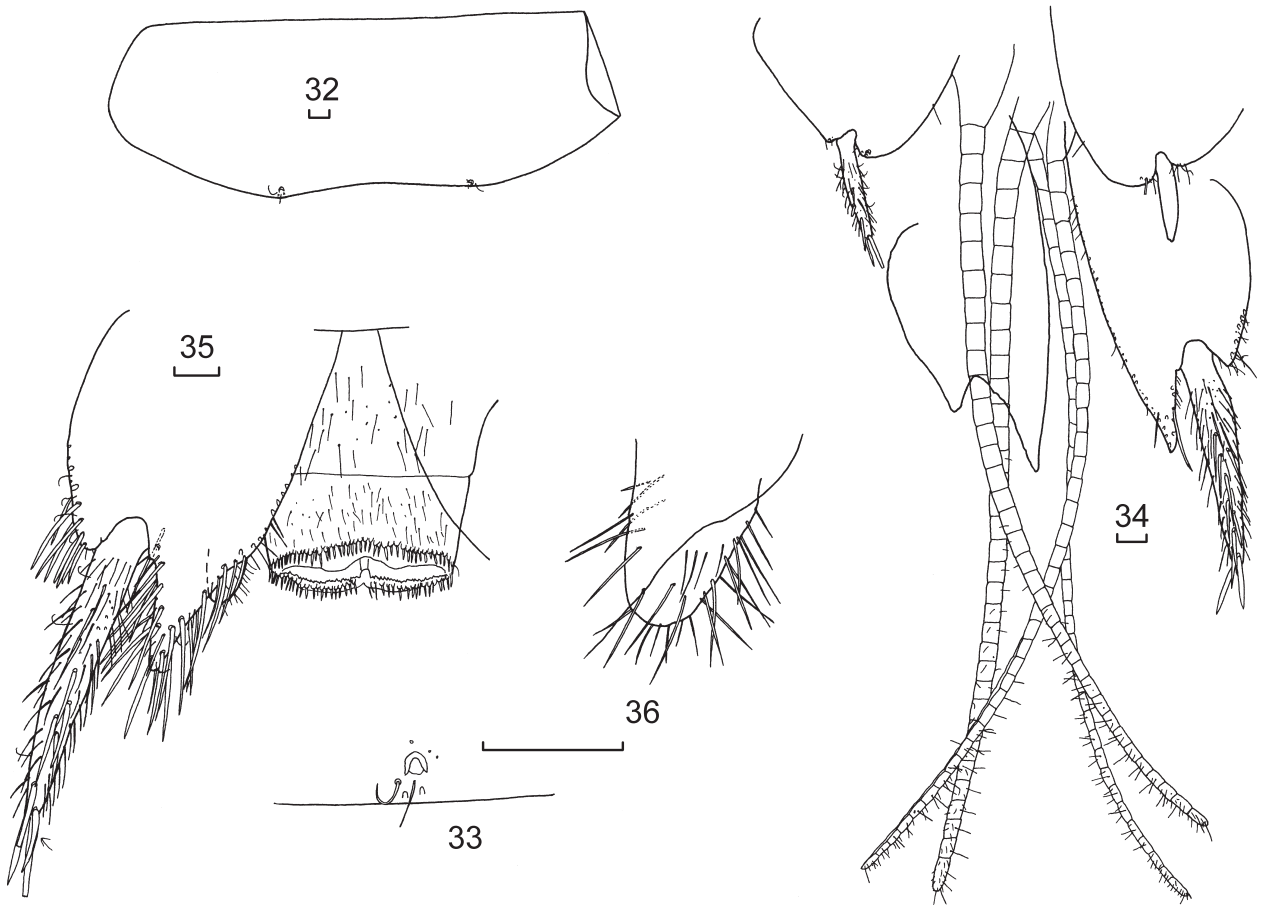


Figures 24–31. *Heterolepisma coorongooba* sp. nov. holotype ♀ *(24)* presternum, prothoracic sternum and PI; *(25)* mesothoracic sternum; *(26)* metathoracic sternum and PIII; *(27)* urotergite III; *(28)* idem, sublateral comb; *(29)* idem, submedial comb; *(30)* urotergite IX, infralateral combs; *(31)* urotergite X. Scale bars = 0.1 mm.

Macrochaetae: Bifid apically, or simple, light to darker brown in colour. Often quite thick e.g., the stronger macrochaetae of the pronotal collar measured about 50 microns in diameter in the holotype (HW 1.30) compared to 38 microns in the similar-sized holotype of *H. sclerophylla* (HW 1.28) from Broulee (compare Figs 7, 8). They also appear to be more densely packed.

Scales: Unevenly rounded or ovoid, with numerous parallel ribs that do not extend beyond the margin (Fig. 9); in alcohol dorsal scales with dark brown ribs; ventrally mostly hyaline. Lanceolate scales not observed. Scales absent from flagellum of antennae, mouthparts and terminal filaments.

Head: Wider than long (Fig. 10) with marginal rows about three macrochaetae wide along the sides of the vertex



Figures 32–36. *Heterolepisma coorongooba* sp. nov. holotype ♀, unless otherwise indicated by specimen number *(32)* urosternite III; *(33)* idem, posterior comb; *(34)* coxites VIII and IX, ovipositor and styli; *(35)* coxite IX, paramere and penis (K.260993); *(36)* paramere (K.260993). Scale bars = 0.1 mm.

decreasing to two wide in front of the antennae and six strong curved macrochaetae along the anterior margin, the lateral rows extend back above the eyes, as well as a small 1+1 peri-antennal groups not quite isolated from the marginal rows at the level of each antenna. Clypeus with numerous setae, some long and thin, others more robust arranged in 1+1 lines in the proximal lateral regions but not forming combs. Labrum with thin setae only. Eyes dark, composed of about 12 ommatidia.—Antennal scape with a subdistal rosette of setae, very conspicuous from above (Fig. 11), and numerous setae along the sides and over the ventral face; pedicel short, 0.51 times the length of the scape (range 0.47–0.55), with many setae mostly distally and on the ventral face; repeating intervals of distal end of antennae (Fig. 12) of eight annuli, the most apical annulus of each interval (T-annulus) with a trichobothrium and at least one small inconspicuous rod-like basiconic sensillum (type B of Adel, 1984), the second and forth annuli also each with a sausage-shaped type C sensillum (confirmed also to be present on same annuli in holotype of *H. sclerophylla*).—Mandibles typical for genus with well-developed molar and incisor areas; a group of about nine strong setae distally adjacent to the pectinate molar area and a bush of 50+ setae and macrochaetae externally.—Maxilla (Fig. 13) with three large macrochaetae externally proximal to the palp, the lacinia with three strong teeth, one shorter than the rest, seven lamellate processes and a row of eight simple setae, the galea longer than the lacinia with setulae on the outer face. Palp with rosettes of distinctly stronger setae (some "carrot-shaped") subapically on the three basal articles, all articles with numerous fine setae, apical article of maxillary palp 4.6 times longer than wide (range 4.1–4.9) and 1.23 times longer than penultimate article (range 1.16–1.27), the ultimate article in both sexes with three "branched" papillae, those in the female less robust than those in the male.—Labium (Fig. 14) short and broad with rows of strong setae on the prementum and submentum; glossae and paraglossae quite broad with short curved setulae; labial palp short, apical article eccentric suboval, 1.1 times as long as wide (range L/W 0.8–1.3) with 2+3 papillae of compact type in a "cluster formation" where the slightly larger distal papillae curve around the two smaller proximal papillae and a curved club-like thin-walled basiconic sensillum and at least one rod-like basiconic sensillum, which can also be confirmed as present on the holotype of *H. sclerophylla*.

Thorax: Pronotum (Fig. 15) with strong setal collar of short, apically bifurcated setae and cilia with a row of longer macrochaetae spaced along the back of the collar including 1+1 long thin simple setae about one third in from each side, the density and length of the smaller more marginal macrochaetae of the collar decreases only slightly towards the middle but not the size and density of the larger spaced macrochaetae in the posterior row; lateral margins also with numerous shorter but quite robust, apically bifurcate

setae as well as several larger more erect submarginal macrochaetae; trichobothrial areas open and in contact with the lateral margins, the anterior one (Fig. 16) located just anterior to the mid-point along the margin, with or without a large submarginal macrochaeta, when present laterad to the trichobothrium (this macrochaeta missing on the left side of the holotype) and with a cilium and a few setulae; posterior trichobothrial area (Fig. 17) near posterior lateral corner with two submarginal macrochaetae between the trichobothrium and the margin as well as a few setae and setulae; posterior margin slightly concave with 1+1 combs (Fig. 18) each of two macrochaetae, the more postero-mediad lying flatter than the other, each comb associated with a setula and a few cilia.—Mesonotum with lateral chaetotaxy similar to pronotum but less dense (Fig. 19), except the submarginal macrochaetae anterior to the trichobothrial areas are grouped into combs of two, each associated with a cilium and a few setulae, the more posterior of these on the left side of the holotype of only one macrochaeta, both trichobothrial areas (Fig. 20) of similar configuration to those of pronotum except anterior area has a macrochaeta mediad of the trichobothrium. The posterior combs of the mesonotum are unusual in that they both consist of only a single macrochaeta in the illustrated holotype (Fig. 21) whereas in the paratype K.261204 one consists of two macrochaetae and the other of only one and in the other paratypes (K.261213 and K.261210) two macrochaetae are present on both sides.—Metanotum (Figs 22, 23) similar to mesonotum; both posterior combs consist of two equal sized insertion points. The trichobothrium appears to be absent from the right posterior area of the holotype.

