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Closing a biogeographic gap: a new pettalid genus from South Australia (Arachnida : Opiliones : Cyphophthalmi : Pettalidae) with a UCE-based phylogeny of Cyphophthalmi

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ABSTRACT

Pettalidae is a family of mite harvestmen that inhabits the former circum-Antarctic Gondwanan terranes, including southern South America, South Africa, Madagascar, Sri Lanka, Australia and New Zealand. Australia is home to two pettalid genera, *Austropurcellia*, in northern New South Wales and Queensland, and *Karripurcellia*, in Western Australia, until now showing a large distributional gap between these two parts of the Australian continent. Here we report specimens of a new pettalid from South Australia, *Archaeopurcellia eureka*, gen. et sp. nov., closing this distributional gap of Australian pettalids. Phylogenetic analyses using traditional Sanger markers as well as ultra-conserved elements (UCEs) reveal that the new genus is related to the Chilean *Chileogovea*, instead of any of the other East Gondwanan genera. This relationship of an Australian species to a South American clade can be explained by the Antarctic land bridge between these two terranes, a connection that was maintained with Australia until 45 Ma. The UCE dataset also shows the promise of using museum specimens to resolve relationships within Pettalidae and Cyphophthalmi.

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Keywords: arachnid, biogeography, Gondwana, mite harvestmen, phylogenomics, UCEs, ultra-conserved elements, vicariance.

Introduction

The family Pettalidae, because of its low dispersal ability and high ecological constraints, has emerged as one of the best examples of Gondwanan vicariance, as it inhabits the terranes once surrounding Antarctica that today constitute mostly the temperate region of the former Gondwana. Extant pettalids are found in southern South America (southern Chile), South Africa, Madagascar, Sri Lanka, Australia and New Zealand (Juberthie and Massoud 1976; Boyer and Giribet 2007; Giribet *et al.* 2016; Oberski *et al.* 2018; Baker *et al.* 2020a). The family is currently composed of 10 genera and 81 species (one of these with two subspecies) (Giribet 2020; Kury *et al.* 2021; Boyer *et al.* 2022). Each of the 10 pettalid genera is restricted to small areas within a single landmass, with the 2 species of *Chileogovea* Roewer, 1961 found exclusively in Chile; the monotypic *Managotria* Shear & Gruber, 1996 in Madagascar; the 4 species of *Pettalus* Thorell, 1876 in Sri Lanka; 11 species of *Parapurcellia* Rosas Costa, 1950 and 5 of *Purcellia* Hansen & Sørensen, 1904 are found in South Africa; and a final 3 genera found in New Zealand, *Rakaia* Hirst, 1926, with 18 species, *Aoraki* Boyer & Giribet, 2007 with 13 species or subspecies, both in the North and South islands, and the monotypic *Neopurcellia* Forster, 1948, from the South Island.

Australia is also home to the family Pettalidae, with *Austropurcellia* Juberthie, 1988, including 25 species in the Wet Tropics of Queensland and northern New South Wales (Juberthie 1988, 1989, 2000; Baker 2012; Boyer and Reuter 2012; Popkin-Hall and Boyer 2014; Jay *et al.* 2016; Oberski *et al.* 2018), and *Karripurcellia* Giribet, 2003 with 2 species in south-western Western Australia (Giribet 2003; Karaman 2012; Schwentner and Giribet 2018) (Fig. 1). Given the large gap in the distribution ranges of these two

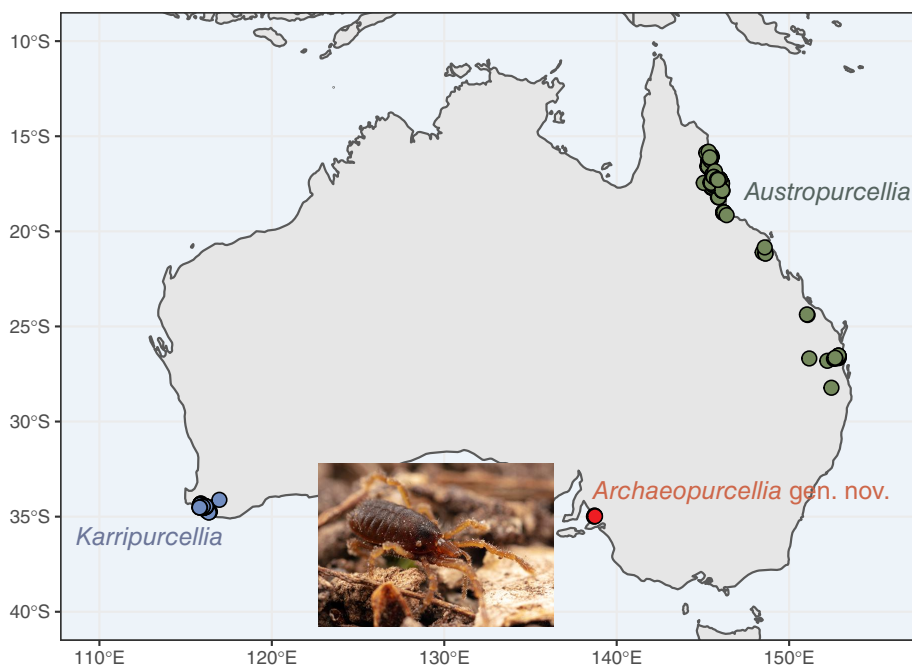


Fig. 1. Distribution map of all known records of *Karripurcellia* (blue), *Austropurcellia* (green) and *Archaeopurcellia*, gen. nov. (red), available in GBIF (GBIF.org, accessed 28 January 2022, GBIF Occurrence Download <https://doi.org/10.15468/dl.sq3757>). Photo of live specimen courtesy of James Dorey.

genera, the lack of pettalids in other suitable locations of Australia (notably the temperate, mesic areas in the south-east occurring in Tasmania, Victoria and South Australia) has puzzled zoologists for a long time.

Recent field work by the second author has resulted in a considerable number of specimens from a few sites in Cleland Conservation Park, Adelaide, South Australia. Anatomical study and phylogenetic work using traditional Sanger-based markers as well as ultra-conserved elements (UCEs) show that the specimens from Cleland constitute a new pettalid species unrelated to the other Australian genera. Here we describe the new genus and species and provide the biogeographic context for the new taxon.

Materials and methods

Abbreviations

MCZ IZ, Invertebrate Zoology collection, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; SAMA, South Australian Museum Arachnid collection, Adelaide SA, Australia. All specimens were collected by desiccating extraction funnels (Berlese funnels) and transferred to 96% EtOH or RNAlater. This material was collected under permit Y27000-1 to M. Shaw.

Anatomical methods

Specimens prepared for Automontage and for SEM were sonicated for 3 min in a Branson 200 Ultrasonic cleaner and dissected under an Olympus SZX16 stereomicroscope.

