Contents lists available at ScienceDirect



Molecular Phylogenetics and Evolution



journal homepage: www.elsevier.com/locate/ympev

A revised dated phylogeny of scorpions: Phylogenomic support for ancient divergence of the temperate Gondwanan family Bothriuridae



Prashant P. Sharma^{a,*}, Caitlin M. Baker^b, Julia G. Cosgrove^b, Joanne E. Johnson^c, Jill T. Oberski^c, Robert J. Raven^d, Mark S. Harvey^e, Sarah L. Boyer^c, Gonzalo Giribet^b

^a Department of Integrative Biology, University of Wisconsin-Madison, 430 Lincoln Drive, Madison, WI 53706, USA

^b Museum of Comparative Zoology & Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

^c Biology Department, Macalester College, 1600 Grand Avenue, St. Paul, MN 55105, USA

^d Department of Terrestrial Biodiversity, Queensland Museum, Grey Street, South Bank, Brisbane, Queensland, Australia

^e Department of Terrestrial Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC 6986, Western Australia, Australia

ARTICLE INFO

Keywords: Bothriuroidea Molecular dating Arachnida Taxonomic sampling Stability

ABSTRACT

The scorpion family Bothriuridae occupies a subset of landmasses formerly constituting East and West temperate Gondwana, but its relationship to other scorpion families is in question. Whereas morphological data have strongly supported a sister group relationship of Bothriuridae and the superfamily Scorpionoidea, a recent phylogenomic analysis recovered a basal placement of bothriurids within Iurida, albeit sampling only a single exemplar. Here we reexamined the phylogenetic placement of the family Bothriuridae, sampling six bothriurid exemplars representing both East and West Gondwana, using transcriptomic data. Our results demonstrate that the sister group relationship of Bothriuridae to the clade ("Chactoidea" + Scorpionoidea) is supported by the inclusion of additional bothriurid taxa, and that this placement is insensitive to matrix completeness or partitioning by evolutionary rate. We also estimated divergence times within the order Scorpiones using multiple fossil calibrations, to infer whether the family Bothriuridae is sufficiently old to be characterized as a true Gondwanan lineage. We show that scorpions underwent ancient diversification between the Devonian and early Carboniferous. The age interval of the bothriurids sampled (a derived group that excludes exemplars from South Africa) spans the timing of breakup of temperate Gondwana.

1. Introduction

The higher level phylogeny of scorpions is presently in a state of flux. Previous analyses of various morphological character systems recovered different topologies of basal relationships, with specific reference to the composition of the two basal branches of scorpions, the parvorders Buthida and Iurida (Soleglad and Fet, 2003; Coddington et al., 2004; Prendini and Wheeler, 2005; Fet and Soleglad, 2005; Prendini et al., 2006; Volschenk et al., 2008). A recent phylogenomic analysis of basal scorpion relationships supported an alternative composition of Buthida and Iurida altogether, and further challenged the monophyly of some superfamilies (e.g., Chactoidea, Iuroidea) (Sharma et al., 2015).

Among the lineages with the most unanticipated placement in the phylogenomic tree was the scorpion family Bothriuridae. The peak of bothriurid diversity occurs in South America, which harbors 141 (94%) of the total 150 described species. Two genera, *Brandbergia* and *Lisposoma*, are endemic to Namibia, and a single genus, *Cercophonius*,

occurs in Australia, with an additional dubious record from the east Indian Himalayas (Lourenço, 1996; Ojanguren-Affilastro, 2013). Geographically therefore, bothriurid diversity reflects a temperate Gondwanan distribution, and the family has thus been of considerable interest from the perspective of historical biogeography.

