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Cryptic speciation in a biodiversity hotspot: multilocus molecular data reveal new velvet worm species from Western Australia (Onychophora : Peripatopsidae : *Kumbadjena*)

Shoyo Sato [A,F], *Rebecca S. Buckman-Young* [A,B], *Mark S. Harvey* [C,D,E] *and Gonzalo Giribet* [A]

[A]Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA.
[B]Current address: Department of Integrative Biology, University of Wisconsin–Madison, 250 N. Mills Street, Madison, WI 53706, USA.
[C]Department of Terrestrial Zoology, Western Australia Museum, Welshpool, WA 6106, Australia.
[D]School of Biological Sciences, The University of Western Australia, Crawley, WA 6009, Australia.
[E]School of Natural Sciences, Edith Cowan University, Joondalup, WA 6027, Australia.
[F]Corresponding author. Email: shoyosato@g.harvard.edu

Abstract. There is a yet uncovered multitude of species to be found among Western Australian Onychophora. *Kumbadjena*, one of the two genera that reside in this region, has been previously suggested to house an extensive species complex. Morphology alone has not been able to elucidate the diversity in this genus and has instead muddled species delineations. Topologies and species delimitation analyses resulting from the sequences of two mitochondrial ribosomal markers (12S rRNA and 16S rRNA), one nuclear ribosomal marker (18S rRNA), and one mitochondrial protein-coding gene (cytochrome *c* oxidase subunit I) are indicative of several undescribed species. Fixed diagnostic nucleotide changes in the highly conserved sequences of 18S rRNA warrant distinction of three new species of *Kumbadjena*: *K. toolbrunupensis*, sp. nov., *K. karricola*, sp. nov., and *K. extrema*, sp. nov. The geographic distributions of the proposed species suggest that *Kumbadjena* is another example of short-range endemism, a common occurrence in the flora and fauna of the region. The extensive biodiversity and endemism in the region necessitates conservation to preserve the species and processes that promote speciation harboured by Western Australia.

Additional keywords: conservation, endemism, molecular diagnosis.

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Introduction

Onychophora are soft bodied, many-legged relatives of arthropods, commonly known as velvet worms or peripatus. They are an ancient phylum that diversified before the breakup of Gondwana in the Mesozoic (Murienne *et al*. 2013), and with a terrestrial fossil record since the Carboniferous (Garwood *et al*. 2016). Onychophora are prone to desiccation and hence have an affinity towards, but are not tethered to, warm tropical climates, and often can be found living in permanently moist habitats (Oliveira *et al*. 2012; Wright 2012). The phylum encompasses two families, Peripatidae and Peripatopsidae, containing 84 and 128 species respectively (Oliveira *et al*. 2012, 2013; Oliveira and Mayer 2017; Daniels *et al*. 2013, 2016; Ruhberg and Daniels 2013) – although 20 of these are considered *nomina dubia* (Oliveira *et al*. 2012). The Australian onychophoran fauna consists exclusively of peripatopsids and is home to the highest number of species in this family for any country (75) with species being concentrated along the eastern seaboard. Research has focussed mainly on the higher number of species in this eastern Australia group while western species have been largely ignored (Briscoe and Tait 1995; Reid 1996; Reinhard and Rowell 2005; Bull and Sunnucks 2014). Indeed, only four named species have been recorded from Western Australia (Reid 2002; Oliveira *et al*. 2012) (Fig. 1*a–e*).

Due to their low vagility, restriction to moist habitats, and isolated ranges, Onychophora are a unique case of short-range endemism (Harvey 2002). Many of the taxa in which endemics are the norm are ‘Gondwanan relics’ (Hopper *et al*. 1996), of which Onychophora are a representative. The pre-Miocene Australian landscape was uniform and primarily mesic with many more moist habitats than present today (Hill 1994). The aridification of Australia in the Miocene (Byrne *et al*. 2011)

Fig. 1. Representative specimens of Western Australian peripatopsids: (*a*) *Occiperipatoides gilesii* (MCZ IZ-141469), John Forrest NP, photo M. S. Harvey; (*b*) *Kumbadjena shannonensis* (MCZ IZ-131438), Bibbulmun Track, photo G. Giribet; (*c*) *Kumbadjena karricola*, sp. nov. (paratype MCZ IZ-131432), Beedelup NP, photo G. Giribet; (*d*, *e*) *Kumbadjena toolbrunupensis*, sp. nov. (paratype MCZ IZ-131433), Stirling Range NP, photo G. Giribet.

resulted in the contraction of favourable habitat for certain taxa like Onychophora, replacing it in many locales with xeric communities. The last remnants of this bygone era can be found on the east coast and the south-west of Australia. The fragmented populations found in the refugia created by aridification can spawn and hold isolated species (Hopper *et al*. 1996). The explosion of speciation in the south-west is hypothesised to have been caused by population subdivision due to the climatic stress of aridification (Hopper 1979). Harvey (2002) notes that these habitats are predisposed to the proliferation of endemics.

Only six species of Onychophora were described from Australia before 1985 (Tait *et al*. 1990). An investigation into the diversity of Australian onychophoran fauna was mobilised when the monotypic genus *Cephalofovea* Ruhberg 1988, was split into three species by Reid *et al*. (1995) on the basis of electron microscopy examination of morphology, cytogenetics and allozyme data. Subsequently, Briscoe and Tait (1995) conducted a 21-allozyme-locus study of Australian peripatopsids, exposing a previously unknown medley of Australian Onychophora. Much work was needed to disentangle this hidden diversity. A review of Australian Onychophora by Reid (1996) described 22 new genera and 41 new species, again predominantly from eastern Australia. In a note in that *tour de force* study, Reid suggested that *Occiperipatoides occidentalis* Fletcher, 1895 from Western Australia could be split into five putative species, suggesting an extensive species complex. Reid (2002) formalised this by transferring *O. occidentalis* to a newly named genus, *Kumbadjena* Reid, 2002, with *K. occidentalis* (Fletcher 1895) as its type species. An additional two species, *K. shannonensis* Reid, 2002 and *K. kaata* Reid, 2002 were ascribed to the genus

following extensive taxon sampling and scanning electron microscopy. However, the attempt to draw definitive lines between the two additional, putative species expounded in 1996 did not come to fruition. Instead, the addition of *Kumbadjena* specimens from across its range only served to obscure species delineations and many individuals could not be assigned to any of the three described species. Since then, morphology alone has not been able to elucidate the hidden diversity of *Kumbadjena.* Due to the highly conserved morphology of this genus, the intraspecific morphological variation is comparable to the variation among interspecific individuals. After conducting preliminary scanning electron microscopy in addition to the extensive microscopic work done by Reid (2002), the analysis of key morphological traits typically used to distinguish Onychophora species failed to establish unambiguous diagnostic characters between all potential species. Therefore, here we turn to molecular data to resolve the species diversity of this clade and present the first molecular investigation into south-western Australian Onychophora with 26 individuals of the genus *Kumbadjena* examined for four genetic loci.

Materials and methods

Molecular methods

Sequences of two mitochondrial ribosomal markers (12S rRNA and 16S rRNA), one nuclear ribosomal marker (18S rRNA), and one mitochondrial protein-coding gene (cytochrome *c* oxidase subunit I, hereafter COI) were obtained for 10 *Kumbadjena* individuals along with three species each of *Euperipatoides* and *Ooperipatus*, and two individuals of *Occiperipatoides* from Murienne *et al*. (2013). Five new *Kumbadjena* individuals were collected from Western Australia for this study and an additional 16 specimens were obtained from the Western Australia Museum and sequenced (Table 1, Fig. 2). The paucity of specimens included in this molecular analysis is a reflection of the natural history of the members of this phylum. Collecting fresh individuals of *Kumbadjena* was particularly difficult due to their small body size even among Onychophora, low population densities, and cryptic habits. The individuals sampled in this study represent all molecular-quality specimens currently in museum collections and are the result of several trips by the authors in addition to older specimens deposited in the Western Australia Museum. The additional specimens included in Fig. 4 are historical specimens or specimens not collected via methods amenable for standard molecular work. A purely molecular species description was preferred in light of this poverty of sampling.

DNA was extracted from a single oncopod using the DNEasy tissue kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocol. 12S rRNA was amplified and sequenced using onychophoran specific primers developed for this study. 16S rRNA was amplified and sequenced with the universal primer pair 16Sa–16Sb. COI was amplified and sequenced in two fragments using the primer pairs LCO1490–HCOoutout and ExtA–ExtB. Previous studies have noted the difficulty in amplifying and sequencing 18S rRNA due to enlarged variable regions V2, V7, and V9 in Onychophora (Giribet and Wheeler 2001; Murienne *et al*. 2013). Consequently, sequencing of 18S rRNA was only partially successful and resulted in an incomplete data matrix. It was amplified and sequenced in three fragments using the primer pairs 18S1F–18S4R, 18S3F–18Sbi, and 18Sa2.0–18S9R (see Table 2 for list of primers, sequences, and references). PCR amplifications were completed in 25-μL reactions with Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Chicago, IL, USA) and cleaned with an incubation of 1 μL ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). Sequencing reactions were performed in 10-μL reactions using BigDye ver. 1 chain-termination chemistry on an ABI3730xl (Applied Biosystems Inc., Carlsbad, CA, USA). Raw sequences were assembled into contigs in Geneious 11.0.3 (https://www.geneious.com: Kearse *et al*. 2012). All new sequences have been submitted to GenBank (see Table 1 for accession numbers).