We imaged the male holotype (SAMA OP2) using a Leica DFC500 digital camera mounted on a Leica M205C stereomicroscope with a $1.0\times$ plan-apochromatic objective. The Leica Application Suite (ver. 3.8, see <https://www.leica-microsystems.com/products/microscope-software/p/leica-application-suite/downloads/>) was used to assemble a series of 25–30 images for each view. A female paratype (MCZ IZ-162243) was imaged in dorsal, ventral and lateral views using a JVC-KY-F75U digital camera mounted on a Leica MZ 12.5 stereomicroscope (Leica Biosystems, Nußloch, Germany) with a Plan 1 objective. The software package Auto-Montage Pro (ver. 5.02.0096, Synoptics Group, Cambridge, UK) was used to produce and assemble a series of 5–7 images taken at different focal planes. A male and the same female specimen (MCZ IZ-162243) were then used for SEM. The appendages of the left side of this male specimen were dissected and mounted in retrolateral view (a few appendages were mounted in prolateral view unintentionally) on a SEM stub using a carbon adhesive tab (Electron Microscopy Sciences, Hatfield, PA, USA). The male specimen and the female were mounted on their dorsal side, exposing the venter. A third specimen (male, MCZ IZ-162249) was mounted on its ventral side for dorsal imaging. The specimens were coated with 10 nm of Pt–Pd (80:20) in a HAR 050 EMS 300T D dual head sputter coater at the Center for Nanoscale Systems, Harvard University. Specimens were then imaged using a Zeiss FESEM Ultra Plus using an SE2 detector with an EHT target of 5 kV. Images were processed and edited in Adobe Photoshop 2022 (ver. 32.3.2).

The spermatopositor of a male paratype (MCZ IZ-162243) was dissected out and imaged on an LSM 880 confocal microscope (Carl Zeiss Microscopy, Jena, Germany) using a

20×/0.8 NA Plan-Apochromat objective. Autofluorescence was excited with a 561-nm laser and signal was collected from 570 to 730 nm. An axial volume of 40 µm was obtained in 75 slices. Post-acquisition, a maximum intensity projection was performed to enable visualisation of structures.

Molecular methods

In order to investigate the phylogenetic position of the new species, we assembled two genetic data sets. For the first approach we amplified for two specimens of the new species a series of Sanger markers routinely used in Opiliones systematics for the past two decades (e.g. Giribet et al. 1999, 2002; Boyer and Giribet 2007; Boyer et al. 2007; Sharma and Giribet 2011). These include 18S rRNA, 28S rRNA and 16S rRNA. We also obtained a sequence of cytochrome *c* oxidase subunit I (*COI*) from a UCE library. We then combined these Sanger-sequenced markers and the UCE-derived *COI* sequence with a curated version of the data set of Giribet et al. (2016). The new Sanger sequences were submitted to GenBank under accession numbers ON790664–ON790668.

The three Sanger markers were each aligned with a server version of MAFFT (ver. 7, see <https://mafft.cbrc.jp/alignment/server/>; Kuraku et al. 2013; Katoh et al. 2019) using the Auto strategy, with a gap opening penalty of 1.53, and the remaining default parameters (% mafft –inputorder –auto input). Each individual data set was then trimmed with BMGE (ver. 1.12, see <https://bioweb.pasteur.fr/packages/pack@BMGE@1.12>; Criscuolo and Grimaldo 2010) on a web server (Lemoine et al. 2019) to remove regions of ambiguous alignment which were reduced as follows: 16S rRNA from 564 to 466 positions; 18S rRNA from 1763 to 1762; and 28S rRNA from 2157 to 2075 positions. No trimming was necessary for the cytochrome *c* oxidase subunit I dataset. We concatenated the untrimmed and the BMGE alignments using SequenceMatrix (ver. 1.8, see <http://www.ggvaidya.com/taxondna/>; Vaidya et al. 2011).

With these ‘Sanger data’ we conducted a series of maximum likelihood analyses in IQ-TREE (ver. 1.6.12, see <http://www.iqtree.org/release/v1.6.12>; Nguyen et al. 2015) using the built-in ModelFinder (Kalyaanamoorthy et al. 2017) + a FreeRate heterogeneity, both, for the individual genes as well as for the concatenated data. For the concatenated datasets (untrimmed and trimmed) we specified an edge-unlinked partition model for each individual partition.

Because the standard Sanger markers have provided little resolution of the internal pettalid phylogeny, we aimed to explore pettalid relationships using a genomic approach. A recent phylotranscriptomic analysis has shown improved resolution of pettalid relationships (Baker et al. 2020a), but, because of a lack of samples suitable for RNA work, we decided to undertake another approach to subsample the genome, using target capture of UCEs (Faircloth et al. 2012; McCormack et al. 2012). UCEs have been routinely applied to study Opiliones (e.g. Derkarabetian et al. 2018, 2021a) by

leveraging a probe set developed for all arachnids (Starrett et al. 2017). This method allows the inclusion of old museum samples with degraded DNA (Derkarabetian et al. 2019).

For the UCE dataset, we selected 34 Cyphophthalmi samples plus 3 outgroups (Supplementary Table S2). This dataset included 20 pettalid, 3 neogoveid, 1 ogoveid, 1 troglisironid, 5 sironid and 4 stylocellid samples. UCE sequence capture followed standard protocols used in recent UCE studies of Opiliones (e.g. Derkarabetian et al. 2019, 2021a). DNA from fresh specimens was extracted using the Qiagen DNeasy Blood and Tissue kit (Valencia, CA, USA), whereas historical specimens with degraded DNA were extracted using the extraction protocol specified in Derkarabetian et al. (2019), derived from Tin et al. (2014). Library preparation used either the Kapa HyperPrep kit (Roche Sequencing) preceded by sonication on the Covaris S220 Ultrasonicator, or the Kapa HyperPlus kit with a fragmentation time of 3 min, both of which were at half reaction of the manufacturer's protocol. Pools were hybridised at 60°C for 24 h using the Arachnida 1K1 UCE probe set (Faircloth 2017; Starrett et al. 2017), and sequencing was done on the Illumina NovaSeq with 150-bp paired end reads at The Bauer Core Facility at Harvard University.

Demultiplexed UCE data were processed through the standard PHYLUCE pipeline (Faircloth 2016) using parameters and options typically employed in previous studies (e.g. Derkarabetian et al. 2019, 2021a). After individual matrices for each locus were created, we used CIALign (<https://github.com/KatyBrown/CIALign>; Tumescheit et al. 2022) to filter out obviously divergent non-homologous sequences from each matrix, with the –remove_divergent_minperc option set to 0.7. All loci were then manually inspected in Geneious Prime (ver. 2022.1.1, see <https://www.geneious.com>), retaining only the loci with at least 50% taxon occupancy for phylogenetic analyses, for a total of 840 loci. Phylogenies were estimated on a concatenated partitioned data set with IQ-TREE (ver. 1.6, see <http://www.iqtree.org/release/v1.6.12>; Nguyen et al. 2015) using the optimal partitioning strategy found using ModelFinder (MFP + MERGE; Kalyaanamoorthy et al. 2017), the fast relaxed clustering algorithm (rclusterf), and 1000 ultrafast bootstrap replicates (Hoang et al. 2018).