Presternum narrow, with transverse row of strong setae and numerous cilia and setulae (Fig. 24).—All thoracic sterna and coxae with hyaline scales. Prothoracic sternum pointed cordiform, almost as long as wide at its base (range L/W 0.91–1.00) and reaching to about two thirds the length of the coxa, rounded apically and with a medial furrow (Fig. 24), most of lateral margins with numerous small marginal setae and cilia, with 6–7 larger submarginal macrochaetae forming weak combs parallel to the edges in the distal third.—Mesosternum (Fig. 25) 1.08 times longer than broad (range 1.01–1.12) with an acutely rounded apex, with setae and cilia along the distal quarter of the margins, with 1+1 distal combs of four apically bifurcate macrochaetae and 1+1 subposterior more pointed macrochaetae.—Metasternum (Fig. 26) 0.79 times longer than wide (range 0.77–0.80) with less pointed, even slightly concave, apex, each comb of four macrochaetae.

Legs fairly long (Figs 24, 26), tibia L/W ratio of legs PI 3.0 (range 2.9–3.2), PII 3.4 (range 2.8–4.0), PIII 3.9 (range 3.5–4.2); tarsi L/W ratio PI 7.0 (range 6.3–8.0), PII 7.0 (range 6.3–7.7), PIII 8.5 (range 7.4–9.7). Legs increasingly longer from front to back, mean ratio PI/PIII (tibia 0.62, tarsus 0.72). PI with transverse comb of about six macrochaetae laterally on the precoxa. Coxa of all legs covered with hyaline scales and with strong macrochaetae and numerous cilia and setulae in a row about two macrochaetae wide along the external margin, a strong seta on the inner margin subapically and group of about six curved setae at the apex over the articulation. Trochanter lacking scales, with fine and one stronger seta over the surface. Femur with numerous setae over most of the surface and along the margin; anterior distal end with two strong, quite deeply bifurcate stout macrochaetae and another simple stout macrochaeta more proximal; posterior margin with several strong macrochaetae as illustrated. Tibia with numerous long setae over the ventral surface, with three stout macrochaetae on or near the anterior margin and six or seven stout macrochaetae along the posterior margin; apical spur with several setae. Tibia of PIII with a long thin, laterally projecting trichobothria-like seta inserted dorsal to the proximal stout macrochaeta on the anterior margin, which is about two times as long as the tibia is wide. Tarsus with four articles, all with numerous setae, some on the ventral surface quite long and strong. Pretarsus with long curved lateral claws and a strong curved shorter medial claw.

Table 7. Number of macrochaetae per bristle comb—*Heterolepisma coorongooba* sp. nov.

segment	urotergite			urosternites
	lateral	sublateral	submedial	
I	1–3	2	—	—
II	3	2–3	2	1
III	2–4	3	1–2[b]	1
IV	4	0–4	1–2[b]	1
V	4	3–4	1–2[b]	1
VI	3–4	3–4	2	1
VII	3–4	3	1–2[b]	1
VIII	3–4	—	1–2[b]	1–2
IX	2[a]	—	—	—

[a] Small infralateral setae
[b] More posterior seta insertion smaller

Abdomen: Urotergite I usually with 2+2 combs each of one to three macrochaetae (usually two) located quite close together, urotergites II-VII with 3+3 combs of macrochaetae as in Table 7 (Figs 9, 27–29) noting that the more posteromedial insertion point of each submedial comb was occasionally, quite a bit smaller than the other insertion point, but mostly of about the same size; each comb also associated with up to five marginal setae, five setulae and four cilia. Urotergite VIII with 2+2 combs, lacking the sublateral comb; urotergite IX (Fig. 30) with two infralateral setae on each side as well as a setula and a few cilia. Urotergite X short, parabolic in both sexes (Fig. 31), L/W at base about 0.52 (range 0.51–0.55) with many strong setae along entire margin and obscure 1+1 submarginal macrochaetae in the posterolateral corners.

Urosternite I glabrous, urosternites II–VIII (Fig. 32) with 1+1 single macrochaetae (Fig. 33), each associated with 0–2 small marginal setae as well as a few cilia and/or setulae. Coxites of segment VIII in ♀ (Fig. 34) with one or two small macrochaetae, one or two small marginal setae and a cilium mediad of the stylus base and some small setae, setulae and a cilium laterad of the stylus base. Styli in two pairs in the ♀ (VIII–IX); all styli with several noticeably longer and stronger setae apically (Fig. 34) as well as stronger setae along the middle of the ventral face. Styli IX 2.4 times as long as styli VIII (range 2.2–2.8).

Coxite IX of ♀ (Fig. 34), the internal process acute apically, about three times longer than the external process (range 2.9–3.2) and 1.5 times as long as broad at its base (range 1.5–1.6), not reaching to half the length of the stylus; external and internal margins of internal process and external margin and apex of outer process with many moderately

strong setae directed both up and down.—Ovipositor (Fig. 34), very long and thin (up to 2.34 HW), surpassing the apex of stylus IX by more than the length of the stylus (excluding terminal macrochaetae), composed of 34–39 divisions. Distal divisions of gonapophyses VIII and IX with only short fine setae and setulae.

Cerci not well preserved in slide material, with basal divisions shorter than long, gradually becoming longer distally, equally wide as long by about the eighth division after which they become even longer with more annuli each with a rosette of setae and some with trichobothria with the large macrochaetae restricted to the most distal annulus of each division; the most distal surviving divisions with up to four annuli, this annulus without pigment.—Medial filament of similar arrangement.

Male: As for female except only one pair of styli (segment IX). Coxites IX (Fig. 35) with acute inner process about 1.3 times longer than wide at its base and about three times longer than the external process, reaching to just under half the length of the stylus. Both process also with several strong setae mostly apically emerging from both the dorsal and ventral surfaces of the processes close to or on the margin. Parameres a little longer than wide, with about thirty fine setae (Fig. 36). Penis typical for genus with numerous glandular setae apically, each set on a protuberance.

Subadult stages: the single juvenile specimen available, K.377726 (HW 0.63) had a single pair of styli (IX) and no indication of urosternite VIII dividing into separate coxites nor any nascent genitalia (ovipositor or parameres). The thoracic sternites conformed to those of the adults and the long thin setae on the tibia of PIII was present and very long (about three times the width of the tibia), tergite X was round but shorter than in the adults and the feathered papilla of the maxillary palp could not be seen.

Habitat. *Heterolepisma coorongooba* was collected from leaf litter protected from rain under a fallen but still elevated, log.

Etymology. The species name is derived from the proper noun Coorongooba referring the creek that flows through the valley from where it was collected.

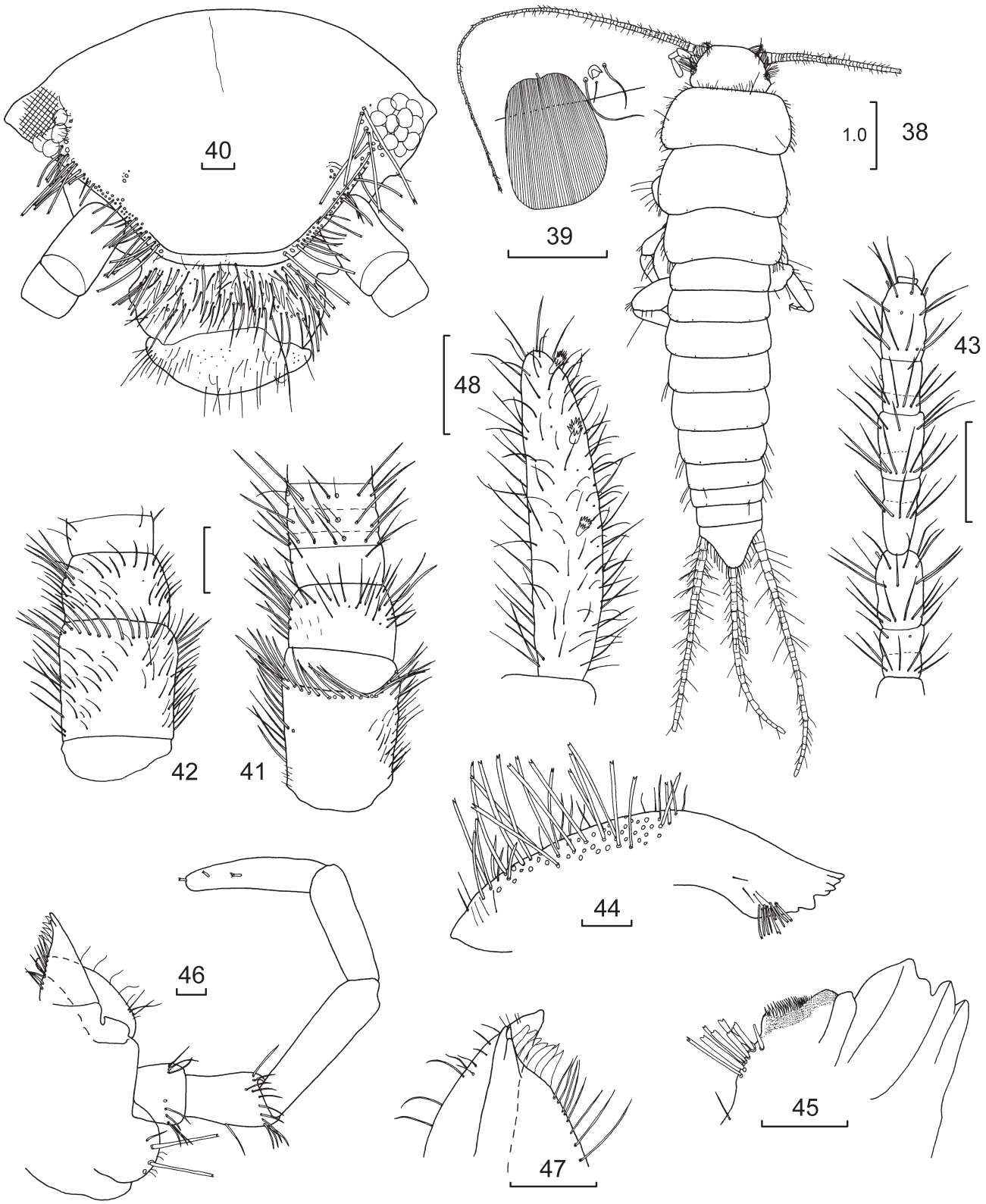
Comments. The morphology of this species is very close to that of *H. sclerophylla*, differing from it only in the absence of the most anterior pair of styli in both sexes. It differs from the Broulee lineage in the length of the ovipositor (2.17–2.34 times HW versus 1.49–1.95), but not so much in the number of divisions (34–39 versus 32–37) and the more robust macrochaetae. The Megalong, North Nowra and Glenbrook lineages also have more robust macrochaetae and a longer ovipositor (but more divisions) leaving the fewer styli as the only unambiguous defining character.