Phylogeny

All sequences were individually aligned using MAFFT implemented in Geneious (https://www.geneious.com: Kearse *et al*. 2012). The G-INS-i algorithm was utilised in the alignments of 12S rRNA, 16S rRNA, and COI. E-INS-i was used for the 18S rRNA alignment (Katoh and Standley 2013) to account for extensive gaps and missing data in this gene. Nucleotide evolution models were determined using jModelTest (Posada 2008) and implemented in both maximum-likelihood and Bayesian methods for tree reconstruction. Trees were constructed using RAxML-NG 0.5.1b (Kozlov 2018), BEAST 2.4.7 (Bouckaert *et al*. 2014), and MrBayes (Ronquist *et al*. 2012). Four single-gene topologies were generated using all three methods to test congruence among gene trees. Maximum-likelihood trees were obtained from 1000 random starting trees and bootstrap support values were determined with 1000 replicate trees. MrBayes was run with 1 million generations and a sampling frequency of every 1000 trees. Convergence of the chains was confirmed via Tracer 1.7 (Rambaut *et al*. 2013) and a burn-in of 50% was used for a total of 1000 trees. Four BEAST runs, each with 1 million generations, were implemented, sampling every 1000 trees. Convergence was confirmed in Tracer 1.5 and trees from all four runs were combined in LogCombiner 2.4.7 (Bouckaert *et al*. 2014) with a burn-in of 50% for a total of 20 000 trees for later analysis in bGMYC. Final trees were combined in TreeAnnotator 2.4.7 (Bouckaert *et al*. 2014) to obtain a single ultrametric tree for use in GMYC (Reid and Carstens 2012). Sequences were concatenated in SequenceMatrix 1.8 (Vaidya *et al*. 2011) to produce maximum-likelihood and Bayesian trees to which to compare the four single-gene phylogenies. The same parameters as the gene trees were used in RAxML, MrBayes, and BEAST to create species trees from the concatenated dataset partitioned by gene.

Species delimitation

Three species-delimitation methods were employed across all four genes. A Bayesian implementation of the Poisson tree process (bPTP) model (Zhang *et al*. 2013) was run on an online server (http://species.h-its.org/ptp/) with 500 000 generations on a rooted tree with specified outgroups. Convergence of the chains was confirmed via the online server. A General Mixed Yule

Table 1. Voucher numbers, locality data, and GenBank accession numbers for loci of samples included in analysis for specimens used in this study

Missing GPS coordinates represent missing records for vouchers. New sequences for this paper are shown in bold. We were unable to obtain a sequence of 18S rRNA in *Euperipatoides leuckartii*. NP, National Park; SF, State Forest; WAM, Western Australian Museum; MCZ, Museum of Comparative Zoology, Harvard University

Taxon	Voucher no.	Locality	Latitude	Longitude	GenBank accession nos			
					12S	16S	18S	COI
Kumbadjena karricola, **sp. nov.**	MCZ-IZ131432	Beedelup NP, WA	–34.34187	115.86191	**MH040614**	**MH040637**	**MH040666**	**MH040676**
Kumbadjena karricola, **sp. nov.**	WAM-T111965	Treen Brook SF, WA	–34.44583	115.98333	**MH040609**	**MH040632**	**MH040661**	**MH040677**
Kumbadjena karricola, **sp. nov.**	WAM-T111967	Treen Brook SF, WA	–34.44583	115.98333	**MH040607**	**MH040630**	**MH040658**	**MH040678**
Kumbadjena karricola, **sp. nov.**	WAM-T138513	Beedelup NP, WA	–34.43611	115.88833	**MH040598**	**MH040621**	**MH040644**	**MH040679**
Kumbadjena karricola, **sp. nov.**	WAM-T142371	Beedelup NP, WA	–34.43611	115.88830	**MH040596**	**MH040619**	**MH040640, MH040641**	**MH040680**
Kumbadjena karricola, **sp. nov.**	MCZ-IZ131367[A]	Bicentennial Tree, Warren NP, WA	–34.49500	115.97527	KC754485	KC754536	KC754580	KC754654
Kumbadjena kaata	WAM-T113491	Porongurup NP, WA	–34.68194	117.84861	**MH040606**	**MH040629**	**MH040656, MH040657**	**MH040681**
Kumbadjena kaata	WAM-T129595	Porongurup NP, WA	–34.68194	117.84861	**MH040604**	**MH040627**	**MH040654**	**MH040682**
Kumbadjena kaata	WAM-T142370	Porongurup NP, WA	–34.68195	117.84860	**MH040597**	**MH040620**	**MH040642, MH040643**	**MH040683**
Kumbadjena extrema, **sp. nov.**	MCZ-IZ131400[A]	Limeburners Rd, WA	–35.09056	117.91083	KC754487	KC754538	MH040667	KC754656
Kumbadjena extrema, **sp. nov.**	WAM-T111963	Mt Shadforth, WA	–34.96750	117.26500	**MH040611**	**MH040634**	**MH040660**	**MH040684**
Kumbadjena extrema, **sp. nov.**	WAM-T132593	Limeburners Rd, near Torndirrup NP	–34.09083	117.91111	**MH040603**	**MH040626**	**MH040652, MH040653**	**MH040685**
Kumbadjena extrema, **sp. nov.**	WAM-T132795	Gilge Rd, NW of Cape Howe NP, WA	–34.05416	117.48027	**MH040602**	**MH040625**	**MH040650, MH040651**	**MH040686**
Kumbadjena extrema, **sp. nov.**	WAM-T132796	Gilge Rd, NW of Cape Howe NP, WA	–34.05416	117.48027	**MH040601**	**MH040624**	**MH040648, MH040649**	**MH040687**
Kumbadjena extrema, **sp. nov.**	WAM-T133783	Limeburners Rd, near Torndirrup NP	–35.09083	117.91110	**MH040599**	**MH040622**	**MH040645**	**MH040688**
Kumbadjena occidentalis	MCZ-IZ131369[A]	Bridgetown Jarrah Park, Bridgetown, WA	–34.02928	115.98266	KC754484	KC754535	KC754579	KC754653
Kumbadjena occidentalis	MCZ-IZ131365	Leeuwin–Naturaliste NP, WA	–	–	**MH040618**	**MH040639**	**MH040674**	**MH040689**
Kumbadjena occidentalis	MCZ-IZ131368[A]	Boranup Drive, Karri Forest, WA	–34.16444	115.07623	KC754486	KC754537	KC754581	KC754655
Kumbadjena occidentalis.	MCZ-IZ131398	South of Gracetown, WA	–33.90889	115.00667	**MH040615**	**MH040638**	**MH040668**	**MH040690**
Kumbadjena shannonensis	MCZ-IZ131359[A]	Shannon NP, WA	–34.59558	116.40918	KC754488	KC754539	KC754582	KC754657
Kumbadjena shannonensis	MCZ-IZ131438	Bibbulmun track at Shannon River, WA	–34.76407	116.37721	**MH040612**	**MH040635**	**MH040664**	**MH040691**
Kumbadjena shannonensis	WAM-T111964	Shannon NP, WA	–34.53555	116.34638	**MH040610**	**MH040633**	**MH040662**	**MH040692**
Kumbadjena shannonensis	WAM-T111966	Treen Brook SF, WA	–34.44583	115.98333	**MH040608**	**MH040631**	**MH040659**	**MH040693**
Kumbadjena sp.	WAM-T133782	Pyongurup Peak, Stirling Range NP, WA	–34.36500	118.32890	**MH040600**	**MH040623**	**MH040646, MH040647**	**MH040694**
Kumbadjena toolbrunupensis, **sp. nov.**	MCZ-IZ131433	Toolbrunup Peak, Stirling Range NP, WA	–34.39038	118.05500	**MH040613**	**MH040636**	**MH040665**	**MH040695**
Kumbadjena toolbrunupensis, **sp. nov.**	WAM-T113492	Toolbrunup Peak, Stirling Range NP, WA	–34.39027	118.05500	**MH040605**	**MH040628**	**MH040655**	**MH040696**
Occiperipatoides gilesii	MCZ-IZ131370[A]	WA	–31.91206	116.1965	KC754660	KC754542	KC754491	KC754583
Occiperipatoides gilesii	MCZ-IZ131396[A]	Mt Cook, WA	–32.4245	116.30878	MH040616	KC754543	MH040669	KC754661
Ooperipatus birrgus	MCZ-IZ131375[A]	Southeast Forest NP, NSW	–37.01667	149.38333	KC754501	KC754553	MH040673	KC754671
Ooperipatus caesius	MCZ-IZ131379[A]	Mount Buffalo NP, Vic.	–36.71667	146.8333	KC754501	KC754554	MH040671	KC754672
Ooperipatus porcatus	MCZ-IZ131378[A]	Mt Useful, Vic.	–37.71667	146.5333x	KC754502	KC754554	MH040672	KC754673
Euperipatoides kanangrensis	MCZ-IZ131395[A]	Kanangra Boyd NP, NSW	–33.98333	150.13333	MH040617	KC754530	MH040670	KC754648
Euperipatoides leuckartii	MCZ-IZ131377[A]	Mt Tomah, NSW	–33.55000	150.41667	KC754481	KC754531	–	KC754649
Euperipatoides rowelli	MCZ-IZ131334[A]	Tallanga SF, NSW	–35.43330	149.55000	KC754482	KC754532	MH040675	KC754650

[A]Specimens from Murienne *et al.* (2013).