Results and discussion

A new genus of Pettalidae from South Australia

The anatomy of the new species differs markedly from that of the other genera of Australian Pettalidae (see taxonomic section below). Phylogenetically, the Sanger-based phylogeny (Fig. 2) places the new genus as sister group to the South African genus *Purcellia* (with a bootstrap support, BS, of 60–64%) whereas the UCE-based tree (Fig. 3) places the new genus as the sister group to the Chilean genus *Chileogovea* with high support (100% BS). This result is interesting not

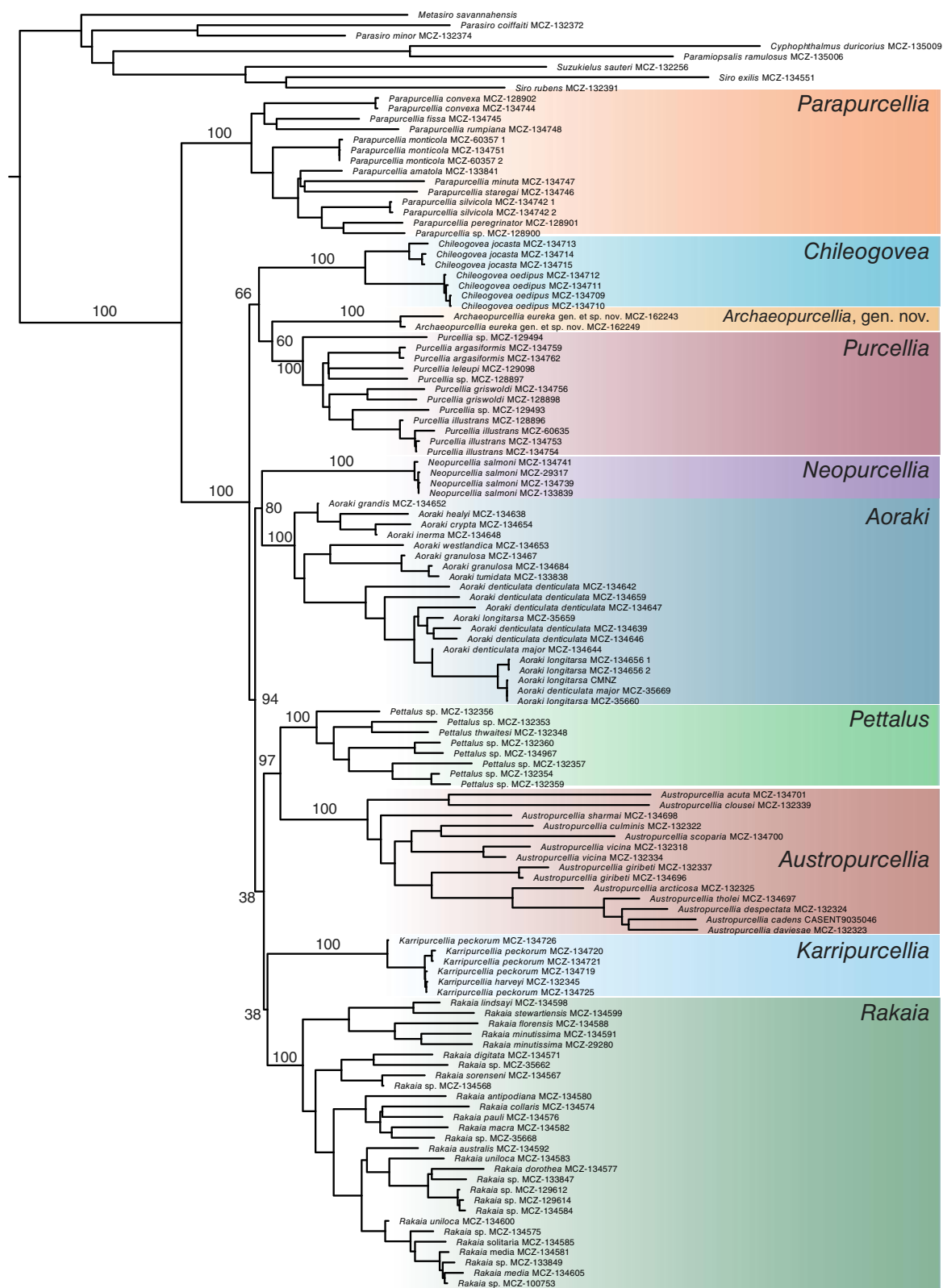


Fig. 2. Phylogenetic tree of the legacy rRNA + *COI* sequence data, untrimmed, concatenated and analysed in IQ-TREE under a partitioned edge-unlinked model. Bootstrap support values are provided for pettalid genera and deeper branches only. *Limulus*, used as outgroup, is not depicted.

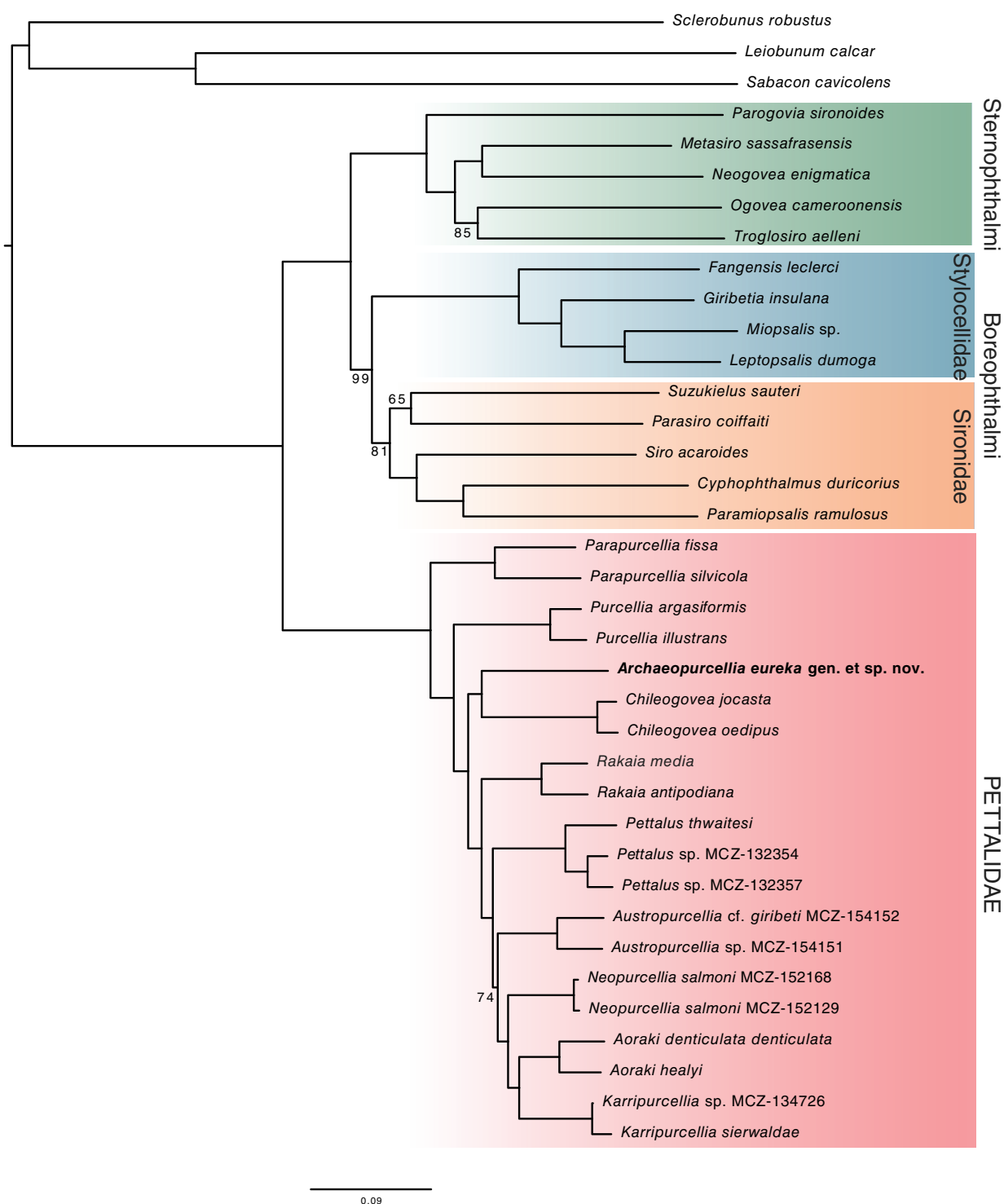


Fig. 3. Phylogenetic results of the 840 concatenated UCE loci analysed in IQ-TREE. Only nodes with a bootstrap support <100% show support value.