Heterolepisma cooloola sp. nov.

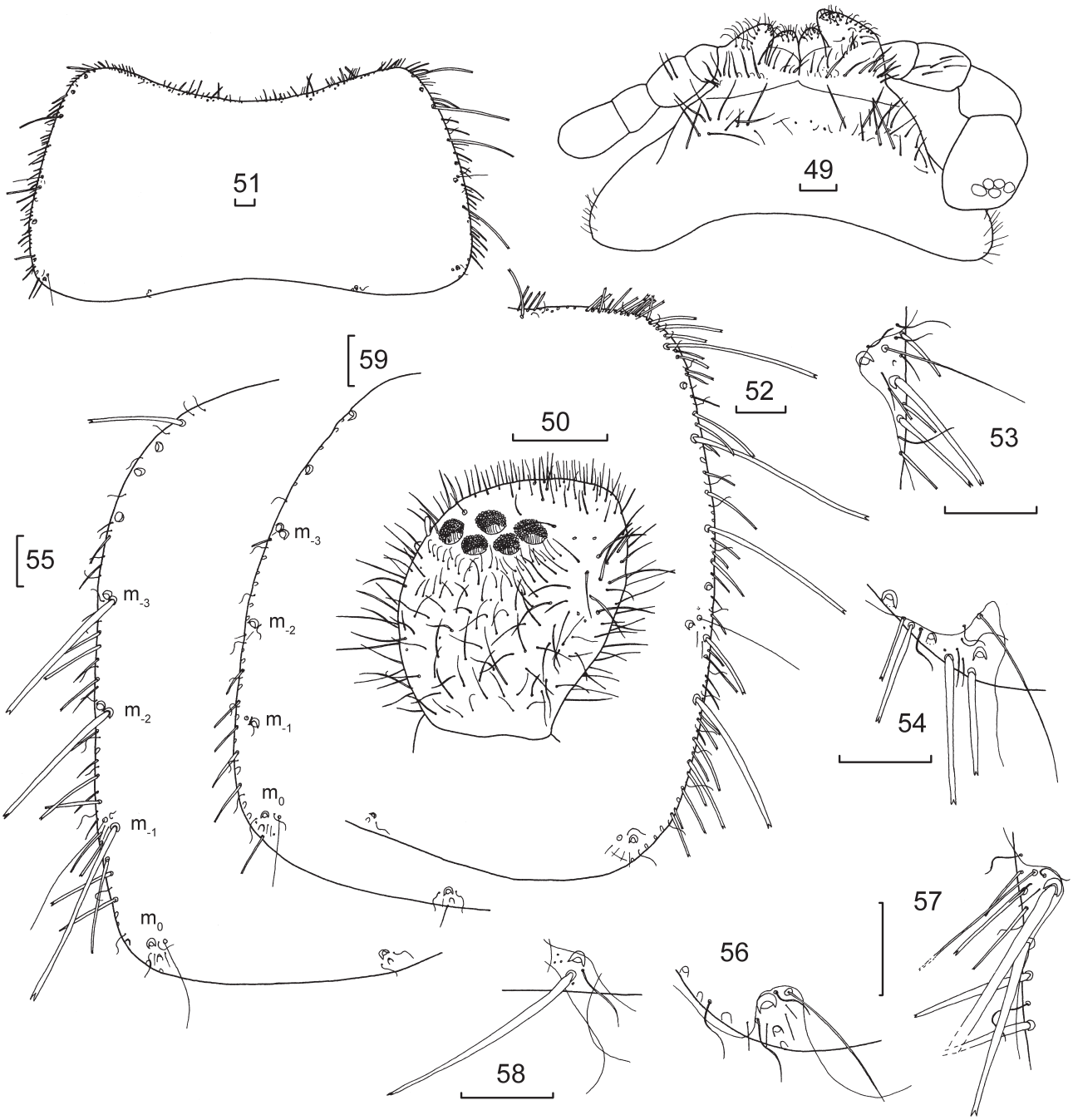
Figs 37–85

Holotype. ♀ (HW 1.20) (QM 207011 on two slides) QLD: Cooloola, Freshwater track rainforest patch, 25.9492°S 153.0927°E 71 m asl, 7.vii.2013, Graeme Smith. **Paratypes**: 14♀♀, 14♂♂, 21 subadult specimens including 1♂ (HW 1.08) (QM 207012 on two slides) same data as holotype; 1♂ (HW 1.06) (AMS K.261176, K.261177 on two slides) same data as holotype; 1 juvenile (HW 0.74) (AMS K.261178, K.261179 on two slides) same data as holotype; 1♀ (HW 1.15) (AMS K.261186, K.261187 on two slides) same data as holotype; 1♀ (HW 1.09) (AMS K.377742 in ethanol) same data as holotype; 1♂ (HW 1.20) (AMS K.377739 in ethanol) same data as holotype; 5♂♂, 5♀♀, 4 juvenile ♀♀, 3 juveniles (AMS K.377744 all together in ethanol) same data as holotype; 1♀ (HW1.05) (AMS K.261126, K.261127 on two slides) same locality as holotype, 27.i.2016, Graeme Smith; 1♂ (HW 0.98) (AMS K.261128, K.261129 on two slides) same data as previous; 1♀ (HW 1.23) (AMS K.261130, K.261131 on two slides) same data as previous; 1♀ (HW 1.08) (gbs004893 in 100% ethanol) same data as previous; 1♂ (HW 1.05) (AMS K.261132, K.261133 on two slides) same data as previous; 1♀ (HW 0.93) (AMS K.261134, K.261135 on two slides) same data as previous; 1♂ (HW 0.85) (gbs004896 in 100% ethanol) same data as previous; 1♀ (HW 1.01) (gbs004897 in 100% ethanol) same data as previous; 1♂ (HW 0.85) (AMS K.377754 in 100% ethanol) same data as previous; 1♀ (HW 0.95) (gbs004899 in 100% ethanol) same data as previous; 1♀ (HW 0.95) (gbs004900 in 100% ethanol) same data as previous; 1 subadult ♀ (HW 0.90) (gbs004901 in 100% ethanol) same data as

Figure 37. *Heterolepisma cooloola* sp. nov. from leaf litter at Carlo Point.