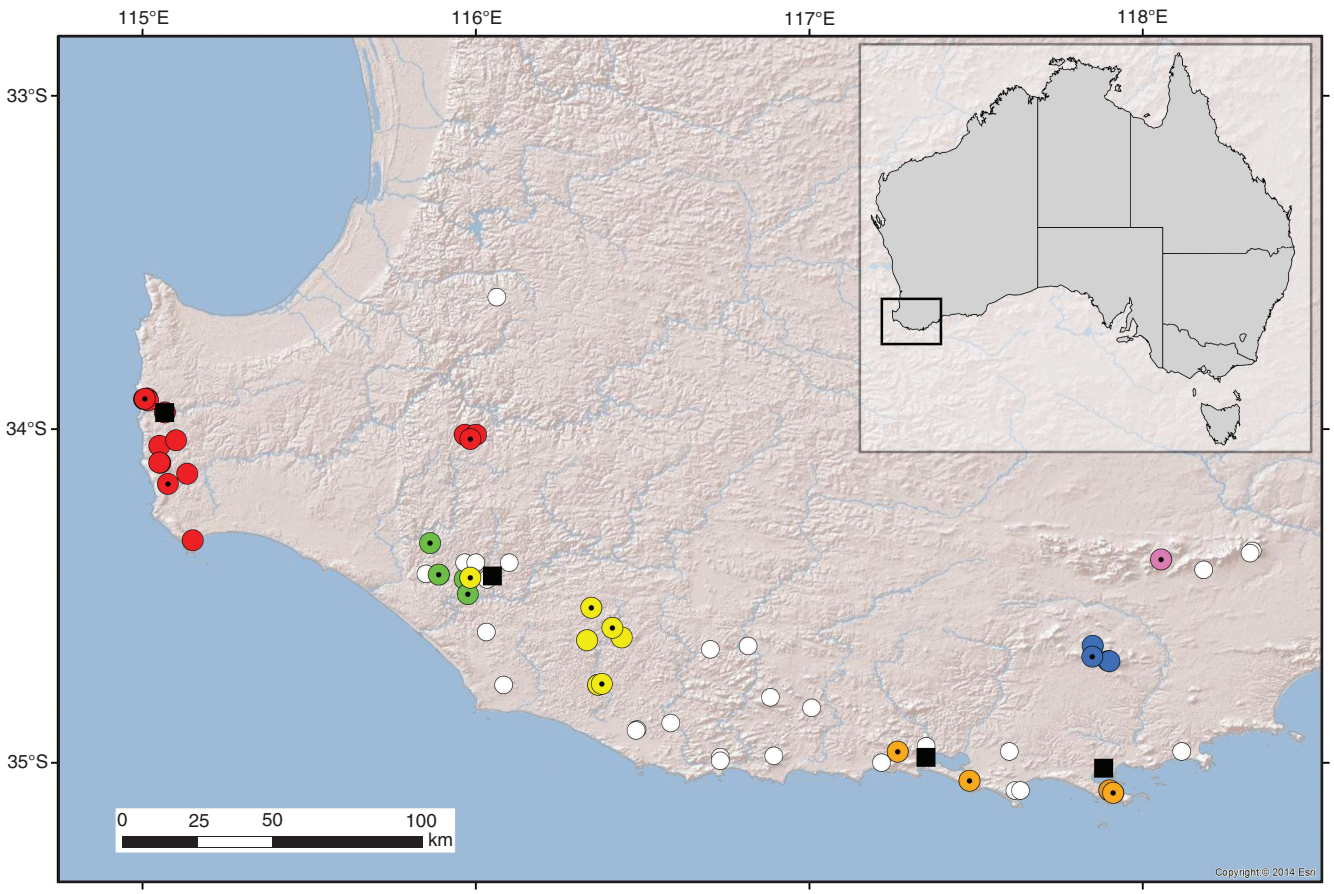

Fig. 2. Locations of all *Kumbadjena* specimens in the Western Australia Museum and Museum of Comparative Zoology collections. Colours correspond with proposed species. Median dots correspond with specimens sequenced for analysis in this study. White circles represent unidentified specimens in the WAM collections. Black squares denote major landmarks (from west to east: Margaret River, Pemberton, Denmark and Albany). Inset: map of Australia showing location of study area.

Table 2. List of primers used in this study

Primers	Primer sequence	Reference
12Sbi_Ony[A]	5′–RAT GAC GGG CGA TAT GTA–3′	This study
12S_OnyA[A]	5′–CAG CAG YWG CGG TTA TAC G–3′	This study
16Sa	5′–CGC CTG TTT ATC AAA AAC AT–3′	Xiong and Kocher (1991)
16Sb	5′–CTC CGG TTT GAA CTC AGA TCA–3′	Edgecombe *et al.* (2002)
HCOoo	5′–GTA AAT ATA TGR TGD GCT C–3′	Schwendinger and Giribet (2005)
LCO1490	5′–GGT CAA CAA ATC ATA AAG ATA TTG G–3′	Folmer *et al.* (1994)
ExtA	5′–GAA GTT TAT ATT TTA ATT TTA CCT GG–3′	Schulmeister *et al.* (2002)
ExtB	5′–CCT ATT GAW ARA ACA TAR TGA AAA TG–3′	Schulmeister *et al.* (2002)
18S1F	5′–TAC CTG GTT GAT CCT GCC GTA G–3′	Giribet *et al.* (1996)
18S4R	5′–GAA TTA CCG CGG CTG CTG G–3′	Giribet *et al.* (1996)
18S3F	5′–GTT CGA TTC CGG AGA GGG A–3′	Giribet *et al.* (1996)
18Sbi	5′–GAG TCT CGT TCG TTA TCG GA–3′	Whiting *et al.* (1997)
18Sa2.0	5′–ATG GTT GCA AAG CTG AAA C–3′	Whiting *et al.* (1997)
18S9R	5′–GAT CCT TCC GCA GGT TCA CCT AC–3′	Giribet *et al.* (1996)

[A]Onychophora-specific primers developed for this study.

Coalescent (GMYC) model (Fujisawa and Barraclough 2013) was implemented on a single ultrametric tree obtained from BEAST via an online server of the Exelixis Laboratory (http://species.h-its.org/gmyc/) with a single threshold model. A Bayesian implementation of GMYC (bGMYC) (Reid and Carstens 2012) was executed in R on the 20 000 trees obtained from BEAST with a Markov Chain Monte Carlo run of 5000, burn-in of 4000 and thinning of 100 for each tree. Convergence

was confirmed in R using the built-in convergence function in the bGMYC package.

Results and discussion

Phylogeny

The nucleotide evolution models determined by jModelTest (Posada 2008) and implemented in all phylogenetic analyses were TIM2+G, TVM+I+G, TPMuf+I, and TVM+I +G for 12S rRNA, 16S rRNA, 18S rRNA, and COI respectively. Maximum likelihood (–ln*L* = 15779.315736) and both Bayesian analyses produced the same topology for the concatenated dataset (Fig. 3). The Western Australia clade formed a well supported monophyletic group in all concatenated analyses (bootstrap support (BS) = 100%, posterior probability (PP) = 1). *Kumbadjena* was also recovered as monophyletic with maximum support (BS = 100%, PP = 1) with *Occiperipatoides* as its sister genus within the Western Australia clade. All analyses of the concatenated dataset supported seven distinct subclades (*Kumbadjena* sp., *K. toolbrunupensis*, sp. nov., *K. extrema*, sp. nov., *K. shannonensis*, *K. kaata*, *K. occidentalis*, *K. karricola*, sp. nov.), each with a bootstrap support of 100% and a PP of 1. All individual gene trees were congruent with the concatenated topologies regarding the validity of the seven major subclades (Fig. 3), with the exception of 18S rRNA, which does not contain enough signal to resolve the species tree.

Relationships within the major subclades were variable and poorly resolved. One difference was found in the COI ML (maximum likelihood) topology where *Kumbadjena* sp. was recovered as sister group to the eastern Australia clade. However, this node has low support (BS = 52%). This individual is recovered as the sister group to all other *Kumbadjena* in all other analyses with high support. The branch lengths of the topologies obtained in the two Bayesian analyses of the COI dataset corresponded to those of the ML topology, thus making incomplete lineage sorting unlikely. The incongruence found in this analysis of COI is more likely an artefact of the analysis. There were several inconsistencies between gene trees obtained from 16S rRNA with regard to the relationships between the major subclades. In the BEAST and RAxML topologies, *K. shannonensis* and *K. kaata* formed a distinct clade while in other analyses, *K. kaata* was sister group to a clade formed by *K. occidentalis* and *K. karricola*, sp. nov. There was no resolution in the MrBayes topology regarding the relationships between the *K. toolbrunupensis*, sp. nov./*Kumbadjena* sp., *K. occidentalis*/*K. extrema*, sp. nov., *K. kaata*, and *K. shannonensis* clades. Furthermore, the MrBayes topology obtained from COI sequences recovered a completely pectinate relationship between all major subclades whereas *K. toolbrunupensis*, sp. nov. and *Kumbadjena* sp. form a distinct clade in all other analyses. The slight discrepancies across gene trees could be the result of the lack of sufficient information in the chosen genes to resolve the same topologies. In addition, this could be confounded by incomplete lineage sorting, which is exceedingly troublesome in phylogenies of recent divergence times constructed with a single locus and small sample size (Maddison and Knowles 2006). This issue could possibly be remedied by sampling more individuals, but specimens of *Kumbadjena* are rare and difficult to obtain.