only because the new genus appears unrelated to the two other Australian genera, but also because it is the sole East Gondwanan pettalid that does not cluster within the East Gondwanan clade (i.e. the remaining samples from Australia,

New Zealand and Sri Lanka). This East Gondwanan clade has been identified here as well as in prior studies (Giribet *et al.* 2012; Oberski *et al.* 2018; Baker *et al.* 2020a). Instead, the new genus appears within a grade of West Gondwanan genera:

Parapurcellia, *Purcellia* and *Chileogovea*. Both our UCE analysis and the phylotranscriptomic analysis of Baker *et al.* (2020a) resolve *Parapurcellia* as the sister group to all other pettalids (as do our Sanger-based phylogenies here), followed by *Purcellia* and then *Chileogovea* (or *Chileogovea* plus the new genus in our study) as the sister group to East Gondwanan taxa. Instead, our Sanger-based phylogenies place *Chileogovea*, *Archaeopurcellia*, gen. nov., and *Purcellia* in a clade (with BS = 66–68%) that is the sister group to the East Gondwanan clade (BS = 94–95%).

The position of the new genus is of interest for multiple reasons. Geographically, *Archaeopurcellia*, gen. nov., closes a large distributional gap between *Karripurcellia* and *Austropurcellia*, something that has puzzled pettalid workers for a long time, and once more shows that the absence of pettalids in places with suitable habitat may be an artefact of undersampling obscure saproxylic taxa. Tasmania and Victoria could easily harbour pettalids, as they are home to other groups that often co-occur with pettalids across temperate Gondwana, including Opiliones in the families Neopilionidae (Giribet *et al.* 2021), Triaenonychidae (Baker *et al.* 2020b; Derkarabetian *et al.* 2021b) and Acropsopilionidae (Groh and Giribet 2015), the spider family Malkaridae (Hormiga and Scharff 2020), and the velvet worm family Peripatopsidae (Giribet *et al.* 2018; Baker *et al.* 2021).

In South Australia, *Archaeopurcellia*, gen. nov., has not necessarily been overlooked because of an overall lack of sampling effort. As a well-known mesic refuge, Cleland Conservation Park has been repeatedly targeted by several prominent collectors of mites and other arachnids for over 80 years and it is a significant type locality (e.g. Lee 1973). *Archaeopurcellia*, gen. nov., is patchily distributed and is clearly absent from various apparently suitable sites within Cleland Conservation Park but also from many other suitable catchments in the region. Its conservation status requires assessment because of its restricted range and impending environmental changes such as available habitat being modified by invasive European blackberry. Biologists visiting these vulnerable sites should employ good phytosanitary hygiene, including disinfecting footwear to slow the spread of *Phytophthora*.

From a biogeographic point of view, the close relationship of an Australian species to a South American clade can easily be explained by the Antarctic land bridge between these two terranes, a connection that was maintained with Australia until 45 Ma (White *et al.* 2013; van den Ende *et al.* 2017). Since the diversification of *Chileogovea* has been estimated to be Cretaceous to Paleogene (Baker *et al.* 2020a), it is clear that the sister group relationship of *Archaeopurcellia*, gen. nov., and *Chileogovea* occurred during a time when South America and Australia were connected through Antarctica. This old connection is also found in the clade of cold and temperate triaenonychids, with the genera *Calliuncus* Roewer, 1931, *Callihamina* Roewer, 1942 and *Callihamus* Roewer, 1931 from South Australia and other parts of

temperate southern Australia nesting within the Chilean ‘*Nuncia*’ (Baker *et al.* 2020b; Derkarabetian *et al.* 2021a, 2021b; Porto *et al.* 2022).

Cyphophthalmi phylogeny

Our UCE dataset allows us to evaluate Cyphophthalmi phylogeny for the first time using genome-scale data (Fig. 3) including multiple representatives of all currently recognised families. Nearly all suborder-level phylogenies of Cyphophthalmi to date have been based on morphological data (Giribet and Boyer 2002), a few PCR-amplified markers (Boyer *et al.* 2007; Oberski *et al.* 2018) or a combination of both (Giribet *et al.* 2012), but genomic-level sampling has remained limited to a handful of species in four families (Fernández *et al.* 2017; Baker *et al.* 2020a). As in recent analyses, Pettalidae (infraorder Scopulophthalmi) appears as the sister group to all other Cyphophthalmi, which divide into Sternophthalmi (Neogoveidae, Ogoveidae, Troglosironidae; 100% BS) and Boreophthalmi (Sironidae, Stylocellidae; 99% BS) (Fig. 3).

Although the two Boerophthalmi families appear reciprocally monophyletic, this is not the case for the Sternophthalmi families, as Ogoveidae and Troglosironidae constitute a clade (albeit with BS = 85%) that nests within Neogoveidae, which is therefore paraphyletic. Paraphyly of Neogoveidae with respect to Troglosironidae has been obtained in earlier studies using Sanger-based approaches (Boyer *et al.* 2007), but this was not the case in subsequent studies including Ogoveidae and denser sampling (Giribet *et al.* 2012; Oberski *et al.* 2018). Inclusion of further genera in the UCE dataset may be required to further test the monophyly of Neogoveidae.

The first split within Sternophthalmi is among the African *Parogovia* Hansen, 1921 and the remaining species, including American neogoveids (*Metasiro* Juberthie, 1960 and *Neogovea* Hinton, 1938), plus the African Ogoveidae and the New Caledonian Troglosironidae. The association of a New Caledonian endemism to a clade of West Gondwanan Cyphophthalmi has been difficult to reconcile with current views on biogeography, but anatomically the presence of a sternal secretory organ in adult males and a toothed claw on legs II are characteristics of this entire clade (the toothed claw is missing in Ogoveidae) that were once considered convergences. The broad biogeographic range and the morphological disparity observed in the members of this clade are most probably a consequence of the old age of the group, estimated to be Carboniferous to Permian (Giribet *et al.* 2012; Oberski *et al.* 2018).

Within Boreophthalmi, Stylocellidae is well supported, but support for some of the deepest nodes within Sironidae is low (81% BS). Stylocellid phylogeny has received little attention in recent times after the seminal work of R. Clouse (e.g. Clouse *et al.* 2009; Clouse and Giribet 2010; Schmidt *et al.* 2020). Our phylogeny places the genus *Fangensis* Rambla, 1994 as the sister group to all other genera, with *Giribetia* Clouse,

2012 appearing as the sister group of the remaining genera, thus rejecting the monophyly of Fangensinae proposed by Clouse (2012). Within Sironidae, the Japanese *Suzukielus* Juberthie, 1970 is sister group to the European *Parasiro* Hansen & Sørensen, 1904; the amphi-Atlantic *Siro* Latreille, 1795 constitutes the sister group to an exclusively European clade that splits between the Iberian genus *Paramiopsalis* Juberthie, 1962 and the Balkan genus *Cyphophthalmus* Joseph, 1869. This resolution is similar to that of Sanger-based studies with broader taxon sampling (Giribet et al. 2017; Karaman et al. 2022), with the main difference being that in the former study *Parasiro* or *Suzukielus* sometimes fell outside Sironidae, and in the latter the root is artificially placed between *Cyphophthalmus* and the remaining sironids.