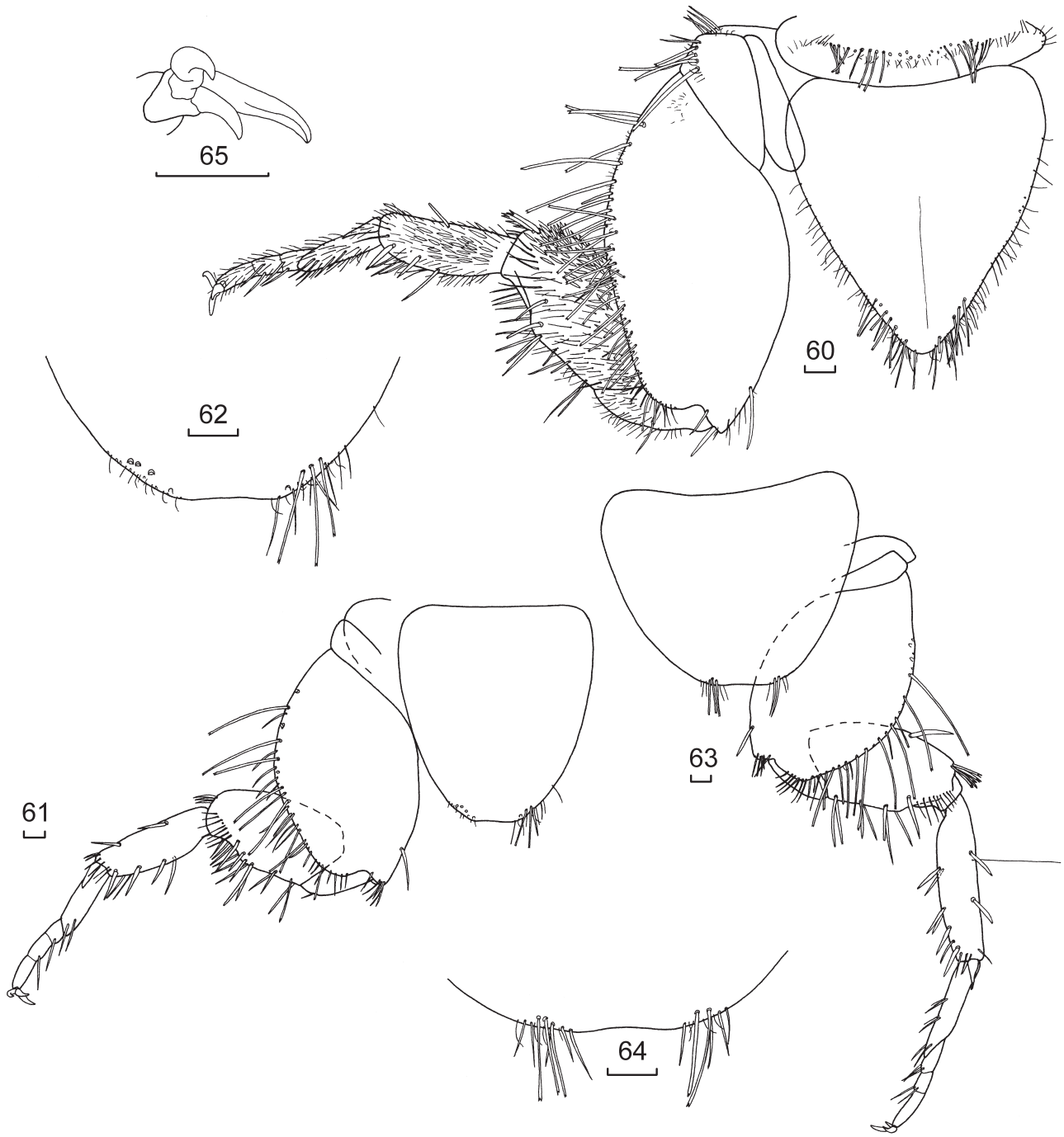
Figures 38–48. *Heterolepisma cooloola* sp. nov. holotype ♀, unless indicated otherwise by specimen number*(38)* habitus (K.377754); *(39)* posterior comb of pronotum with scale; *(40)* head; *(41)* scape, pedicel and basal intervals of flagellum, from above; *(42)* idem, from below; *(43)* antenna, most distal surviving interval; *(44)* mandible; *(45)* idem, detail of molar and incisor regions; *(46)* maxilla, only larger setae of palp illustrated; *(47)* idem, lacinia and galea (QM 207012); *(48)* ultimate article of maxillary palp of ♂ (QM 207012). Scale bars = 0.1 mm unless otherwise indicated.



Figures 49–59. *Heterolepisma cooloola* sp. nov. holotype ♀ *(49)* labium, only large setae of palp illustrated; *(50)* idem, ultimate article of palp; *(51)* pronotum; *(52)* idem, right margin; *(53)* idem, detail of right anterior trichobothrial area; *(54)* idem, detail of left posterior trichobothrial area; (*55*) lateral margin of mesonotum; *(56)* idem, posterior trichobothrial area; *(57)* idem, anterior trichobothrial area; *(58)* idem, right posterior comb; *(59)* lateral margin of metanotum. Scale bars = 0.1 mm.

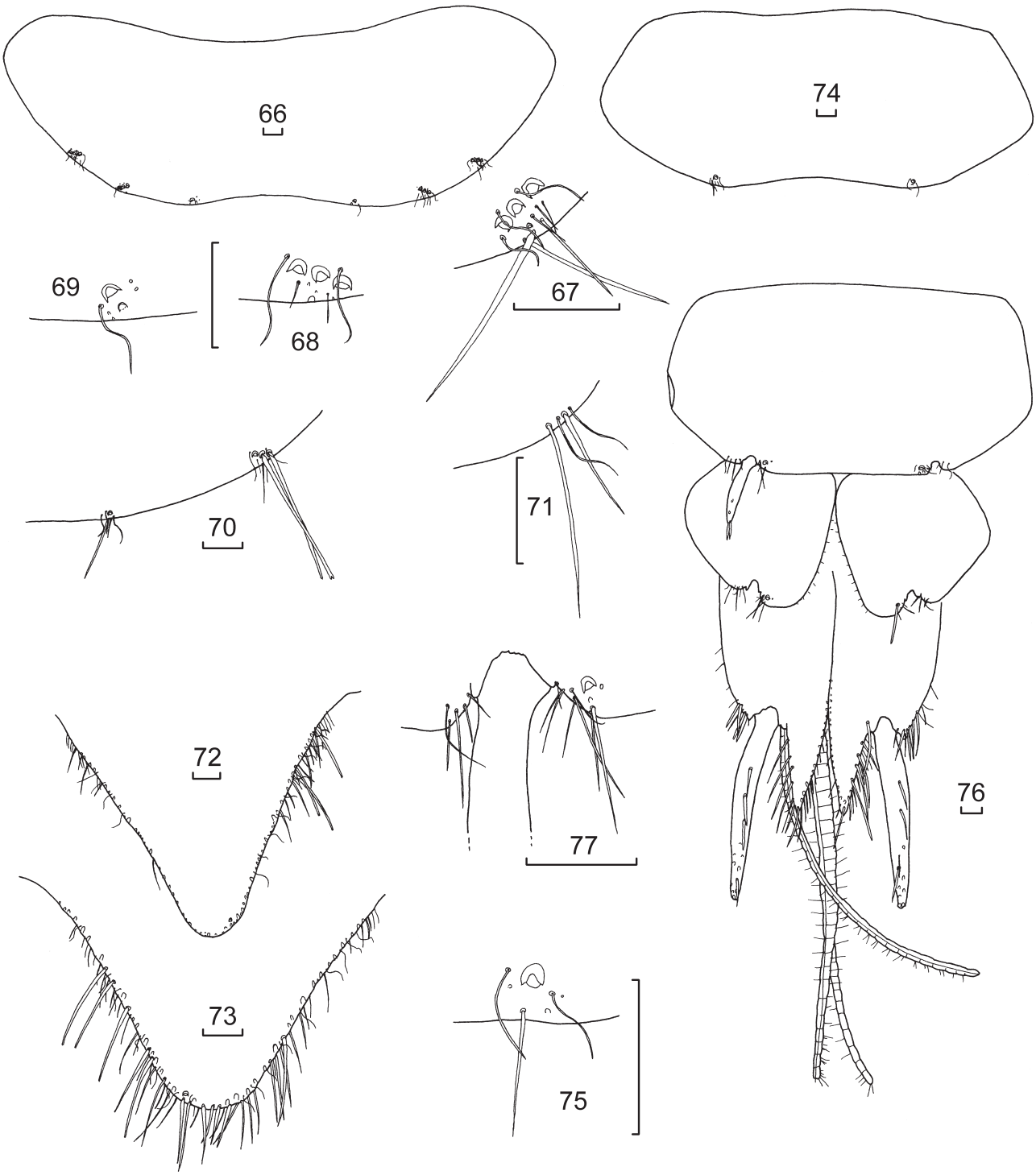
previous; 1♂ (HW 0.95) (gbs004902 in 100% ethanol) same data as previous; 1♂ (HW 0.88) (gbs004903 in 100% ethanol) same data as previous; 1 juvenile ♀ (HW 0.83) (gbs004904 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.75) (gbs004905 in 100% ethanol) same data as previous; 1 juvenile ♀ (HW 0.75) (gbs004906 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.70) (gbs004907 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.70) (gbs004908 in 100% ethanol) same data as previous; 1 juvenile ♀ (HW 0.83) (gbs004909 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.68) (gbs004910 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.75) (gbs004911 in 100% ethanol) same data as previous; 1 juvenile ♀ (HW 0.68) (gbs004912 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.70) (gbs004913 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.58) (gbs004914 in 100% ethanol) same data as previous; 1 juvenile (HW 0.48) (gbs004915 in 100% ethanol) same data as previous.

Other material examined. 1♂ (HW 0.95) (AMS K.377743 in ethanol) QLD: Cooloola, near start of Freshwater track, 25.9439°S 153.0816°E 45 m asl, 7.vii. 2013, Graeme Smith; 1♀ (HW 0.98) (AMS K.377746 in ethanol) same data as previous; 1♂ (HW 1.01) (AMS K.377747 in ethanol) same data as previous (in ethanol); 1♂ (HW 1.01) (AMS K.377341 in ethanol) QLD: Carlo Point, 25.8975°S 153.0620°E 22 m asl, 6.vii.2013, Graeme Smith; 1♂ (HW 1.03) (AMS K.377728 in ethanol) same data as previous; 3♀♀ (HW