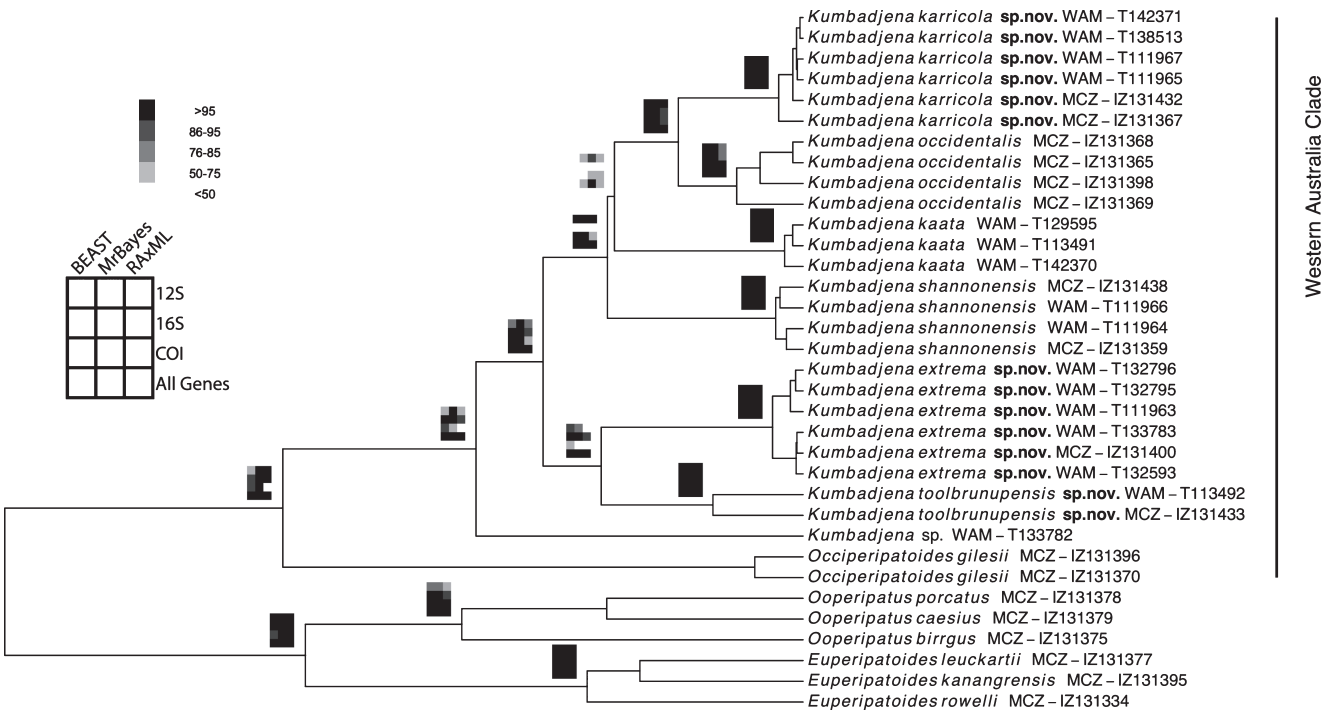

Fig. 3. Summary of sensitivity analysis of important nodes from two Bayesian methods (BEAST, MrBayes) and one maximum-likelihood methodology (RAxML) across three genes (12S rRNA, 16S rRNA, COI) and a concatenated dataset including all four genes (12S rRNA, 16S rRNA, 18S rRNA, COI) overlaid on top of a single tree obtained from BEAST using a concatenated dataset.

The slowly evolving nuclear gene 18S rRNA had little power to resolve the shallower nodes in the tree. In Onychophora, this gene contains conserved blocks and hypervariable regions and hence is often reserved for tackling deeper phylogenetic questions (e.g. Wheeler *et al*. 1993; Abouheif *et al*. 1998; Giribet and Wheeler 2001; Aris-Brosou and Yang 2002; Kjer 2004). The 18S rRNA analyses produced very little resolution of the relationships among the *Kumbadjena* species. Both the RAxML and MrBayes trees were poorly resolved, with many polytomies. The BEAST tree did not contain polytomies but all nodes had low support and differed drastically from all other topologies. Results of the 18S rRNA analyses alone are not shown.

Species delimitation

Species-delimitation analyses were highly congruent across genes and methodologies (Fig. 4). Analyses on 18S rRNA predicted one species of *Kumbadjena* (bPTP and GMYC) or separated all individuals (bGMYC) and was subsequently not represented in the final figures due to its poor resolution at this level. Closely related species are expected to have not more than one to a few fixed changes in nucleotide sequence for the nuclear rRNAs (e.g. Edgecombe and Giribet 2008; Vélez *et al*. 2012) and the phylogenetic signal from these diagnostic characteristics was outweighed by non-synapomorphic nucleotide changes in the *Kumbadjena* sequences, thus providing little power to resolve topology. The topology obtained from 18S rRNA is what is expected for such closely related taxa as addressed in this study.

All eastern Australian outgroup species were identified as valid in all analyses. The two *Occiperipatoides* individuals constituted one species in the bPTP analysis of 12S rRNA and GMYC for all genes. The two individuals were split into two species in all other analyses, but sampling was not targeted to resolve this species. *Kumbadjena* sp. was a singleton and sister group to all other *Kumbadjena* spp. in all but one analysis. The three-specimen *K. kaata*, containing WAM T129595, WAM T113491 and WAM T142370, were also recovered as a species in all analyses. The two-specimen *K. toolbrunupensis*, sp. nov., consisting of WAM T113492 and MCZ IZ-131433, were supported in all but two analyses. The two specimens were split in the bPTP and bGMYC delimitations of COI. A six-specimen group (*K. extrema*, sp. nov.) was recovered as one species in most analyses. The GMYC analysis of 12S rRNA and bGMYC for both 16S rRNA and COI split the two clusters of three into separate species. The four specimens of the *K. shannonensis* group were supported as a species in all but two analyses. Specimen MCZ IZ-131438 was sister species to the other three in the group in the bGMYC analysis of 16S rRNA. Additionally, bGMYC of COI split *K. shannonensis* into two species with two specimens each. Another six-specimen group (*K. karricola*, sp. nov.) was recovered in most analyses. bGMYC of 16S rRNA and COI split this species into four and two species respectively. Major incongruence across methods occurs in the four-specimen *K. occidentalis*. bPTP and GMYC analyses of 16S rRNA recovered the clade as one species while *K. occidentalis* MCZ IZ-131369 was found to be sister species to the other three

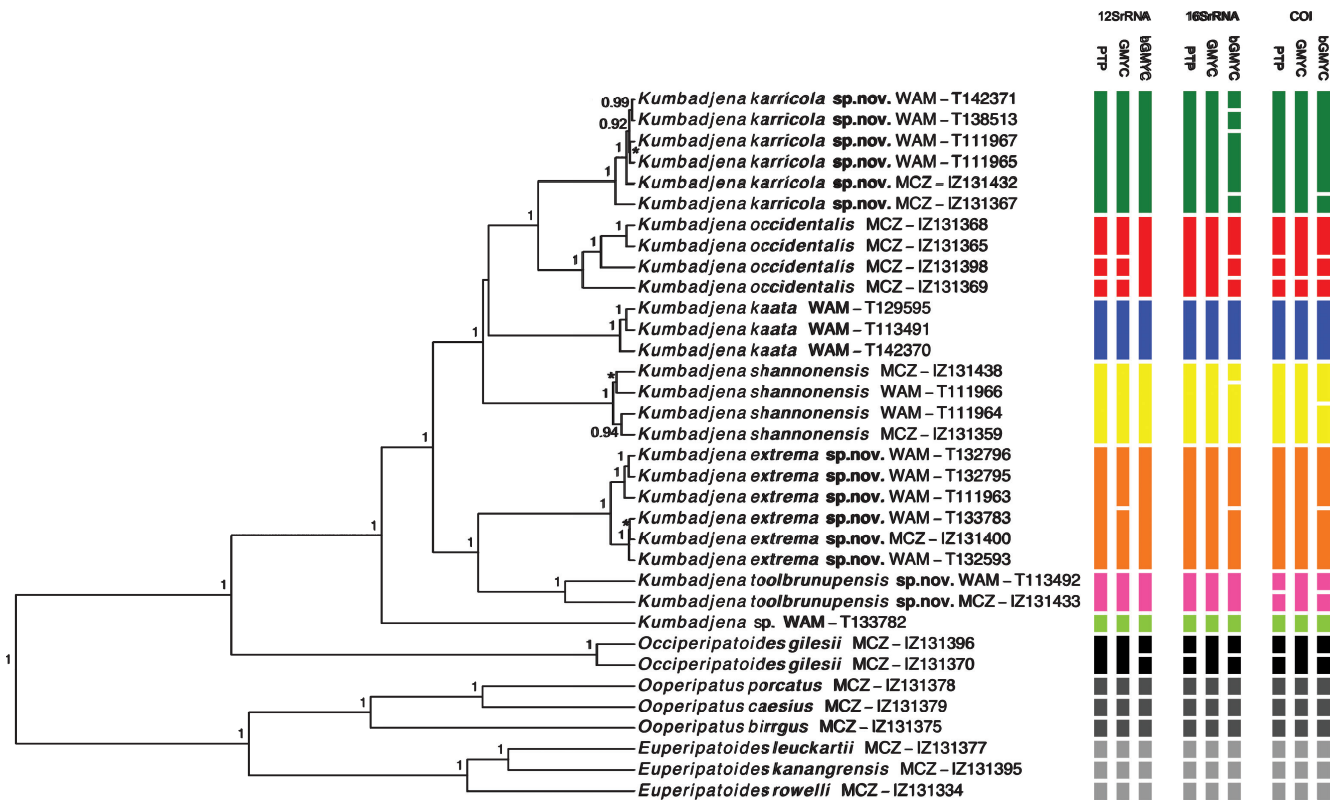

Fig. 4. Summary of species delimitation analyses (PTP, GMYC, bGMYC) across three genes (12S rRNA, 16S rRNA, COI) overlaid on top of a single tree obtained from BEAST using a concatenated dataset. Colours correspond to proposed species and geographic location on map (Fig. 2).

individuals in the GMYC analysis of COI. Furthermore, GMYC and bPTP of 12S rRNA, bGMYC of 16S rRNA and both bPTP and bGMYC of COI split *K. occidentalis* MCZ IZ-131369 and *K. occidentalis* MCZ IZ-131398 into two species separate from the rest of the *K. occidentalis* clade.