Overall, our UCE phylogeny shows promise for resolving genus-level and family-level relationships within Cyphophthalmi. It also illustrates the best available strategy for generating a phylogeny that includes nearly all genera of Cyphophthalmi at genomic-level resolution, as it allows for inclusion of museum samples not previously included in any molecular phylogeny as well as dense sampling within each family. A larger dataset including nearly all cyphophthalmid

genera is currently underway and should resolve some of the potential conflicts shown by the subset represented here, which focused on pettalids.

Taxonomic section

Archaeopurcellia, gen. nov.

(Fig. 1, 4–8)

ZooBank: urn:lsid:zoobank.org:act:A794F31C-8BD8-43E8-8FE8-6CB9A28CDFCC

Diagnosis

Medium-sized pettalid with type 2 ozophores (*sensu* Juberthie 1970). Chelicerae without a dorsal crest. Male posterior region bilobed with a scopula on the internal side of each lobe; corona analis with a divided tergite IX and free sternites 8 and 9, the latter much smaller; anal plate without modifications or scopulae. Eyes and eye lenses entirely

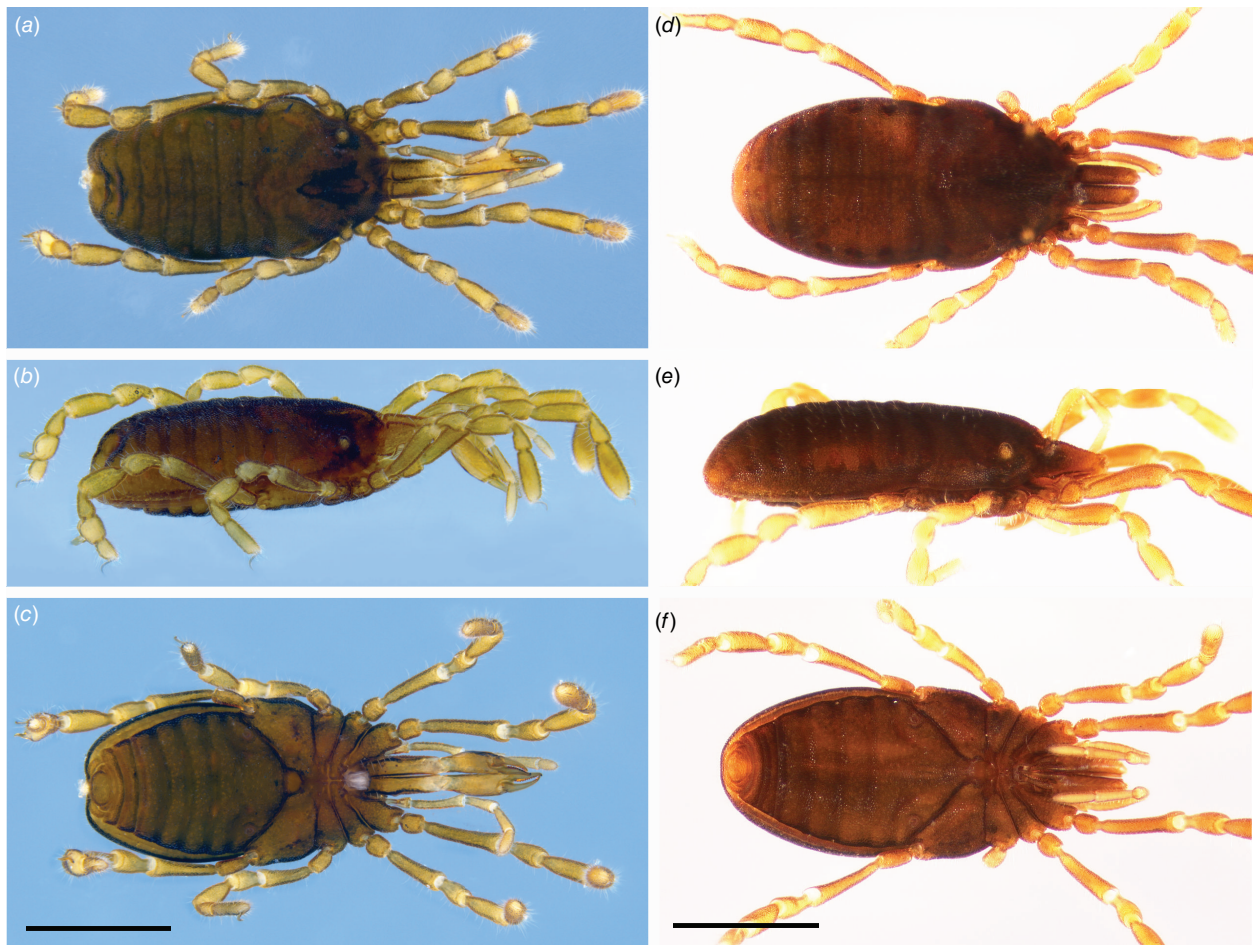


Fig. 4. *Archaeopurcellia eureka*, gen. et sp. nov.: (a–c) Holotype, male (SAMA OP2), (d–f) paratype, female (MCZ IZ-162243).