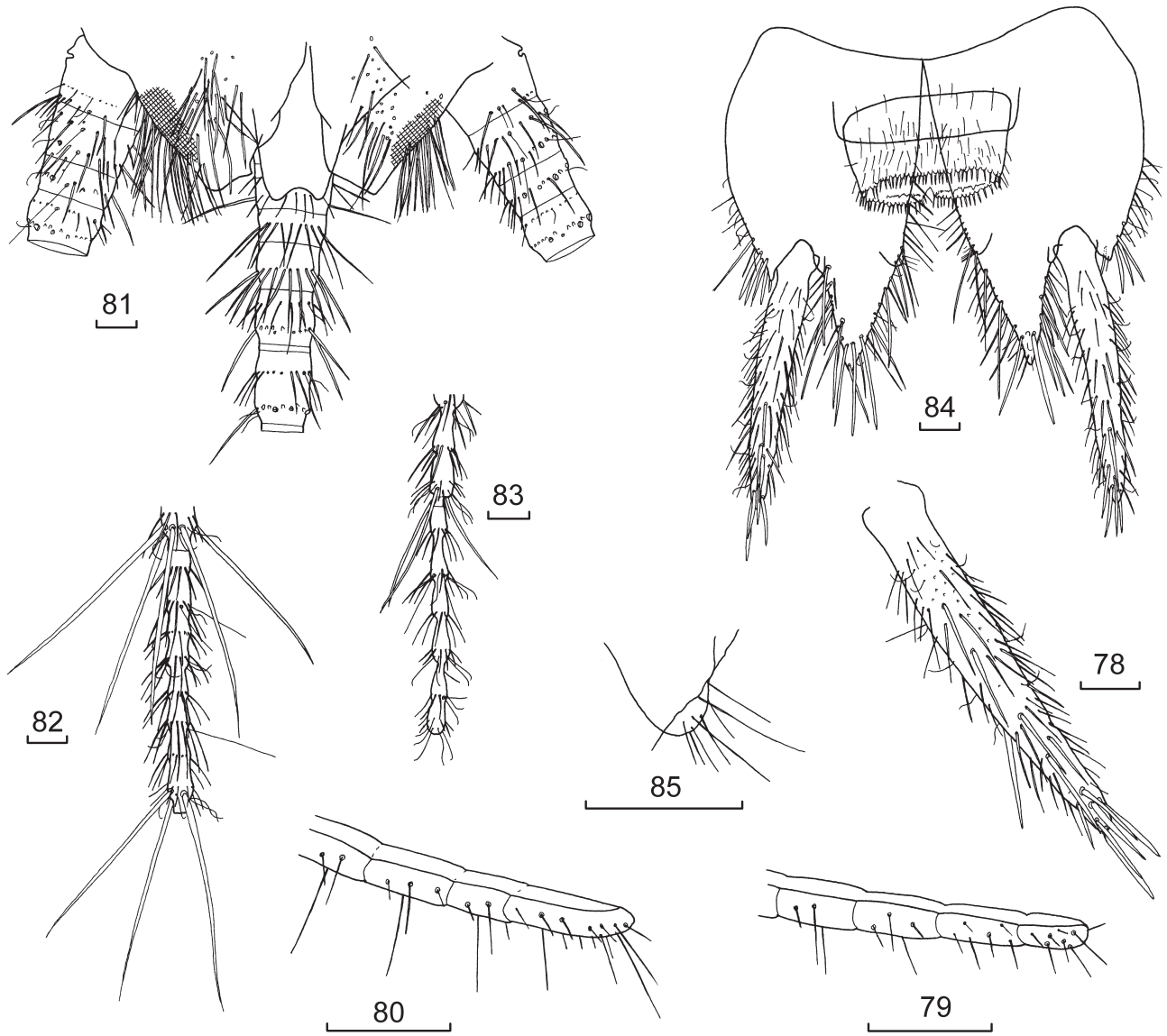
Figures 60–65. *Heterolepisma cooloola* sp. nov. holotype ♀ *(60)* presternum, prothoracic sternum and PI; *(61)* mesothoracic sternum and PII; *(62)* apex of mesothoracic sternum; *(63)* metathoracic sternum and PIII; *(64)* apex of metathoracic sternum; *(65)* pretarsus of PIII. Scale bars = 0.1 mm.

1.11, 0.98 and 0.69) (AMS K.377740 all together in ethanol) same data as previous; 1♀ (HW1.15) (AMS K.377748 in ethanol) QLD: Cooloola, 25.9960°S 153.0716°E 63 m asl, 7.vii. 2013, Graeme Smith; 1♂ (HW 1.03) (AMS K.261188, K.261189 on two slides) same data as previous; 24 specimens (AMS K.377745 all together in ethanol) same data as previous; 1♀ (HW 1.15) (AMS K.261180, K.261181 on two slides) QLD: Carlo Point, 25.8991°S 153.0615°E near sea level, 27.i.2016, Graeme Smith; 1♀ (HW 1.10) (K.261136, K.261137 on two slides) same data as previous; 1♂ (HW 0.95) (gbs004869 in 100% ethanol) same data as previous; 1♀ (HW 1.10) (gbs004870 in 100% ethanol) same data as previous; 1♀ (HW 1.13) (gbs004871 in 100% ethanol) same data as previous; 1♀ (HW 0.98) (gbs004872 in 100% ethanol) same data as previous; 1♂ (HW 1.05) (AMS K.261138, K.261139 on two slides) same data as previous; 1♂ (HW 0.98) (gbs004874 in 100% ethanol) same data as previous; 1♂ (HW 1.00) (gbs004875 in 100% ethanol) same data as previous; 1♀ (HW 0.90) (gbs004876 in 100% ethanol) same data as previous; 1♂ (HW 0.95) (AMS K.261140, K.261141 on two slides) same data as previous; 1♀ (HW 0.83) (gbs004878 in 100% ethanol) same data as previous; 1♀ (HW 0.93) (AMS K.261182, K.261183 on two slides) same data as previous; 1 juvenile ♀ (HW 0.88) (gbs004880 in 100% ethanol) same data as previous; 1 juvenile ♂ (HW 0.83) (gbs004881 in 100% ethanol) same data as previous; 1 juvenile (HW 0.75) (gbs004882 in 100% ethanol) same data as previous; 1♂ (HW 0.90) (AMS K.377753 in 100% ethanol) QLD: Carlo Point, 25.8991°S 153.0616°E near sea level, 27.i.2016, Graeme Smith.



Figures 66–77. *Heterolepisma cooloola* sp. nov. holotype ♀, unless otherwise indicated by specimen number *(66)* urotergite IV; *(67)* urotergite V, right lateral comb; *(68)* idem, left sublateral comb; *(69)* idem, left submedial comb; *(70)* right side of urotergite VIII (K.261189); *(71)* infralateral comb of urotergite IX; *(72)* urotergite X; *(73)* urotergite X of paratype (QM 207012); *(74)* urosternite IV; *(75)* posterior comb of urosternite V; *(76)* urosternites VII, VIII, IX and ovipositor; *(77)* base of stylus VII. Scale bars = 0.1 mm.

Diagnosis. This species differs from other described species of *Heterolepisma* that also have 2+2 combs on urotergite I and three pairs of styli in the female and only two in the male, by the presence of lanceolate scales on the femora, tibiae and clypeus, the straight anterior margin of the head devoid of macrochaetae, the single macrochaeta mediad of the anterior trichobothrium on the pronotum, the mesosternum is also slightly different with a wider glabrous apex and the combs more compact, the posterior margin of the metasternum is rounded with a comparatively wide glabrous gap with small 1+1 combs of two to three macrochaetae. The parabolic shape of urotergite X is narrower and the terminal filaments evenly pigmented.



Figures 78–85. *Heterolepisma cooloola* sp. nov. holotype ♀ unless otherwise indicated by specimen number *(78)* stylet IX (QM 207012); *(79)* apex of anterior gonapophysis; *(80)* apex of posterior gonapophysis; *(81)* bases of terminal filaments; *(82)* most distal surviving divisions of cerci; *(83)* most distal surviving divisions of median filament; *(84)* coxites IX, styli and penis of male (QM 207012); *(85)* paramere. Scale bars = 0.1 mm.

Description

Appearance: Medium to large silverfish, scale covering in life uniform or slightly mottled grey with brown antennae, terminal filaments slightly darker than antennae with only a small portion of each annulus bearing the larger macrochaetae lighter in colour (Fig. 37).

Body length: H+B up to 9.9 mm (♀) 8.25 mm (♂); maximum HW 1.20 mm; thorax: length up to 2.85 mm (or 0.26–0.32 H+B); width up to 1.93 mm, usually slightly widest at the mesonotum; antennae damaged in all specimens, maximum preserved length of antenna 5.6 mm (or 0.68 H+B); terminal filaments damaged in all specimens, maximum preserved length of cercus 3.6 mm (or 0.48 H+B); maximum preserved length of median dorsal appendage 5.00 mm (or 0.60 H+B). Body neither elongate nor broad (Fig. 38) with thorax slightly wider than abdominal segment I, the following abdominal segments about the same width until the fourth or fifth after which it tapers posteriorly.

Pigmentation: Pigment light chestnut-brown in alcohol preserved specimens, stronger around peri-antennal and supra-ocular lines of macrochaetae and along the band of setae on the clypeus (especially laterally); pedicel and scape very lightly pigmented distally, rest of flagellum uniformly lightly pigmented becoming somewhat darker distally; all articles of maxillary palp with pigment except the most distal article, densest on article three especially distally; labium with lines of pigment around macrochaetae across the mentum, present on distal three articles of labial palp being stronger on the edges, pigment of ultimate article mostly in basal half but with a noticeable line above the more distal row of papillae. Nota with some pigment anteriorly and along margins. Legs with pigmentation along outer edge of precoxa of PI, along the length of the outer margin among the macrochaetae and only very faintly along the inner margin, distally; trochanter with light patch on margin distally; femur pigmented, darkest distally along dorsal margin and around bulge on ventral margin; tibia pigmented along edges being

a little darker distally; first tarsal article pigmented distally. Urotergite X with very faint pigment along anterior lateral margins. Styli IX slightly pigmented in distal three quarters; other styli with very little or no pigment. Ovipositor with yellowish hue. Terminal filaments lightly pigmented basally becoming a lot darker distally. Some individuals show greater or lesser levels of pigmentation, with less pigmentation in juvenile specimens.

Macrochaetae: Bifid apically, or simple, hyaline, light to darker brown.