Taking the most conservative estimates across genes and analytical methods, this study supports at least seven tentative species within *Kumbadjena*, clearly delimiting the three currently described species along with an additional four corresponding to the seven distinct subclades. Corroborating Reid's (1996, 2002) proposal of an extensive species complex, this study demonstrates genetic structure indicative of several undescribed species within *Kumbadjena*. Substantiating the proposed 'hotspot' of south-western Australia (Beard *et al*. 2000; Myers *et al*. 2000; Rix *et al*. 2015), there is hidden diversity in the onychophoran fauna of the region that demands a more thorough investigation. The biogeographic distributions of the proposed species are indicative of Onychophora being an exemplar taxon of short-range endemism and of the landscape in south-western Australia harbouring many endemics (Harvey 2002; Rix *et al*. 2015).

Owing to the notoriously conserved morphologies and paucity of fixed species-specific diagnostic characters in Onychophora, many species have been erected using molecular methods. For example, Trewick (1998) used allozyme data to describe four new species of *Peripatoides* that constitute a species complex. Trewick (1999, 2000) subsequently suggested the existence of, but not did not describe, several additional species using mitochondrial gene data. Molecular data have also guided the description of numerous cryptic species in the *Peripatopsis capensis* species group (McDonald *et al*. 2012), the *P. balfouri* species complex (Daniels *et al*. 2013) and the *Opisthopatus cinctipes* species complex (Daniels *et al*. 2016). Given the conserved anatomy of *Kumbadjena*, there are compelling reasons that justify using molecular markers to diagnose species (Cook *et al*. 2010), and thus we rely on the highly conserved 18S rRNA marker to identify fixed diagnostic molecular characters that allow us to recognise three of the supposed four new taxa. Similar fixed diagnostic molecular characteristics were not found in 12S rRNA, 16S rRNA, or COI and thus these genes were not considered for the final species descriptions. Here, we provide descriptions of three new species (*K. karricola*, sp. nov., *K. extrema*, sp. nov., *K. toolbrunupensis*, sp. nov.) using diagnostic molecular characters, and append molecular diagnostic characters to the original species descriptions of *K. kaata*, and *K. shannonensis* from Reid (2002). *Peripatopsis hamerae* Ruhberg & Daniels, 2013 was described solely on molecular sequences from one individual. However, choosing to stay consistent in methodology, we opted for a more stringent approach and preferred not to describe a species based on a singleton (*Kumbadjena* sp. WAM T133782), as it is not possible to assess what characters are fixed at the species level. We favour conserved markers that are fixed at the species level (Giribet *et al*. 2009; Vélez *et al*. 2012) over the use of genetic distances or highly variable mitochondrial markers. In our case, our fixed molecular species apomorphies are congruent with the species-delimitation techniques. Sequences were aligned to a complete reference sequence of 18S rRNA (GenBank accession no. MH040663) obtained using Illumina sequencing (Illumina, San Diego) to determine position of fixed molecular diagnostic characters.

Biogeography

The geographic ranges of each of the putative species are clearly distinguished (Fig. 2). *Kumbadjena* sp. (light green in Fig. 2), the sister group to all other species, resides in the easternmost part of the genus' range along with another basal lineage, *K. toolbrunupensis*, sp. nov. (pink). Both can be found within Stirling Range National Park. The distributions of these lineages, sister group to the other clades, suggest an eastern origin of the genus with subsequent diversification towards the western parts of the range in a stepping-stone fashion. There is overlap between *K. karricola*, sp. nov. (green) and *K. shannonensis* (yellow) though these two species are not each other's sister groups. *K. occidentalis* (red) was restricted to Bridgetown, Western Australia (Reid 2002), but addition of individuals from Leeuwin–Naturaliste National Park and other westernmost populations greatly expands the original species range. *K. kaata* (blue) is restricted to Porongurup National Park. *K. extrema*, sp. nov. (orange) resides on the southern coast of Western Australia and is congruent biogeographically with the south-easternmost extent of the high-rainfall region proposed by Hopper and Gioia (2004).

The Stirling Range has long been regarded as a biodiversity hotspot, and recent studies on various short-range endemic invertebrate taxa have demonstrated that speciation on separate upland terrains is a common occurrence. The most frequent pattern includes separate species on Toolbrunup Peak, Talyuberlup Peak/Mt Magog, and the eastern massif (including Bluff Knoll, Ellen Peak, Pyungurup Peak, etc.), although slight variations are known. This pattern is evident for the millipede genus *Atelomastix* (Edward and Harvey 2010), assassin spiders of the genus *Zephyrarchaea* (Rix and Harvey 2012), the migid trapdoor spider genus *Bertmainius* (Harvey *et al*. 2015), and a spiny trapdoor spider *Cataxia* (Rix *et al*. 2017). In those studies where molecular data are available, the Stirling Range taxa are either each other's closest relatives derived from a single common ancestor (*Bertmainius* and *Zephyrarchaea*: Rix and Harvey 2012; Harvey *et al*. 2015), or have clades that are sister to taxa outside of the Stirling Range (*Cataxia*: Rix *et al*. 2017). Specimens of *Kumbadjena* have been collected from only three localities in the Stirling Range, despite many years of dedicated short range endemism research: the base of Toolbrunup Peak, the base of Pyongurup Peak, and Wedge Hill (Fig. 4). The Wedge Hill specimens (WAM T38461, T130941) were collected in the 1990s and are unsuitable for molecular studies. Sequence data from the other two populations (Fig. 4) demonstrate that *Kumbadjena* sp. WAM-T133782 from Pyongurup is the sister group to all other species of *Kumbadjena*, and *K. toolbrunupensis*, sp. nov. from Toolbrunup is sister to *K. extrema*, sp. nov. from the Albany region of the south coast. In this regard, the *K. toolbrunupensis*, sp. nov–*K. extrema*, sp. nov. distribution pattern is similar to that of the trapdoor spider sister taxa *Cataxia colesi* from Toolbrunup and *C. bolganupensis* from the Porongurups.

The most widespread *Kumbadjena* species, *K. occidentalis*, has been recorded from the Bridgetown and Leeuwin–Naturaliste regions (Fig. 2). This distribution can be explained by the occurrence of extensive, historically contiguous forest ecosystems (largely dominated by *Eucalyptus marginata*, *E. diversicolor*, and *Corymbia calophylla*), that strongly resemble the widespread distribution of *Bertmainius opimus* (see Harvey *et al*. 2015). However, both have significant genetic substructuring that is consistent with historical bottlenecks and/or lack of dispersal. This is another species that is worthy of more extensive sampling to tease apart population-level structuring.

Hopper and Gioia (2004) distinguished areas of high rainfall and floral diversity along the coast of south-western Australia with inland areas being more arid with the exception of high-elevation areas such as the Porongurups and Stirling Ranges. The vegetation zones are formed from giant eucalypts (e.g. *Eucalyptus diversicolor* and *E. jacksonii*). Flowering plant diversity is particularly high in these areas with ~3600 species, 80% of which are endemic (Hopper and Gioia 2004). The distributions of *Kumbadjena* species corroborate the areas of higher rainfall, as proposed by Hopper and Gioia (2004), and in conjunction with other studies (e.g. Cooper *et al*. 2011; Rix *et al*. 2015) provide evidence of the importance of this biogeographic region for biodiversity and conservation. The reliance of Onychophora on moist habitats has possibly confined them to the high-rainfall area along the coast of south-western Australia and also to the upland areas such as the Stirling and Porongurups Ranges. This substantiates the hypotheses supported by the biogeographic distributions of other fauna such as *Bertmainius* mygalomorph spiders (Cooper *et al*. 2011; Harvey *et al*. 2015; Rix *et al*. 2017), assassin spiders (Rix and Harvey 2012), millipedes (Moir *et al*. 2009) and frogs (Edwards *et al*. 2007, 2008); see Rix *et al*. (2015) for further discussion.

Geography plays an integral role in the speciation of *Kumbadjena*. The short-range endemism of Onychophora is clearly evidenced by the ranges of the different species of *Kumbadjena.* Each clade has a limited distribution while some species have overlapping distributions suggesting isolation despite this overlap. The overlap is due to the secondary contact and not sympatry, since the overlapping species do not form sister pairs. Further sampling of this taxon from throughout its range could uncover yet more species and help refine species' ranges. Indeed, a more robust sample is required to rigorously test the hypotheses proposed in this paper with morphological and molecular data. Limited sampling can affect the accuracy of the species-delimitation analyses, and most advise multiple individuals from the same localities for more comprehensive results (Puillandre *et al*. 2012; Roy *et al*. 2014). For example, PTP assumes equal sampling of multiple individuals for proper analysis (Zhang *et al*. 2013), which is an issue for some purported species presented here.

Conservation

The possibility of a recent divergence of the species within *Kumbadjena* can have profound effects on the delimitation of species. Confounding factors, such as incomplete lineage sorting, can cause equivocal species delineation (Rittmeyer and Austin 2012). Morphological evidence seems to suggest a recent/ongoing divergence of these species where some individuals share morphological characteristics with more than one described species and thus cannot properly be assigned (Reid 2002). This suggests a tantalising possibility that we can capture a snapshot in the ongoing process of speciation in the area and increasing biodiversity of the region. Furthermore, the implications of conservation are immediately apparent as this study offers evidence of the biological significance of south-western Australia, an area that is increasingly compromised by land clearing and further stresses on the environment such as altered fire regimes and invasive species (Environmental Protection Agency 2007). This study supports the growing body of evidence that suggests that the climatic history of Australia, paired with the topographical conditions of south-western Australia, have caused an explosion of speciation in the region (e.g. Hopper 1979; Hopper and Gioia 2004; Edwards *et al*. 2007, 2008; Moir *et al*. 2009; Cooper *et al*. 2011), arguing for increased protection of forests in the area, especially those in upland regions (e.g. Porongurups, Stirling Range).