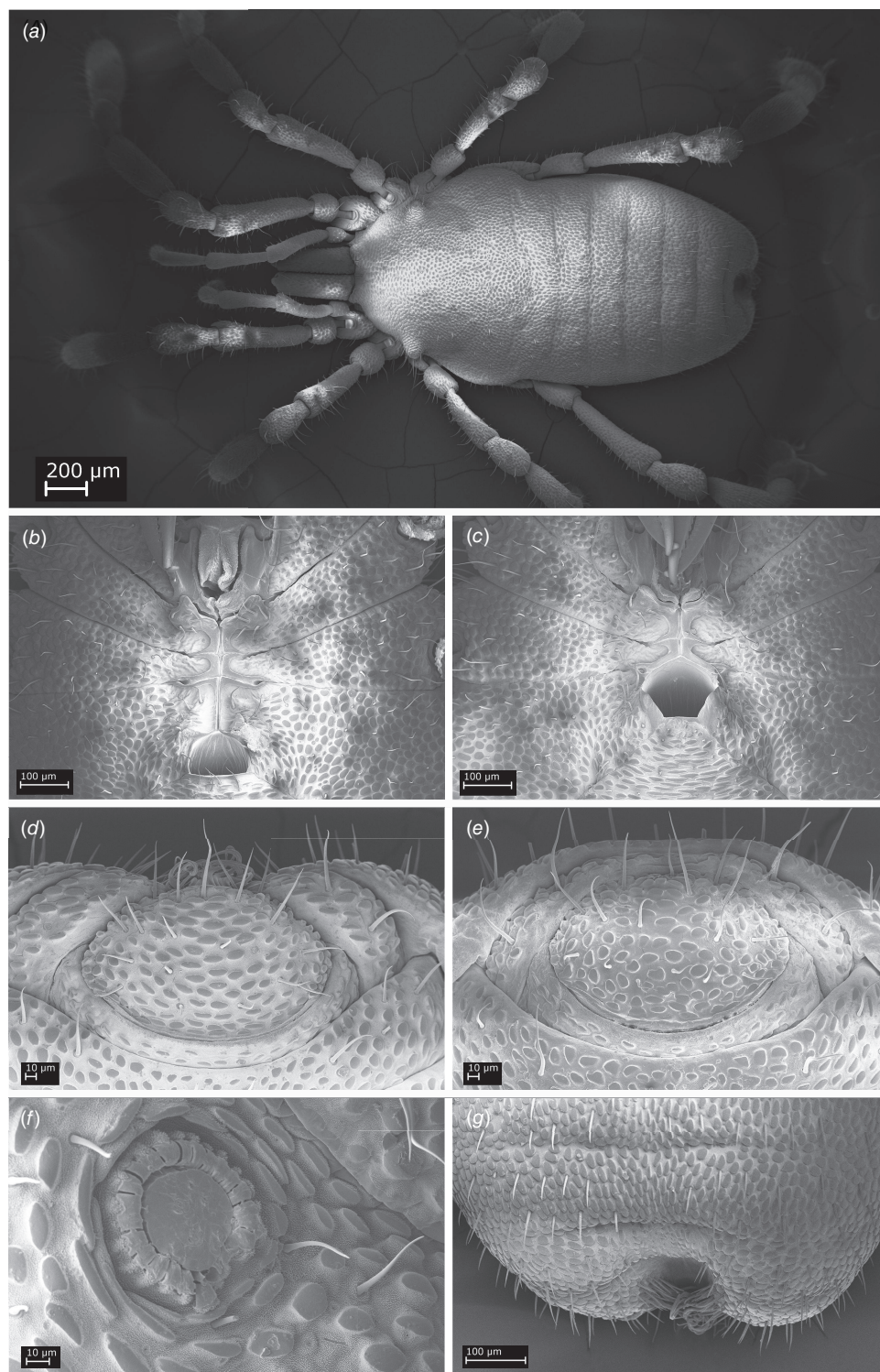


Fig. 5. *Archaeopurcellia eureka*, gen. et sp. nov.: (a) MCZ IZ-162249, male dorsal view; (b) MCZ IZ-162243, male, ventral prosomal complex; (c) MCZ IZ-162243, female, ventral prosomal complex; (d) MCZ IZ-162243, male, ventral view of anal complex; (e) MCZ IZ-162243, female, ventral view of anal complex; (f) MCZ IZ-162243, female, left spiracle; (g) MCZ IZ-162243, male, dorsal view of anal complex.

absent. Male tarsus IV divided, the basalmost article with a wing-like projection bearing the adenostyle. *Archaeopurcellia*, gen. nov., differs from the West Australian *Karripurcellia* in lacking a dorsal crest on chelicerae and in having a distinct solea (Giribet 2003) on tarsus I. *Archaeopurcellia*, gen. nov., differs from most members of the north-east Australian

Austropurcellia is lacking scopulae on the anal plate (Jay *et al.* 2016).

Type species

Archaeopurcellia eureka, gen. et sp. nov.

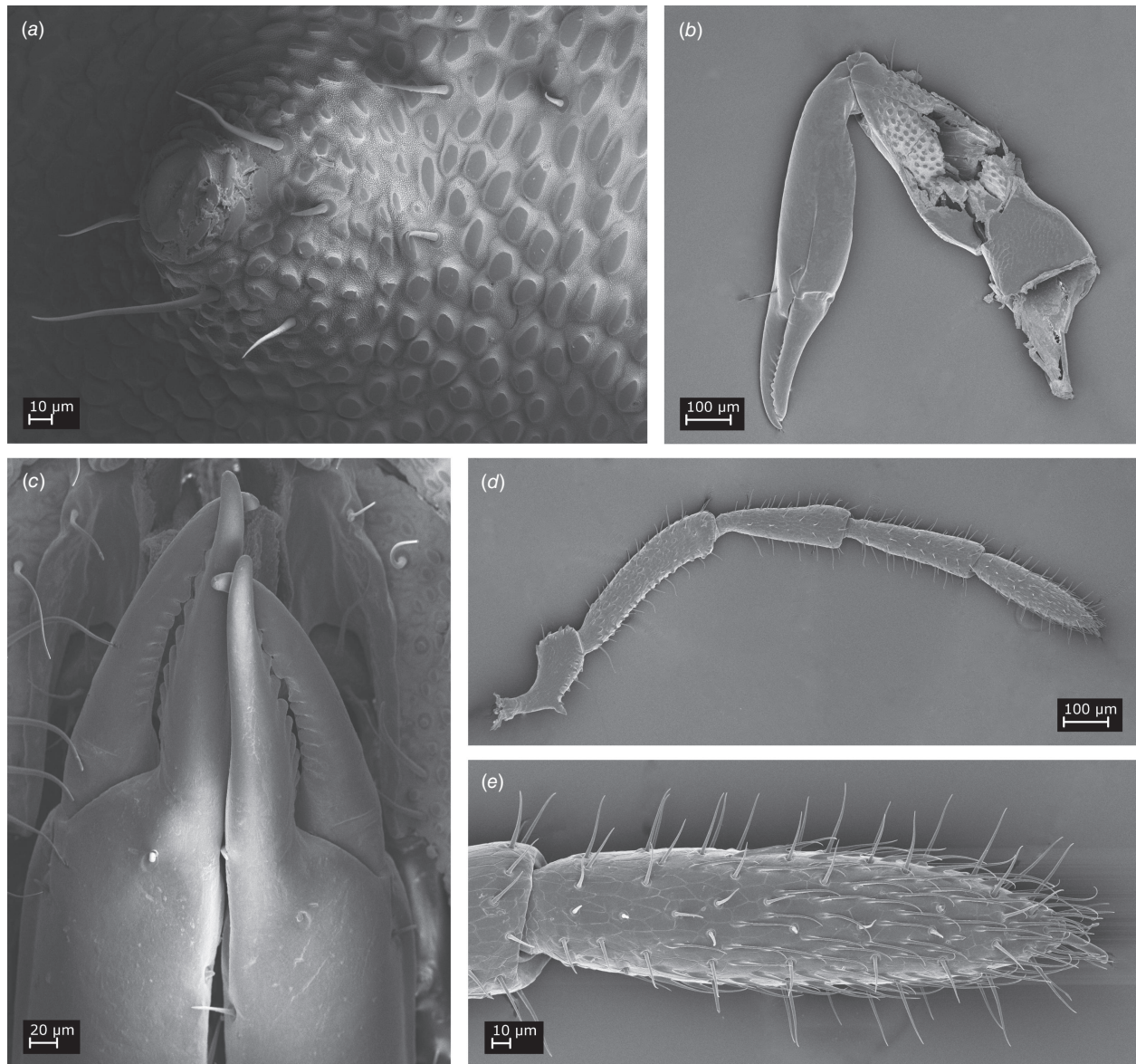


Fig. 6. *Archaeopurcellia eureka*, gen. et sp. nov.: (a) MCZ IZ-162249, male left ozophore; (b) MCZ IZ-162243, male, left chelicere, retrolateral view; (c) MCZ IZ-162243, female, detail of cheliceral teeth; (d) MCZ IZ-162243, male, left palp, prolateral view; (e) detail of palp tarsus, prolateral view.

Distribution

This monotypic genus is only known from a few adjacent sites in Cleland Conservation Park, Adelaide, South Australia.

Remarks

The finding of a pettalid in South Australia is important biogeographically, as it closes a large geographical gap between *Austropurcellia* (in coastal Queensland and northern New South Wales) and *Karripurcellia* in south-western Western Australia. This genus is unique in having a wing-shaped adenostyle.

Etymology

From Ancient Greek, ἀρχαῖος (arkhaîos), meaning ancient, in reference to this deep lineage of Australian pettalid, in combination with the genus *Purcellia*, used to name many pettalid genera.

Archaeopurcellia eureka, sp. nov.