Scales: Unevenly rounded or ovoid, with numerous parallel dark brown ribs, that do not extend beyond the margin (Fig. 39); in alcohol dorsal scales and the more lateral scales of the urosternites with dark brown ribs; ventrally mostly hyaline but those towards the lateral margins with light brown ribs. Lanceolate scales present on clypeus, femora and tibia. Scales absent from flagellum of antennae, mouthparts and terminal filaments.

Head: Wider than long (Fig. 40) with marginal rows about two macrochaetae wide along the sides of the vertex, but without macrochaetae along the anterior margin, the lateral rows extending back along the margin to the eyes and extending as a single short row above the eyes, as well as a small peri-antennal group isolated from the marginal. Clypeus with numerous setae, some long and thin, others more robust but not forming combs; with a few lanceolate scales scattered among the setae. Labrum with thin setae only. Scales on top of head, those along the anterior margin overhanging the margin. Eyes dark, composed of 12 ommatidia.—Antennae long, about ⅔ H+B, scape with a subdistal rosette of setae, very conspicuous from above (Fig. 41), and numerous setae along the sides and over the ventral face (Fig. 42); pedicel short, 0.42 times the length of the scape (range 0.33–0.53), with many setae mostly distally and on the ventral face; the most apical annulus of each interval in the distal end of the flagellum with a few small inconspicuous rod-like basiconic sensilla (type B of Adel, 1984) (Fig. 43).—Mandibles (Figs 44, 45) typical for genus with well-developed molar and incisor areas; a group of about nine strong setae distally adjacent to the pectinate molar area and a bush of 50+ setae and macrochaetae externally.—Maxilla (Figs 46–48) with three large macrochaetae externally proximal to the palp, the lacinia with three strong teeth, one shorter than the rest, seven lamellate processes and a row of eight simple setae, the galea slightly longer than the lacinia with setulae on the outer face. Palp with rosettes of somewhat stronger setae subapically on the two basal articles, all articles with numerous fine setae, apical article of maxillary palp short, being only 0.22 times HW (range 0.20–0.25) and 4.5 times longer than wide (range 3.6–6.0) and 1.3 times longer than penultimate article (range 1.2–1.6), the ultimate article in both sexes with three "branched" papillae, those in the female much less robust and with fewer "arms" than those in the male.—Labium (Fig. 49) short and broad with rows of strong setae on the prementum and submentum, glossae and paraglossae quite broad with short curved setulae; labial palp short, apical article eccentric suboval, 1.05 times as long as wide (range L/W 0.9–1.2) with 2+3 papillae of compact type (Fig. 50) in a "cluster formation" where the slightly larger distal papillae curve around the two smaller proximal papillae and at least one curved club-like thin-walled basiconic sensillum (N.B. one palp of holotype does not show usual shape, possibly as a result of damage in the previous instar).

Thorax: Pronotum (Fig. 51) with narrow setal collar of short, apically bifurcated setae and cilia, quite weak in the medial part of the margin; lateral margins (Fig. 52) also with numerous small to medium sized, apically bifurcate setae as well as several larger more erect submarginal macrochaetae; trichobothrial areas open and in contact with the lateral margins, the anterior one (Fig. 53) located near the mid-point along the margin, almost always with one large macrochaeta located mediad of the trichobothrium (this macrochaeta missing on the left side of the holotype) and with a few cilia and setulae; posterior trichobothrial area (Fig. 54) near posterior lateral corner with a submarginal macrochaeta between the trichobothrium and the margin as well as a few setulae and cilia; posterior margin slightly concave with 1+1 combs each of one macrochaeta with a smaller seta mediad and posterior to it, the insertion of this setae smaller than that of the macrochaeta on most specimens (the smaller insertion missing from the left side of the holotype), the size of the smaller insertion becomes increasingly smaller on posterior segments such that it only appears as a very small submarginal seta on urosternite VIII; these posterior notal combs are associated with two cilia and some setulae.—Mesonotum with lateral chaetotaxy similar to pronotum (Fig. 55), the posterior trichobothrial area (Fig. 56) in the posterolateral corners with a large macrochaeta between the trichobothrium and the margins as well as some marginal and submarginal setae, cilia and setulae; the anterior trichobothrial area (Fig. 57) about ¾ the distance posteriorly along the margin, with a submarginal macrochaeta (m_{-1}) between it and the margin, also with a few setulae and cilia; anterior to this trichobothrial area are two combs (m_{-2}, m_{-3}) each of two macrochaetae, a further two or three submarginal macrochaetae more anterior along the margin; posterior combs as for pronotum (Fig. 58).—Metanotum (Fig. 59) similar to mesonotum (the comb at position m_{-2} on the left side of the holotype composed of only one macrochaeta, suggesting that a degree of variation exists within the species).

Presternum narrow, with transverse row of strong setae and numerous cilia.—All thoracic sterna with hyaline scales. Prothoracic sternum pointed cordiform, only slightly longer than wide at its base (L/W 1.08 range 1.4–1.15) and reaching almost to the end of the coxa, rounded apically and with a medial furrow (Fig. 60), most of lateral margins with numerous small marginal setae and cilia, with 4–6 larger submarginal macrochaetae forming weak combs parallel to the edges in the distal quarter.—Mesosternum (Fig. 61) slightly longer than broad (1.09 range 0.98–1.18) with a truncate or evenly slightly concave posterior margin, with 1+1 distal combs of two to three submarginal macrochaeta associated with some marginal setae and cilia (Fig. 62).—Metasternum (Figs 63, 64) wider than long (L/W 0.75 range 0.69–0.84) but otherwise similar to the mesosternum; the gap between the combs 7.3 times the average width of each comb (range 5.4–11.1).

Legs fairly long (Figs 60, 61, 63), tibia L/W ratio of legs PI 2.6 (range 2.5–3.0), PII 3.0 (range 2.4–3.5, PIII 3.6 (range 3.1–4.1); tarsi L/W ratio PI 6.7 (range 5.4–7.6), PII 6.7 (range 6.0–8.0), PIII 8.8 (range 7.4–10.0). Legs increasingly longer from front to back, mean ratio PI/PIII

(tibia 0.58, tarsus 0.68). PI with transverse comb of about six macrochaetae laterally on the precoxa. Coxa of all legs covered with hyaline scales and with strong macrochaetae and numerous cilia in a row about two macrochaetae wide along the external margin, a stout macrochaeta and some long fine setae on the inner margin subapically and group of about four to six stout curved macrochaetae at the apex over the articulation. Trochanter lacking scales, with fine and one stronger seta over the surface. Femur with numerous lanceolate scales on the anterior half and along the margin, rest of surface with fine setae; anterior distal end with three to six strong, quite deeply bifurcate stout macrochaetae as well as some strong setae; posterior margin with several strong macrochaetae as illustrated. Tibia with numerous setae and lanceolate scales over the ventral surface, with two stout macrochaetae on or near the anterior margin and three or four stout macrochaetae along the posterior margin (some paired with thinner macrochaetae on the dorsal side of the margin; apical spur with several setae. Tibia of PIII with a long thin, laterally projecting trichobothria-like seta inserted dorsal to the proximal stout macrochaeta on the anterior margin, which is more than twice as long as the tibia is wide. Tarsus with four articles, all with numerous setae (without lanceolate scales). Pretarsus with long curved lateral claws and a strong curved shorter medial claw (Fig. 65).

Abdomen: Urotergite I usually with 2+2 combs of 1–2 macrochaetae (sublateral missing on right side of holotype), urotergites II–VII with 3+3 combs of macrochaetae (Fig. 66) as in Table 8, noting that the macrochaeta was sometimes missing from one of the submedial combs; each comb also associated with 0–3 marginal setae, 0–5 setulae plus 1–4 cilia (e.g., Figs 67–69). Urotergite VIII (Fig. 70) with 2+2 combs, lacking the sublateral comb, each comb associated with 0–2 marginal setae, 2–5 setulae and 2–4 cilia; urotergite IX with two long thin infralateral setae on each side as well as a 1–2 cilia (Fig. 71). Urotergite X fairly long and slender parabolic in both sexes (Figs 72, 73), L/W at base about 0.6 (range 0.54–0.70) with many strong setae along entire margin, often without obvious strong submarginal macrochaetae in the postero-lateral corners but sometimes with up to two submarginal insertions visible on each side.

Urosternite I glabrous, urosternites II–VIII (Fig. 74) with 1+1 single macrochaetae (Fig. 75) (although missing from left side of urosternite II on holotype), each associated with 0–1 marginal seta as well as a few cilia and/or setulae. Coxites of segment VII, VIII and IX in ♀ (Fig. 76) with group of several fine setae on the rounded corners on each side of the stylus insertion (Fig. 77). Styli in three pairs in the ♀ (VII–IX); all styli with several noticeably longer and stronger setae apically. Styli IX three times as long as styli VII (range 2.3–3.7) and about two and a half times as long as stylus VIII (range 2.0–2.9) and much more robust (Fig. 78).

Coxite IX of ♀ (Fig. 76), the internal process acute apically, about 4.2 times longer than the external process (range 3.3–6.0) and 1.8 times as long as broad at its base (range 1.6–2.1), reaching almost to half the length of the stylus; external and internal margins of internal process and external margin and apex of outer process with many moderately strong setae directed both up and down.—Ovipositor (Fig. 76) very long and thin (up to 2.30 HW), surpassing the apex of stylus IX by at least the length of the stylus (excluding terminal macrochaetae), composed of about 34–40 divisions. Distal divisions of gonapophyses VIII and IX (Figs 79, 80) with only short fine setae and setulae.