The genus *Kumbadjena* exemplifies the propensity of onychophorans towards producing endemics. Indeed, successful conservation programs should not only encompass species and populations but also the natural processes at the landscape level that promote and sustain speciation and species (Arnold 1995). The sampling and identification of endemics has profound implications for conservation of both species and the processes that forge and harbour these taxa (Harvey 2002). Historically, invertebrates have been neglected in conservation policy, especially in the designation of protected areas (Ferrier *et al*. 1999; McKenzie *et al*. 2000), but, as evidenced by this study, can illuminate historical and ongoing processes of speciation. Proclivity of certain invertebrate taxa to endemism necessitates conservation of the areas in which they reside as they can be congruent with high diversity and endemism in other taxa.

Taxonomy

Family **Peripatopsidae** Evans, 1901

Genus ***Kumbadjena*** Reid, 2002

urn:lsid:zoobank.org:act:3DC8E8E2-B0F2-4C32-9D72-7B09113FC87A
Kumbadjena Reid, 2002: 131–132
Type species: *Peripatus leuckarti* var. *occidentalis* Fletcher, 1895.

Diagnosis (*from Reid 2002*)

Dorsal primary papillar scales ribbed proximally and partially ribbed distally (microcristae fused at tips of scales) in both sexes. Fifteen oncopod pairs; first pair enlarged in males, slightly enlarged in females. Crural papillae oncopods protrude between third (proximalmost) spinous pad and adjacent plica; foramen not obviously demarcated from rest of papillae. Crural papillae oncopods 2–14 very broad basally, conical, not elongate

distally; papillar scales and scale microcristae fused, or partially fused, smooth; foramen a transverse slit opening on inner side of smooth regions. Posterior accessory gland foramen joined, inverted Y-shaped. Posterior accessory glands long and narrow, uniform width. Crural glands extending length of body from first pair of oncopods. Ovoviviparous, ova follicular.

Kumbadjena occidentalis (Fletcher, 1895)

Peripatus leuckarti var. *occidentalis* Fletcher, 1895: 185–186.
Peripatoides occidentalis (Fletcher). – Dakin, 1914*a*: 289–292, text fig. 1; Dakin, 1914*b*: 3–5; Dakin, 1920: 367–389, plates I–V, figs 1–25 (in part).
Occiperipatoides occidentalis (Fletcher). – Ruhberg, 1985: 126; Tait *et al.* 1990: 153–171, table 1; Briscoe and Tait, 1995: 91–102, tables 1 and 3; Reid, 1996: 817–819, figs 97–98.
Kumbadjena occidentalis (Fletcher). – Reid, 2002: 132–139, figs 2–4, 8.

Material examined

Molecular vouchers. **Australia: *Western Australia*:** 1 specimen (MCZ IZ-131368), 14 km S of intersection of Caves Rd and Foreset Grove Rd, south of Margaret River, Boranup Drive (Karri Forest Scenic Drive), 34°09′51.9834″S, 115°04′34.4274″E, 3.iii.2009, G. Mayer; 1 specimen (MCZ IZ-131365), Leeuwin–Naturaliste NP, T. Buckley (no date); 1 specimen (MCZ IZ-131398), Glenbourne Farm, south of Gracetown, 33°54′32″S, 115°00′24″E, 22.x.2001, L. M. Marsh; 1 specimen (MCZ IZ-131369), Brockman Hwy, 20.3 km W of intersection with South Western Hwy, Bridgetown Jarrah Park, Bridgetown, 34°01′45.40″S, 115°58′ 57.57″E, 3.iii.2009, G. Mayer.

Remarks

As explained by Reid (2002), *Peripatus leuckarti* var. *occidentalis* was originally described from specimens collected from Bridgetown, Western Australia (Fletcher 1895), which have not been located. To resolve the identity of this taxon, Reid (2002) designated a neotype male from Bridgetown Jarrah Park (WAM T42554), some 15 km south-west of Bridgetown. We have included a specimen from the neotype locality in the molecular analysis (MCZ IZ-131369), which grouped with specimens from the Leeuwin–Naturaliste region (Boranup Drive, Leeuwin–Naturaliste National Park and Glenbourne Farm), some 80 km to the west (Figs 2, 3). On the basis of these data, we have also assigned all other WAM specimens from the Leeuwin–Naturaliste region to *Kumbadjena occidentalis*.

Kumbadjena kaata Reid, 2002

Kumbadjena kaata Reid, 2002: 139–142, figs 8–10.

Material examined

Molecular vouchers. **Australia: *Western Australia*:** 1 specimen (WAM-T129595), Porongurup NP, deep gully west of Waddy's Hut, 34°40′55.02″S, 117°50′54.96″E, 29.iv.2008, M. G. Rix and M. S. Harvey; 1 specimen (WAM-T113491), Porongurup NP, deep gully west of Waddy's Hut, 34°40′55.02″S, 117°50′54.96″E, 26.iii.2011, M. G. Rix and M. S. Harvey; 1 specimen (WAM-T142370), Porongurup NP, deep gully west of Waddy's Hut, 34°40′55.02″S, 117°50′54.96″E, 29.iv.2008, M. G. Rix and M. S. Harvey.

Diagnosis

GenBank accession nos: MH040656, MH040657, MH040654, MH040642, MH040643.

Kumbadjena kaata is distinguished from all other members of the genus by the underlined synapomorphies in the 18S rRNA. At base 712, there is a substitution of a G to an A. At base 779, there is a substitution of a G to an A. At base 1710, there is a substitution of a C to a G (Fig. 5).

18S: (710)GCATATATTA (770)CGGGCCGCCA (1710) GTCACCTTTC.

Remarks

Kumbadjena kaata was described from several specimens collected in Porongurup National Park (Reid 2002). It has been collected from inside rotting logs and in leaf litter.

Kumbadjena shannonensis Reid, 2002

Kumbadjena shannonensis Reid, 2002: 142–146, figs 1, 8, 11*a*,*b*, 12.

Material examined

Molecular vouchers. **Australia: *Western Australia*:** 1 specimen (WAM-T111964), Shannon NP, Deeside Coast Rd, 500 m off South West Hwy, 34°32′08″S, 116°20′47″E, 17.x.2009, D. Harms and S. M. Harms; 1 specimen (WAM-T111966), Treen Brook SF, Track, 100 m off Vasse Hwy, 34°26′45.0234″S, 115°58′59.8794″E, 14.x.2009, D. Harms and S. M. Harms; 1 specimen (MCZ IZ-131359), Shannon Campground, Shannon NP, 34°35′44′S, 116°24′33′E, 23.i.2006, G. D. Edgecombe and G. Giribet; 1 specimen (MCZ IZ-131438), Dog Pool, Bibbulmun track where crosses Shannon R., 34°45′50.6514″S, 116°22′37.956″E, 12.x.2011, G. Giribet and M. S. Harvey.

Species	Diagnostic molecular characters						
	340	380	710	770	1640	1680	1710
Kumbadjena occidentalis	GATCGCCGGT	CCGCGCCCGA	GCGTATATTA	CGGGCCGCCG	TGTTCCGTGC	GGCGGTACCCTCGGGGAAAC	CTCACCTTTC
Kumbadjena shannonensis	GATCGCCGGT	CCGCGCCCGA	GCGTATATTA	CGGGCCGCCG	TGTTCCGTGC	GGCGGTACCCCTCGGGGAAA	CTCACCTTTC
Kumbadjena kaata	GATCGCCGGT	CCGCGCCCGA	GCATATATTA	CGGGCCGCCA	TGTTCCGTGC	GGCGGTACCCTCGGGGAAAC	GTCACCTTTC
Kumbadjena toolbrunupensis	GATCGCCAGT	TCGCGCCCGA	GCGTATATTA	CGGGCCGCCG	TGTTCCGTGC	GGCGGTACCCTCGGGGAAAC	CTCACCTTTC
Kumbadjena karricola	GATCGCCGGT	CCGCGCCCGA	GCGTATATTA	CGGGCCGCCG	TGTCCCGTGC	GGCGGTACCCTCGGGGAAAC	CTCACCTTTC
Kumbadjena extrema	GATCGCCGGT	CCGCGCCCGA	GCGTATATTA	CGGGCCGCCG	TGTTCCGTGC	GGCGGTACCCTTCGGGGAAA	CTCACCTTTC

Fig. 5. Diagnostic molecular characters in 18S rRNA for distinction of *Kumbadjena shannonensis*, *K. kaata, K. toolbrunupensis,* sp. nov., *K. karricola*, sp. nov., and *K. extrema,* sp. nov. Numbering refers to reference sequence from Illumina (GenBank accession number: MH040663).

Diagnosis

GenBank accession nos: KC754582, MH040664, MH040662, MH040659.

Kumbadjena shannonensis is distinguished from all other species in the genus by the following underlined synapomorphy in 18S rRNA. There is an insertion of a C at base 1690 and a change from T to C with respect to *K. extrema*, sp. nov. (Fig. 5).

18S: (1680)GGCGGTACCCCTCGGGGAAA.