(Fig. 1, 4–8)

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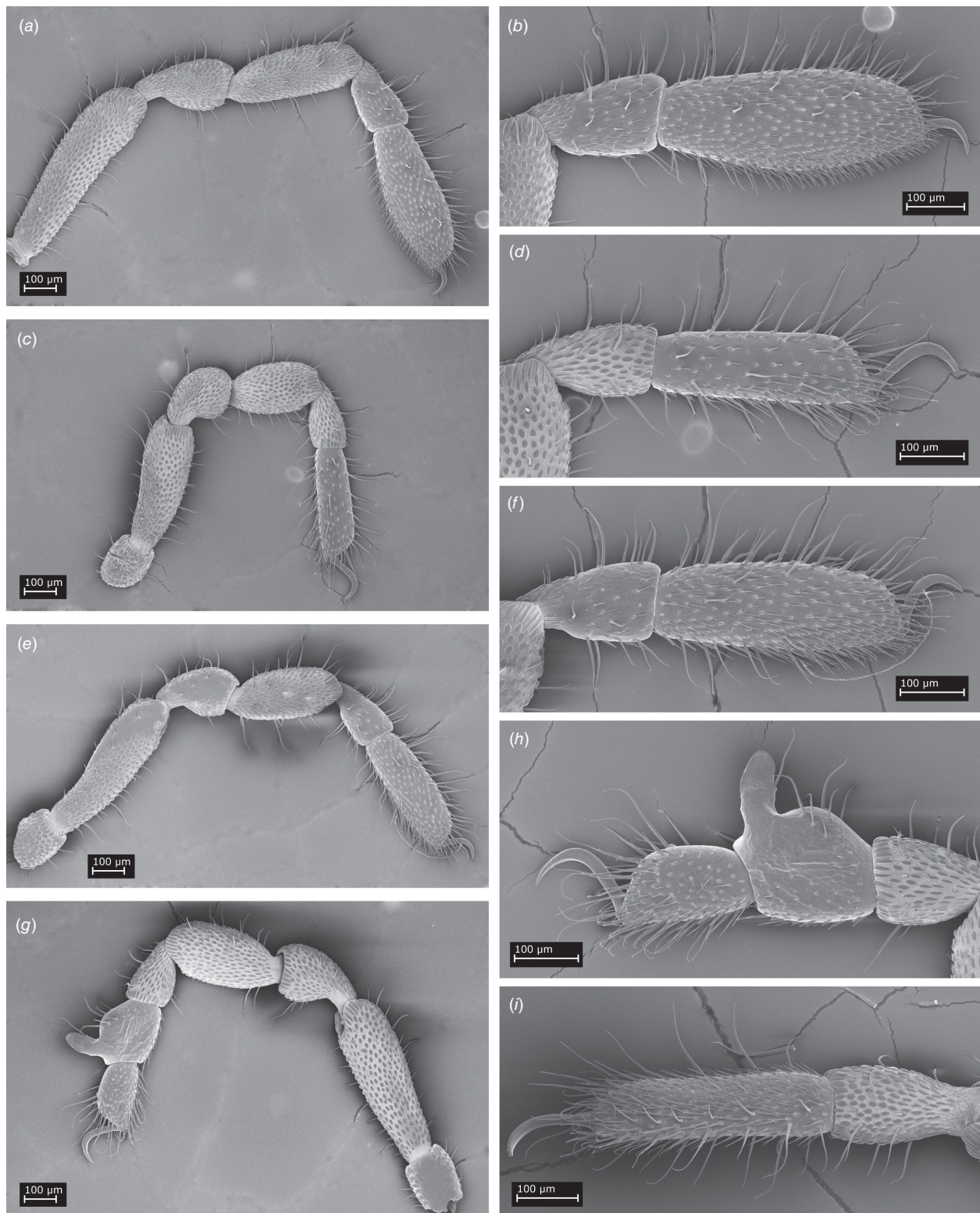


Fig. 7. *Archaeopurcellia eureka*, gen. et sp. nov. (MCZ IZ-162243): (a) male left leg I, prolateral view; (b) male metatarsus and tarsus of leg I, prolateral view; (c) male left leg II, prolateral view; (d) male metatarsus and tarsus of leg II, prolateral view; (e) male left leg III, prolateral view; (f) male metatarsus and tarsus of leg III, prolateral view; (g) male left leg IV, retrolateral view; (h) male metatarsus and tarsus of leg IV, retrolateral view; (i) female metatarsus and tarsus of leg IV, retrolateral view.



Fig. 8. Confocal imaging of spermatopositor, ventral view.

Material examined

Holotype. 1 male (SAMA OP2) from Gully below Wine Shanty Track, Chambers Gully catchment, Cleland Conservation Park, Adelaide, South Australia, 34.9665°S, 138.7061°E; 20 December 2020, Matthew Shaw leg.; next to large fallen trunk in stream gully; MSLITTER 220.

Paratypes. 1 m, 1 f (SAMA OP3, OP4), same collecting data as holotype; 6 m, 5 f, +1 specimen in RNAlater (MCZ IZ-162243) same collecting data as holotype [1 female for UCEs; 1 male imaged with Automontage and mounted for SEM; 1 female imaged with Automontage and mounted for SEM]; 1 m (MCZ IZ-162247) from Gully below Wine Shanty Track, Chambers Gully catchment, Cleland Conservation Park, Adelaide, South Australia, 34.9665°S, 138.7061°E; 20 December 2020, Matthew Shaw leg.; suspended litter on top of large fallen trunk in stream gully; MSLITTER 221. 1 f (SAMA OP5, with exerted ovipositor), 3 m, 1 f (MCZ IZ-162249) from Heptinstalls Spring, Cleland Conservation Park, Adelaide, South Australia, 34.9705°S, 138.7089°E; 16 January 2021, Matthew Shaw leg.; Soil & litter surrounding buried wet log incl. loose litter & sodden, peaty soil; shrubs and *Pteridium*, no tree canopy; MSLITTER 226 [1 male mounted for SEM] 5 m, 2 f (SAMA OP6–OP12), 2 specimens in RNAlater (MCZ IZ-162252) from Cleland Conservation Park, Bartrill Spur Track, Chambers Gully catchment, Adelaide, South Australia, 34.9658°S, 138.7031°E; 12 February 2021, Matthew Shaw leg.; Berlese funnel extract; MSLITTER 229.

Additional material. 2 m, 1 f from Cleland Conservation Park, Bartrill Spur Track, Chambers Gully catchment, Adelaide, South Australia, 34.9658°S, 138.7031°E; 12 February 2021, Matthew Shaw leg.; Berlese funnel extract; MSLITTER 229. 2 m, 2 f (SAMA OP13–OP16) Wine Shanty Tk, Cleland Conservation Park, 34.9689°S, 138.7074°E; 15 May 2021, Matthew Shaw leg.; litter under decayed logs near gully stream, stringybark canopy; MSLITTER 239. 4 f from Cleland Conservation Park, Bartrill Spur Track, Chambers Gully catchment, 34.96582°S, 138.70308°E; 7 August 2021, Matthew Shaw leg.; leaf litter at base of reed and *Gahnia* clumps, stringybark canopy; MSLITTER 250.

Diagnosis

As for genus.

Description of male

Total length of male holotype (Fig. 4a–c) 2.09 mm; width at widest point, at the prosoma, 1.11 mm; length: width ratio 1.88; width across ozophore tips 0.74 mm. Body of a uniform dark brown colour (preserved in ethanol); legs of a lighter brown. Body surface with tuberculate–microgranulate micro-structure (*sensu* Murphree 1988) across its entire surface.