Cerci (Figs 81, 82) with basal divisions shorter than long, gradually becoming longer distally, equally wide as long by about the sixth division after which they become even longer with more annuli each with a rosette of setae and some with trichobothria with the large macrochaetae restricted to the most distal annulus of each division; the most distal surviving divisions with up to eight annuli.—Medial filament of similar arrangement (Figs 81, 83).

Table 8. Number of macrochaetae per bristle comb—*Heterolepisma cooloola* n. sp.

segment	urotergite			urosternites
	lateral	sublateral	submedial	
I	2	0–2	—	—
II	2–3	1–3	1	1
III	2–3	2–3	0–1	1
IV	3–4	3–4	1	1
V	3–4	3–4	1	1
VI	3–4	3–4	1	1
VII	3	1–3	1	1
VIII	1–3	—	0–1	1
IX	2[a]	—	—	—

[a] small infralateral setae

Male: As for female except only two pair of styli (segments VII and IX). Coxites IX (Fig. 84) with acute inner process about 1.8 times longer than wide at its base (range 1.70–1.93) and about four times longer than the external process which has a small preapical constriction, reaching to about half the length of the stylus. Both processes with several strong setae mostly apically emerging from both the dorsal and ventral surfaces of the processes close to or on the margin. Parameres small, slightly longer than wide, with about eight fine setae (Fig. 85). Penis typical for genus with numerous glandular setae apically, each set on a protuberance (Fig. 84).

Subadult stages: Very small specimens (HW 0.68) only have styli on coxites IX; the coxites of segment VIII are already clearly divided in a juvenile ♀ of HW 0.53. The ovipositor is just beginning to appear in a ♀ of HW 0.83 and styli VIII are present but not styli VII; a ♀ with HW of 0.88 had an ovipositor that just attained the end of styli IX but still lacked styli VII; by HW 0.93 all styli were present and the ovipositor was much longer than the end of stylus IX. In a ♂ with HW 0.96 one stylus VIII was developed but the other was only represented by a small triangular appendage. Presumably specimens of both sexes could be considered as sexually mature once HW is greater than 0.93–0.96 mm or once all styli are clearly developed.

Habitat. *Heterolepisma cooloola* was fairly common in the Rainbow Beach area with specimens collected in dry leaf litter accumulating in places largely protected from rainfall such as within burned out ground level tree hollows. It was also taken from the bark of ti-trees, *Casuarina* and *Eucalyptus* in heathland and on the edge of rainforest using pyrethrum sprays.

Etymology. The species name is derived from the proper noun Cooloola referring the locality in which it was collected.

Comments. *Heterolepisma cooloola* is in many ways similar to *H. sclerophylla* (small triangular prothoracic sternum, glabrous urosternite I, urosternites II–VIII with 1+1 macrochaetae, three pairs of styli in the female and two in the male) but in other aspects it shares characters with *H. parva* Smith from Barrow Island (notably the glabrous anterior margin to the frons, a macrochaeta mediad of the anterior trichobothrium on the pronotum and the presence of lanceolate scales). *Heterolepisma parva* is however, one of the species with a medial comb on urosternite I and 1+1 combs of several macrochaetae on urosternites II–VII (VIII), a group that Mendes (pers. comm.) has suggested may be a separate group within *Heterolepisma*. The combined molecular and morphological data support the view that the presence of lanceolate scales and the absence of macrochaetae from the anterior margin of the frons are more significant to phylogeny than the arrangement of styli and the shape of the thoracic sternites in *Heterolepisma*.

Discussion

Molecular data

While previous studies have presented COI sequence data for Zygentoma, they have focussed on higher-level systematics. The present study is the first to use DNA barcodes for species delimitation in Zygentoma, and the first to produce barcode-standard compliant data (Hanner, 2009). Espinasa *et al.* (2007) reported 16 COI sequences from 14 species, all of Nicoletiidae except for two lepismatid specimens (*Thermobia domestica* Packard) and one tricholepidiid (*Tricholepidion gertschi* Wygodzinsky) but since then these authors have utilized 16S data for species level systematics, due to its ease of PCR amplification. Other reported Australian studies have used 12S (e.g., Smith *et al.*, 2012).

Phylogenetic analyses of DNA barcode data suggest that *H. sclerophylla*, as currently defined, is an assemblage of several cryptic species, as there are six well-defined barcode clades (lineages), each with >4% divergence in COI sequences and each geographically restricted. Intra-clade divergences are also large, and despite the well-supported phylogeny there is no clear "barcode gap" (distinction between intra-clade and inter-clade distances) for three of the six NSW populations. This is in contrast to the 28S data, which distinguishes only four lineages from NSW, with essentially no variation within each lineage. Despite this, the 28S data generally aligns well with morphological evidence, clearly identifying *H. cooloola* as a distinct species, supporting also the description of *H. coorongooba* even though it only appears to differ from *H. sclerophylla* in the number of styli. Similar genetic distances are observed in 28S data among *H. sclerophylla* populations from North Nowra, Glenbrook/Burralow/Nattai and Megalong, however the Broulee and Wellington populations have identical 28S sequences. The low levels of variation in 28S sequences among NSW populations accord with the lack of unambiguous morphological differences among these COI lineages.

Unsurprisingly, given that they are both mitochondrial genes and therefore linked markers, the limited 16S data shows a similar pattern to COI with large distances among Megalong and North Nowra lineages in NSW, a large distance between *H. cooloola* and remaining samples, and between *H. cooloola* and the single other Queensland sample.

An analogous situation was reported for the North American nicoletiid silverfish *Texoreddellia texensis* (Ulrich, 1902) which was initially considered to be a widespread and somewhat variable cave-dwelling silverfish. DNA sequences of the 16S gene (Espinasa & Giribet, 2009; Espinasa et al, 2016) have shown that distinct species exist and that, in most cases, subtle morphological differences could be found. Six species of *Texoreddellia* are now described, often with overlapping ranges. It would appear that *Heterolepisma sclerophylla* forms a similar example with several genetically distinct populations, however reliable, non-over-lapping, morphological differences between these populations have mostly not yet been identified. The large variability between individuals within any population, complicated by the continuous moulting, makes quantification of morphological differences difficult and it may be that each separate clade of COI sequences represents a distinct species but we are approaching the practical limits of morphology to separate lepismatid species. Differences in base pairs of 0.9–1.8% in the case of 28S and 7.2% for COI appear to be the lowest levels associated with morphological differences considered indicative of species.

On the other hand, Porco *et al.* (2012) found large intraspecific differences in COI (11.3–21.5%), corroborated by smaller differences in 28S (0.6–9.5 %), within several widespread species of Collembola. Vink and Brown (2014) found up to 8.3% divergence among COI sequences of *Sminthurus viridis* (Collembola) from New Zealand but no variation in 28S D2-region sequences from a small sample of the same specimens and concluded that only one species was present. Resch *et al.* (2014) similarly sequenced both COI and 28S rDNA from Protura, and found that whenever multiple geographic populations were sampled, large intraspecific divergences in COI were observed (up to 21.3%) despite the lack of corroborating morphological characters. Wide variability in COI may be a feature of ancient, low mobility, soil-dwelling taxa.

Unpublished preliminary molecular data suggest it is quite likely that other widespread *Heterolepisma* species, including *H. buntonorum* and an undescribed species related to *H. highlandi* Smith, may also have several genetically distinct lineages. This latter "species" occurs over much the same range as *H. sclerophylla* but is generally only taken on bark using pyrethrum sprays rather than from leaf litter.

Morphology

Wygodzinsky (1961) considered it very probable that *H. howensis* Womersley would prove to be a variety of *H. zealandica* noting that specimens of *H. zealandica* from the type locality possess four pairs of styli while those from the South Island possess only two pairs in both sexes. Tillyard's original illustration (1924) suggests only three pair of styli although he reports them to be present on segments I–IX. Smith (2014) also mentioned the possibility that *H. sclerophylla* might prove to be conspecific with *H. howensis* given the wide variety in morphology observed in the Australian mainland specimens. Although molecular data is currently lacking for the Lord Howe Island and New Zealand specimens, the results of this study suggest that the morphological differences reported are very likely to be indicative of distinct species. This brings into question the

previous tendency to accept small morphological variations within species as a typical feature of the Zygentoma. We believe now that *H. howensis*, with its strongly truncate urotergite X, broader thoracic sternites, single submedial dorsal macrochaetae and a very short ovipositor (reaching only to half the length of stylus IX) is highly likely to be a valid and distinct species. Furthermore, it is quite likely that there is more than a single species of *Heterolepisma* in New Zealand.

The genus *Heterolepisma*, as currently defined, now contains 26 described species with a wide, largely Gondwanan, distribution. The Australian material available display a number of morphological characters that allow them to be easily sorted into species groups but at this stage no clear picture is emerging as to the phylogeny of the genus.

It will be necessary to redescribe many of earliest described species of *Heterolepisma* to include characters that are now considered as important but which were overlooked in the early descriptions, notably the chaetotaxy of the frons, the presence of lanceolate scales, details of the trichobothrial areas, the number of combs on urotergite I and urosternite I. Ideally sequence data should be obtained from types or topotypic examples, however the habitat at the type locality is very likely to have changed greatly over the intervening century. Unless it becomes practical to obtain DNA from specimens stored for more than a century in 70–80% ethanol it may never be possible to obtain sequence data that could be linked unequivocally to the type specimens. Recent advances in "next generation" sequencing, making it possible to "re-assemble" the sequence from the remaining fragments (e.g., Ruane and Austin, 2017), offers some hope that this will not be the case.