Remarks

Kumbadjena shannonensis was described from several specimens collected in Shannon National Park (Reid 2002). We included two specimens from Shannon National Park in our molecular analysis which, surprisingly, grouped with a specimen from Treen Brook State Forest (Figs 2, 3). It has been mostly collected from forest leaf litter.

Kumbadjena karricola, sp. nov.

(Figs 6*a–c*, 9*a*)

urn:lsid:zoobank.org:act:8BFAB92D-8631-4749-883C-F3863335AB75

Material examined

Holotype. **Australia:** ***Western Australia*:** 1 specimen (WAM-T111967), Treen Brook SF, Track, 100 m off Vasse Hwy, 34°26′45″S, 115°59′00″E, 14.x.2009, D. Harms and S. M. Harms.

Paratypes. **Australia:** ***Western Australia*:** 1 specimen (MCZ IZ-131432), Beedelup NP, Seven Day Rd, 34°20′30.7392″S, 115°51′42.8754″E, 11.x.2011, G. Giribet and M. S. Harvey; 1 specimen (MCZ IZ-131367), Bicentennial Tree, Warren NP, 34°29′42″S, 115°58′31″E, 9.vii.2004, G. Giribet, M. K. Nishiguchi, and S. W. Aktipis; 1 specimen (WAM-T142371), Beedelup NP, 34°26′10″S, 115°53′18″E, collected 4.v.2008 by M. G. Rix and M. S. Harvey; 1 specimen (WAM-T138513), Beedelup NP, 34°26′10″S, 115°53′18″S, 4.v.2008, M. G. Rix and M. S. Harvey; 1 specimen (WAM-T111965), Treen Brook SF, Track, 100 m off Vasse Hwy, 34°26′45″S, 115°59′00″E, 14.x.2009, D. Harms and S. M. Harms.

Diagnosis

GenBank accession nos: MH040666, MH040661, MH040658 MH040644, MH040640, MH040641, KC754580.

Kumbadjena karricola, sp. nov. is distinguished from all other species in the genus by the underlined synapomorphy in 18S rRNA (below). At base 1643, there is a substitution of a T to a C (Fig. 5). The number of scale ranks varies: either 7 or 8 (Fig. 9*a*).

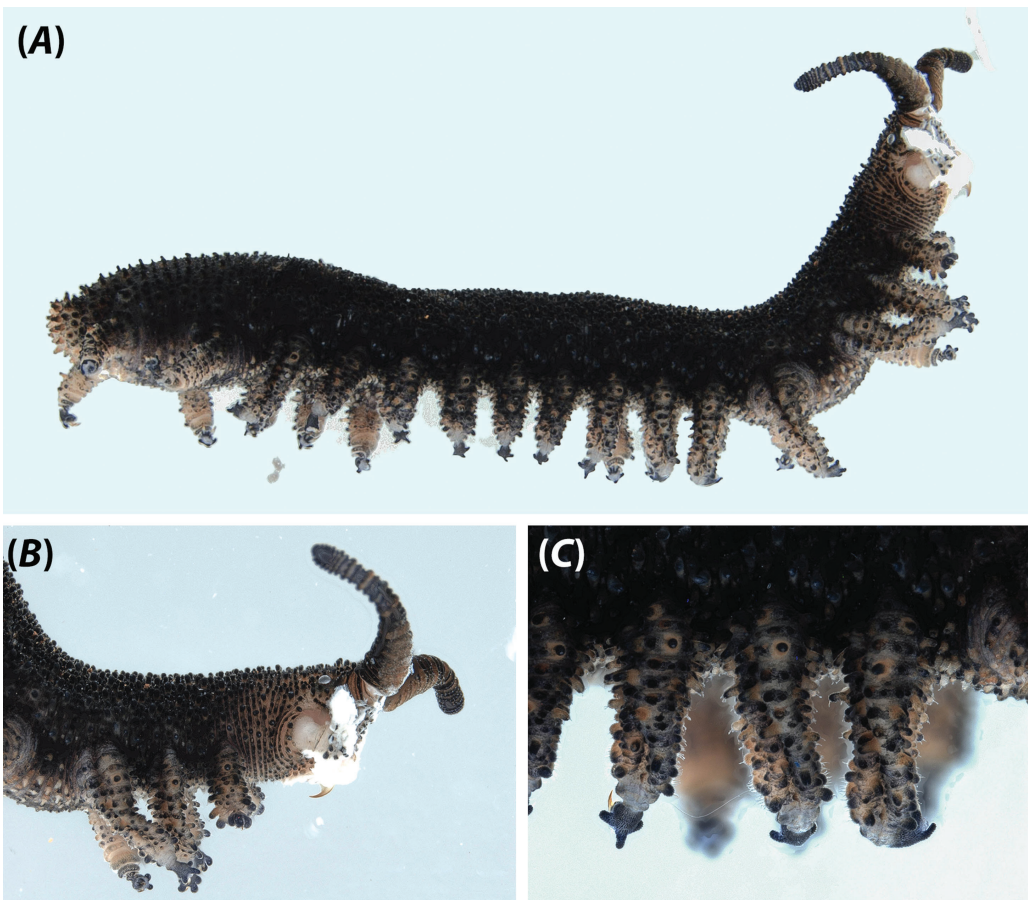

Fig. 6. Holotype of *Kumbadjena karricola*, sp. nov. (WAM-T111967): (*a*) lateral view of the body; (*b*) lateral close-up of the head; (*c*) close-up of oncopods.

18S: (1640)TGTCCCGTGC.

Etymology

This species is named in reference to karri (*Eucalyptus diversicolor*). *Kumbadjena* are often found in association with karri forests, hence *karri* + *cola* (inhabiting).

Remarks

This species is sister group to *K. occidentalis*. It resides in the high-rainfall zones, as defined by Hopper and Gioia (2004), and overlaps in range with *K. shannonensis*.

Kumbadjena extrema, sp. nov.

(Figs 7*a–c*, 9*b*)

urn:lsid:zoobank.org:act:B77B759F-5B5B-4DAB-94FC-096A67E2862B

Material examined

Holotype. **Australia:** ***Western Australia*:** 1 specimen (WAM-T132593), Limeburners Rd, deep gully near Torndirrup NP, 35°05′27″S, 117°54′40″E, 14.iii.2008, M. G. Rix and M. S. Harvey.

Paratypes. **Australia:** ***Western Australia*:** 1 specimen (WAM-T111963), Mount Shadforth Rd, Mt Shadforth, 34°58′03″S, 117°15′54″E, 23.x.2009, D. Harms and S. M. Harms; 1 specimen (WAM-T132795), Gilge Rd, NW of West Cape Howe NP, 35°03′15″S, 117°28′49″E, 16.iii.2008, M. G. Rix and M. S. Harvey; 1 specimen (WAM-T132796), Gilge Rd, NW of West Cape Howe NP, 35°03′15″S, 117°28′49″E, 16.iii.2008, M. G. Rix and M. S. Harvey; 1 specimen (WAM-T133783), Limeburners Rd, deep gully near Torndirrup NP, 35°05′27″S, 117°54′40″E, 14.iii.2008, M. G. Rix and M. S. Harvey; 1 specimen (MCZ IZ-131400), Deep Gully, 35°05′26.016″S, 117°54′38.988″E, 25.ix.2009, K. Edward.

Diagnosis

GenBank accession nos: MH040667, MH040660, MH040652, MH040653, MH040650, MH040651, MH040648, MH040649, MH040645.

Kumbadjena extrema, sp. nov. is distinguished from all other species in the genus by the underlined synapomorphy. At base 1690, there is an insertion of a T or a change from C to T with respect to *K. shannonensis* (Fig. 5). The number of scale ranks is 4 (Fig. 9*b*).

18S: (1680)GGCGGTACCCTTCGGGGAAA.