Ozophores conical, between types 2 and 3 of Juberthie (1970; see also Giribet 2003) (Fig. 5a, 6a). Eyes and eye lenses entirely absent. Mid-dorsal longitudinal opisthosomal sulcus absent (Fig. 5a). Transverse opisthosomal sulci conspicuous (Fig. 5a). Posterior end of body bilobed (Fig. 5a, g), tergites VIII and IX modified.

Ventral thoracic complex (Fig. 5b) typical of pettalids, with ventral surface of prosoma occupied entirely by large coxal segments of legs. Leg I coxae directed forward, so that they embrace palp coxa. Coxae of legs I and II movable, coxae of legs III and IV fused. Ventral prosomal complex of male with coxae of legs II, III and IV meeting in the midline, coxae I not so. Sternum absent. Gonostome semicircular, width (129 μm) greater than length (96 μm) (Fig. 5b).

Spiracles forming an open circle characteristic of most pettalids (Fig. 5f), with maximum diameter 62 μm. Sternites 8 and 9 and tergite IX free, not forming a corona analis (Fig. 5d). Sternite 9 narrow; relative position of sternite 9 and tergite IX of pettalid type. Anal plate oval to fusiform in shape, entirely granulated, without conspicuous modifications, directed ventrally (Fig. 5d); 241 μm wide, 148 μm long. Opisthosomal sternites not depressed; without sternal pore openings.

Tergite IX bipartite; tergite VIII bilobed, with a scopula of trichomes and setae emerging from each inner side of the lobe; tergite VII also bilobed, not as deep (Fig. 5a, g). Anal gland opening absent.

Chelicerae (Fig. 6b, c) without a dorsal crest; with few setae. Granulation restricted to the proximal article. Proximal article 725 μm long, 253 μm deep, without a ventral process. Second article 849 μm long, 168 μm deep, widest near the centre; dentition without an alternation of small and large nodular teeth in the fixed finger (Fig. 6c). Distal article

293 mm long, 82 mm deep, three distalmost teeth larger and less uniform than proximal teeth.

Palp (Fig. 6d) with a ventral process on proximal end of trochanter; without other conspicuous modifications, and ornamentation present on trochanter and second segment. Length/width (mm) (length:width ratio in parentheses) of palpal articles from trochanter to tarsus: 250/113 (2.2); 406/101 (4.0); 306/93 (3.2); 315/85 (3.7); 305/75 (4.0); total length 1.58 mm. Palpal claw 28 mm long (Fig. 6e).

Legs robust (Fig. 7a–i); surfaces of all trochanters, femurs, patellae and tibiae thickly and uniformly granulated; metatarsi I and III only granulated near the base (Fig. 7b, f), metatarsi II and IV completely granulated (Fig. 7d, h, i). Tarsi not appreciably ornamented (Fig. 7a–i). Tarsus I with a distinct solea (Fig. 7b). Dorsum of tarsi I (Fig. 7b) and III (Fig. 7f) with conspicuous solenidia, trichomes and sensilla chaetica (Juberthie 1979; Willemart and Giribet 2010); solenidia absent from tarsi of legs II and IV. Tarsal claws smooth. For leg measurements for each article (length/maximum depth) see Table 1. Leg formula: I > IV > III > II. Male tarsus IV divided, the basalmost article with a wing-like projection bearing the adenostyle (Fig. 7g, h). Adenostyle projection 100 µm long.

Spermatopositor short, 270 µm long, typical of pettalids (Fig. 8). Microtrichal formula 4–6–6 (one penis studied). Ventral side of penis (Fig. 8) with two pairs of microtrichiae forming a tight cluster. Apical lobe semicircular with three pairs of microtrichiae, the lateral ones short. Dorsal side of penis with a group of three long microtrichia on each side, with bases arranged in a ‘V’ and not fused (only seen by transparency in Fig. 8). Gonopore complex without movable fingers.

Description of female

Total length of female paratype MCZ IZ-162243 (Fig. 4d–f) 2.41 mm; width at widest point 1.19 mm; length: width ratio 2.02; width across ozophores 0.78 mm. Ventral prosomal complex (Fig. 5c) with coxae II and III meeting in the midline; coxae I and IV not meeting in the midline; gonostome pentagonal. Opisthosomal sternites without conspicuous modifications. Anal region without modifications (Fig. 5e).

Anal plate oval in shape, entirely granulated, without conspicuous modifications, directed ventrally, with few setae

(Fig. 5e); 251 µm wide, 155 µm long. Tarsus of leg IV smooth, without modifications, densely setose (Fig. 7i). Ovipositor observed protruding outside one specimen (OP 5), with more than 32 segments, longer than 2.56 mm.

Distribution

Known only from a few sites in Cleland Conservation Park, Adelaide, South Australia (Australia).

Remarks

See remarks for genus above. The habitat of this species seems to be restricted to a few immediately adjacent small creeks fed by permanent springs.

Etymology

Used as a noun in apposition, ‘eureka’ is an interjection used to celebrate a discovery or invention. It is a transliteration of an exclamation attributed to Ancient Greek mathematician and inventor, Archimedes. The name refers to the surprising finding of a pettalid species filling a large distributional gap that has puzzled the authors for a long time.

Conclusions

In this article we report a new genus of Cyphophthalmi from South Australia, a genus that for now is monospecific, but that may contain additional species in suitable habitat from South Australia and perhaps Victoria and Tasmania. The new genus is not related (as ingroup or sister group) to the other two Australian genera, and in fact, is not closely related to any of the genera from former East Gondwana. Instead, it appears in a grade of former West Gondwanan genera, as the sister group to the South American genus *Chileogovea*, suggesting an ancestral relationship to this clade through Antarctica, as observed in some clades of triaenonychid Opiliones. This study highlights the need for additional Opiliones prospection in Australia and shows that sparse representation of simple and expected biogeographic patterns, like a division between West and East Gondwana in ancient clades like pettalids, are probably due to undersampling.

Table 1. Measurements for each leg article (µm) (length/maximum depth).

	Tr	Fe	Pa	Ti	Mt	Ta	Total L (mm)
Leg I	–	657/175	323/163	417/152	246/144	460/177	>2.10
Leg II	140/136	405/154	231/162	273/161	218/114	359/109	1.62
Leg III	155/151	525/160	266/160	336/160	218/124	394/141	1.89
Leg IV	202/155	534/177	295/179	361/185	221/139	413(198 + 215)/186	2.02

All leg measurements are in micrometres (except where specified otherwise); parentheses in Ta IV refer to each of the tarsal subsegments. Tr, trochanter; Fe, femur; Pa, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus; L, length.

Supplementary material

Supplementary material is available [online](#).

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Data availability. All genetic data have been deposited in GenBank under accession codes ON790664–ON790668 and UCE data have been deposited under SRA BioProject accession PRJNA860833. DNA sequence alignments and tree files are available in the Harvard Dataverse <https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/8QTTXU>. All Automontage, SEM and confocal images are publicly available online in MCZbase (<https://mczbase.mcz.harvard.edu/guid/MCZ:IZ:162243> and <https://mczbase.mcz.harvard.edu/guid/MCZ:IZ:162249>).

Conflicts of interest. G. Giribet is the Editor-in-Chief of *Invertebrate Systematics*. Despite this relationship, he took no part in the review and acceptance of this manuscript. The authors declare that they have no further conflicts of interest.

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