Acknowledgments. We would like to thank Steve Bunton for assistance in the collection of *Heterolepisma buntonorum* specimens, Dr Rafael Molero-Baltanás and Dr Miquel Gaju-Ricart of the University of Cordoba for their attempts, so far without success, to find useful morphological characters among the antennal sensilla of three otherwise morphologically distinct species *Heterolepisma* using electron microscopy. Students of the Fall 2015 course of BIOL320:Genetics at Marist College performed some of the DNA sequencing of the 16S rRNA.

We would also like to acknowledge support through Australian Government Research Training Program Scholarship, via Federation University in Ballarat. Funding for the COI and 28S work was provided by Federation University and the Australian Museum Research Institute.

References

Adel, T. 1984. Sensilleninventar und sensillenmuster auf den Antennen von *Thermobia domestica* und *Lepisma saccharina* (Insecta: Zygentoma). *Braunschweiger Naturkundliche Schriften* 2: 191–217.

Dinno, A. 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.5.
https://CRAN.R-project.org/package=dunn.test

Dunn, O. J. 1964. Multiple comparisons using rank sums. *Technometrics* 6(3): 241–252.
https://doi.org/10.1080/00401706.1964.10490181

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797.
https://doi.org/10.1093/nar/gkh340

Edgecombe, G. D., G. Giribet, and W. C. Wheeler. 2002. Phylogeny of Henicopidae (Chilopoda: Lithobiomorpha): a combined analysis of morphology and five molecular loci. *Systematic Entomology* 27: 31–64.
https://doi.org/10.1046/j.0307-6970.2001.00163.x

Escherich, K. 1905. Das System der Lepismatiden. *Zoologica (Stuttgart)* 43(18): 1–164.

Espinasa, L., and E. C. Cappuccio. 2008. New genus allocation for the cavernicolous nicoletiids (Insecta: Zygentoma) of Aruba and description of their previously unknown males. *Proceedings of the Biological Society of Washington* 110(2): 292–299.

Espinasa, L., C. Flick, and G. Giribet. 2007. Phylogeny of the American silverfish Cubacubaninae (Hexapoda: Zygentoma: Nicoletiidae): a combined approach using morphology and five molecular loci. *Cladistics* 23: 22–40.
https://doi.org/10.1111/j.1096-0031.2006.00127.x

Espinasa, L., and G. Giribet. 2009. Living in the dark—the taxonomic status, phylogeography and dispersal capabilities of the nicoletiid genus *Texoreddellia* Wygodzinsky, 1973 (Hexapoda, Zygentoma, Nicoletiidae) in Texas and Mexico. *Texas Memorial Museum Speleological Monographs* 7(V): 87–110.

Espinasa, L., N. D. Bartoli, D. M. Centone, C. S. Haruta, and J. R. Reddell. 2016. Revision of genus *Texoreddellia* Wygodzinsky, 1973 (Hexapoda, Zygentoma, Nicoletiidae), a prominent element of the cave-adapted fauna of Texas. *Zootaxa* 4126(2): 221–239.
https://doi.org/10.11646/zootaxa.4126.2.3

Hanner, R. 2009. Proposed standards for BARCODE records in INSDC (BRIs). Technical report, Database Working Group, Consortium for the Barcode of Life.
http://www.boldsystems.org/docs/dwg_data_standards-final.pdf

Irish, J. 1990. Phylogeny of the Lepismatidae (Thysanura), with a revision of the southern African genera. Unpublished Ph.D. thesis, Faculty of Science, University of Pretoria. 337 pp.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock *et al.* 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
https://doi.org/10.1093/bioinformatics/bts199

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
https://doi.org/10.1093/molbev/msw054

Lanfear, R., B. Calcott, S. Y. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analysis. *Molecular Biology and Evolution* 29: 1695–1701.
https://doi.org/10.1093/molbev/mss020

Machida, R. J., and N. Knowlton. 2012. PCR Primers for Metazoan Nuclear 18S and 28S Ribosomal DNA Sequences. *PLoS ONE* 7(9): e46180.
https://doi.org/10.1371/journal.pone.0046180

Mendes, L. F. 1991. 1.1. On the phylogeny of the genera of Lepismatidae (Insecta: Zygentoma). In *Advances in management and conservation of soil fauna,* ed. G. K. Veeresh, D. Rajagopal and C. A. Viraktamath, pp. 3–13. New Delhi Bombay Calcutta: Oxford & IBH Publishing Co. Pvt. Ltd.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA. IEEE, 1–8.
https://doi.org/10.1109/GCE.2010.5676129

Mitchell, A. 2015. Collecting in collections: a PCR strategy and primer set for DNA barcoding of decades-old dried museum specimens. *Molecular Ecology Resources* 15: 1102–1111.
https://doi.org/10.1111/1755-0998.12380

Paclt, J. 1967. Thysanura.Fam. Lepidotrichidae, Maindroniidae, Lepismatidae. *Genera Insectorum* 218e: 1–86.

Porco, D., A. Bedos, Penelope Greenslade, C. Janion, D. Skarżyński, M. I. Stevens, B. Jansen van Vuuren, and L. Deharveng. 2012. Challenging species delimitation in Collembola: cryptic diversity among common springtails unveiled by DNA barcoding. *Invertebrate Systematics* 26: 470–477.
https://doi.org/10.1071/IS12026

Price, M. N., P. S. Dehal and A. P. Arkin. 2010. FastTree 2–Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS One* 5: e9490.
https://doi.org/10.1371/journal.pone.0009490

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratnasingham, S., and P. D. N. Hebert. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364.
https://doi.org/10.1111/j.1471-8286.2007.01678.x

Resch M. C., J. Shrubovych, D. Bartel, N. U. Szucsich, G. Timelthaler *et al.* 2014. Where taxonomy based on subtle morphological differences is perfectly mirrored by huge genetic distances: DNA Barcoding in Protura (Hexapoda). *PLoS One* 9(3): e90653.
https://doi.org/10.1371/journal.pone.0090653

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61(3): 539–542.
https://doi.org/10.1093/sysbio/sys029

Ruane, S., and C. C. Austin. 2017. Phylogenomics using formalin-fixed and 100+ year-old intractable natural history specimens. *Molecular Ecology Resources* 17(5): 1003–1008.
https://doi.org/10.1111/1755-0998.12655

Silvestri, F. 1908. Thysanura. In *Die Fauna Südwest-Australiens. Ergebnisse der Hamburger südwestaustralischen Forschungsreise 1905*, ed. W. Michaelsen and R. Hartmeyer, vol. 2, pp. 47–68. Jena: Gustav Fischer.

Smith, G. B. 2013. A new species of *Heterolepisma* from Barrow Island (Zygentoma: Lepismatidae). *Records of the Western Australian Museum*. Supplement 83: 229–240.
https://doi.org/10.18195/issn.0313-122x.83.2013.229-240

Smith, G. B. 2014. Two new species of *Heterolepisma* (Zygentoma: Lepismatidae) from eastern New South Wales. *General and Applied Entomology: The Journal of the Entomological Society of New South Wales* 42(2013): 7–22 (published 2014).

Smith, G. B. 2016a. On some silverfish taxa from Tasmania (Zygentoma: Lepismatidae and Nicoletiidae). *Records of the Australian Museum* 68(2): 45–80.
https://doi.org/10.3853/j.2201-4349.68.2016.1652

Smith, G. B. 2016b. Revision of the silverfish genus *Anisolepisma* (Zygentoma: Lepismatidae: Acrotelsatinae). *Records of the Australian Museum* 68(6): 269–312.
https://doi.org/10.3853/j.2201-4349.68.2016.1662

Smith, G. B. 2017. The Australian silverfish fauna (order Zygentoma)—abundant, diverse, ancient and largely ignored. *General and Applied Entomology: The Journal of the Entomological Society of New South Wales* 45(Aug 2017): 9–58.

Smith, G. B., S. M. Eberhard, G. Perina, and T. Finston. 2012. New species of short range endemic troglobitic silverfish (Zygentoma: Nicoletiidae) from subterranean habitats in Western Australia's semi-arid Pilbara region. *Records of the Western Australian Museum* 27: 101–116.
https://doi.org/10.18195/issn.0312-3162.27(2).2012.101-116

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
https://doi.org/10.1093/bioinformatics/btl446

Tillyard, R. J. 1924. Primitive wingless insects. Part I. The silverfish, bristletails and their allies (Order Thysanura). *New Zealand Journal of Science and Technology* 7: 232–242.

Villesen, P. 2007. FaBox: an online toolbox for FASTA sequences. *Molecular Ecology Notes* 7: 965–968.
https://doi.org/10.1111/j.1471-8286.2007.01821.x

Vink, C. J., and S. D. J. Brown. 2014. High mitochondrial DNA sequence divergence in *Sminthurus viridis* (Linnaeus) (Collembola: Sminthuridae) from New Zealand. *New Zealand Entomologist* 37: 29–34.
https://doi.org/10.1080/00779962.2013.800698

Vu, V. Q. 2011. ggbiplot: A ggplot2 based biplot. R package version 0.55.
http://github.com/vqv/ggbiplot

Wygodzinsky, P. 1961. Synonymical notes on the Lepismatidae (Thysanura). *Pan-Pacific Entomologist* 37(4): 213–216.

Wygodzinsky, P. 1967. On the geographical distribution of the South American Microcoryphia and Thysanura (Insecta). In *Biologie de L'Amérique Australe,* ed. D. Debouttville and E. Rapoport, vol. 3, pp. 505–524. Paris: Centre National de la Recherche Scientifique.