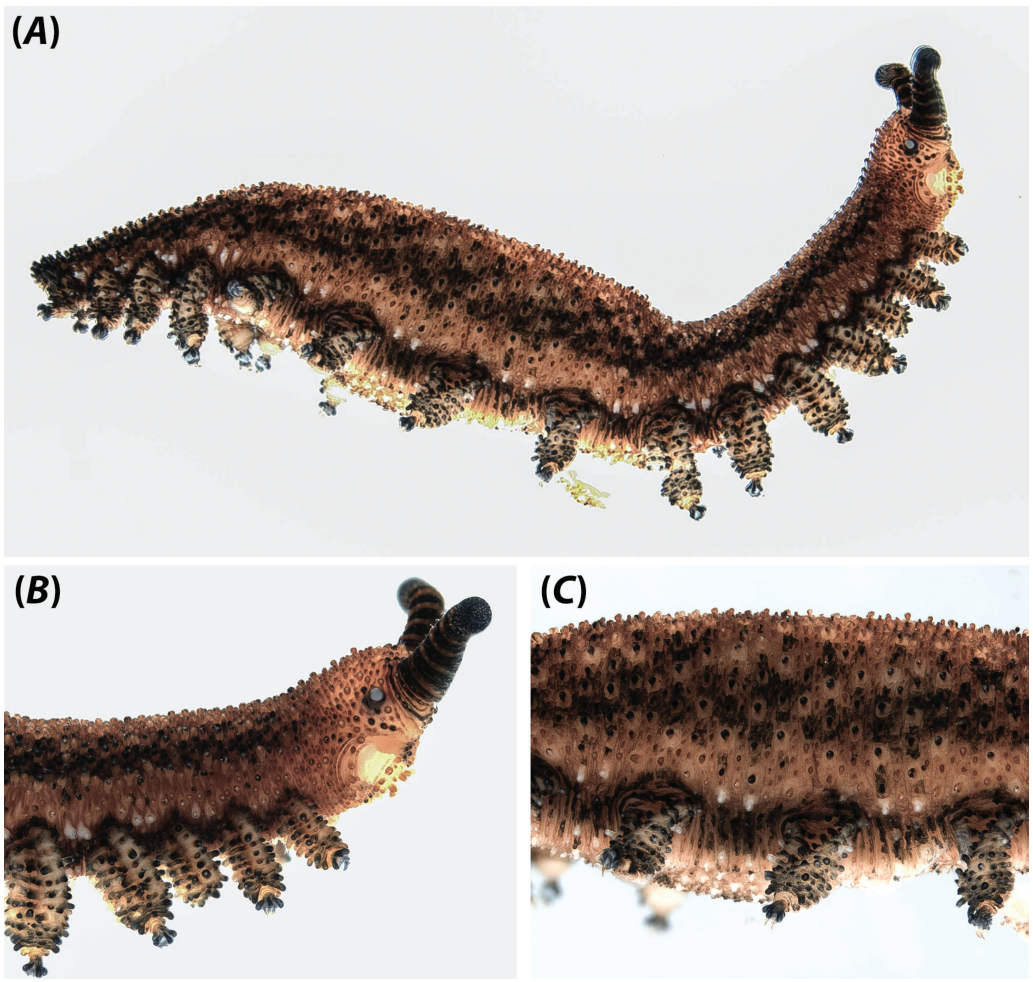

Fig. 7. Holotype of *Kumbadjena extrema*, sp. nov. (WAM-T132593): (*a*) lateral view of the body; (*b*) lateral close-up of the head; (*c*) close-up of oncopods.

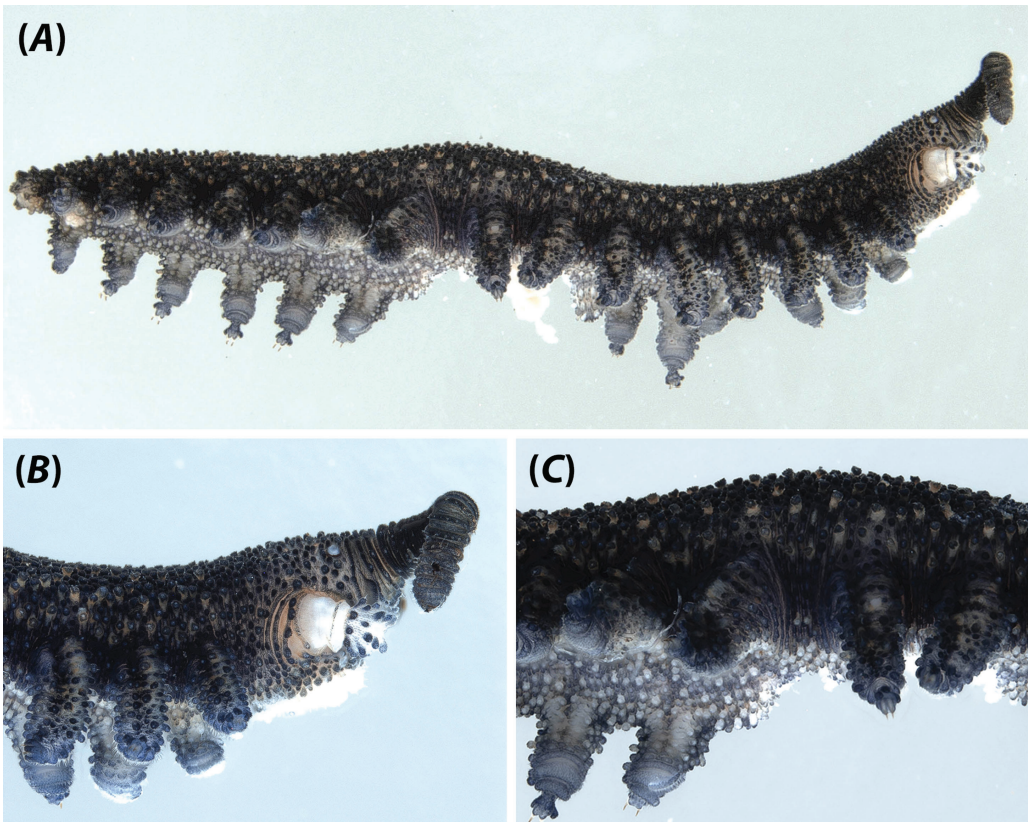

Fig. 8. Holotype of *Kumbadjena toolbrunupensis*, sp. nov. (WAM-T113492): (*a*) lateral view of the body; (*b*) lateral close-up of the head; (*c*) close-up of oncopods.

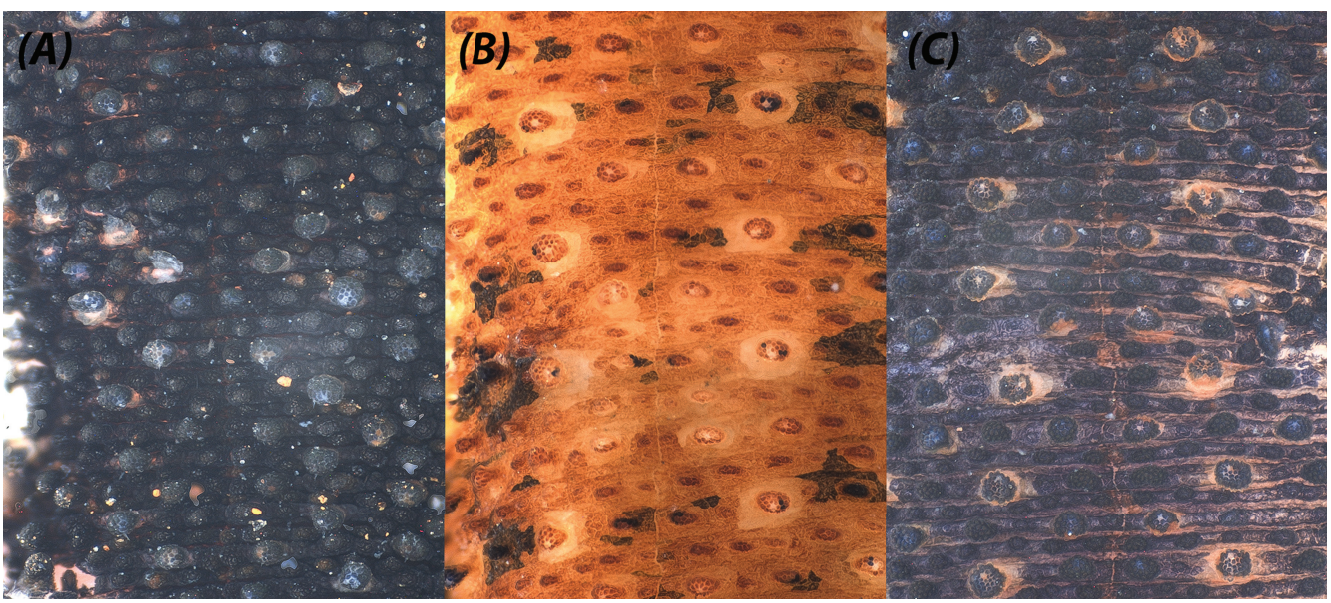

Fig. 9. Light microscopy images of the dorsal integument of the holotypes showing the arrangement of papillae and the scale ranks: (*a*) *Kumbadjena karricola*, sp. nov. (WAM-T111967); (*b*) *Kumbadjena extrema*, sp. nov. (WAM-T132593); (*c*) *Kumbadjena toolbrunupensis*, sp. nov. (WAM-T113492).

Etymology

This specific epithet is in regard to its range in the extreme southernmost portion of Western Australia.

Remarks

This species is sister group to *K. toolbrunupensis*, sp. nov. It resides in karri forests along the southern coast of Western

Australia. The easternmost part of its range corresponds with the eastern border of the high-rainfall area proposed by Hopper and Gioia (2004).

Kumbadjena toolbrunupensis, sp. nov.

(Figs 8*a–c*, 9*c*)

urn:lsid:zoobank.org:act:A5354674-6329-446C-9455-479482ADE0F6

Material examined

Holotype. **Australia:** ***Western Australia*****:** 1 specimen (WAM-T113492), Stirling Range NP, Toolbrunup Peak, 750 m NW of carpark, 34°23′25″S, 118°03′18″E, 26.iii2011, M. G. Rix and M. S. Harvey.

Paratype. **Australia:** ***Western Australia*****:** 1 specimen (MCZ IZ-131433), Toolbrunup Peak, Stirling Range NP, 34°23′25.368″S, 118°03′18.324″E, 14.x.2011, G. Giribet and M. S. Harvey.

Diagnosis

GenBank accession nos: MH040665, MH040655.

Kumbadjena toolbrunupensis, sp. nov. is distinguished from all other species in the genus by the underlined synapomorphies (below). At base 347, there is a substitution from G to A. At base 380, there is a substitution from C to T. (Fig. 5). The number of scale ranks is 6 (Fig. 9*c*).

18S: (340)GATCGCCAGT (380)TCGCGCCCGA.

Etymology

This species is named after its type locality, Toolbrunup Peak in Stirling Range National Park.

Remarks

This species is the sister group to *K. extrema*, sp. nov., and differs by its unusual molecular motif. It is currently known only from a single location at the base of Toolbrunup Peak, in the Stirling Range National Park. The site is a short, shallow gully that also harbours the only known populations of the trapdoor spider *Bertmainius pandus* Harvey, Main, Rix & Cooper, 2015 and the millipede *Atelomastix danksi* Edward & Harvey, 2010 (Edward and Harvey 2010; Harvey *et al*. 2015).

Conflicts of interest

The authors declare no conflicts of interest.

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