On the basis of cladistic analyses of morphology alone, Bothriuridae was previously proposed to be the sister group to chactoids (Lamoral, 1980; Sissom, 1990), and more recently, the sister group to Scorpionoidea (Stockwell, 1989; Prendini, 2000; Soleglad and Fet, 2003). The latter placement was supported in part by characters pertaining to the hemispermatophore morphology originally established by Stockwell (1989) and subsequently adopted by other workers (Prendini, 2000; Soleglad and Fet, 2003; Monod et al., 2017). However, no definitive placement of the family was supported under a total evidence dataset comprising 247 morphological characters and five Sanger-sequenced loci (totaling ~ 4.3 Kb; Mattoni et al., 2010). By contrast, phyloge-nomic assessment of basal scorpion relationships supported placement of the family closer to the base of Iurida, with Iuridae and Bothriuridae

* Corresponding author. E-mail address: prashant.sharma@wisc.edu (P.P. Sharma).

https://doi.org/10.1016/j.ympev.2018.01.003

Received 17 May 2017; Received in revised form 12 December 2017; Accepted 2 January 2018 Available online 31 January 2018

1055-7903/ © 2018 Elsevier Inc. All rights reserved.



Fig. 1. (A) Live habitus of *Centromachetes* sp. from Reserva Nacional Nonguén, Biobío, Chile. Photo by G. Giribet. (B) Live habitus of *Cercophonius sulcatus* from Pemberton, Western Australia. Photo by M.S. Harvey. (C) Traditional phylogeny of Scorpiones, indicating inclusion of Bothriuridae in Scorpionoidea. (D) Simplified phylogenomic tree of Sharma et al. (2015) based on 1557 genes. Colors in tree topology correspond to higher level lineages.

forming a grade with respect to a paraphyletic Chactoidea, and Scorpionoidea nested within Chactoidea (Sharma et al., 2015). Problematically, this analysis included only a single exemplar of Bothriuridae from the New World (*Bothriurus burmeisteri*). The phylogenomic placement of this family could thus be attributable to under-sampling of taxa. This uncertainty hinders hypothesis testing in studies of morphological evolution (e.g., Volschenk et al., 2008; Monod et al., 2017), and may also adversely affect selection of appropriate outgroups for downstream inference of divergence time estimates in biogeographic analysis.

In order to assess the placement of Bothriuridae in Scorpiones, we sequenced the transcriptomes of five additional bothriurids, including exemplars of the East Gondwanan genus *Cercophonius*, and added these to the existing phylogenomic dataset of all major scorpion families (Sharma et al., 2015). We inferred gene orthology *de novo* and inferred relationships using dense (> 75% gene occupancy) matrices with up to 1,464 loci. We specifically aimed to address whether the phylogenetic position of Bothriuridae affected by addition of bothriurid exemplars. To infer the age of Bothriuridae, we estimated divergence times for the entire scorpion tree of life using multiple fossil calibrations constraining outgroup nodes. These analyses were designed to test the correspondence between the divergence times of Bothriuridae and the inferred scenario of diversification on temperate Gondwana.

2. Materials and methods

2.1. Taxon sampling and molecular methods

Specimens of Bothriuridae were collected by hand during the day or with the aid of ultraviolet lamps at night from localities in Australia and Chile (Table 1). These were dissected into RNA*later* solution (Ambion) for subsequent transcriptome sequencing. Additional transcriptomes previously published by us were included for sampling of outgroups and an additional *Bothriurus* (Sharma et al., 2014, 2015). The final taxon set consisted of 43 taxa (30 scorpions and 13 non-scorpion Euchelicerata). Paired-end (150 bp) transcriptomes were generated as previously described (Sharma et al., 2015). Statistics on sequencing yields, and accession numbers are provided in Table 2. Quality filtering, trimming of reads and strand-specific transcriptomic assemblies were conducted as previously described (Sharma et al., 2015).

2.2. Phylogenetic analysis

Predicted open reading frames (ORFs) were assigned to orthologous groups using the Orthologous MAtrix (OMA) algorithm (OMA standalone v.0.99z; Altenhoff et al., 2011, 2013). Peptide sequences less than 50 sites in length were discarded and the shortest sequence in each orthogroup was required to comprise at least 60% of the longest sequence in that corresponding orthogroup. Three initial matrices were assembled using a gene occupancy criterion: Matrix 1 (> 97.5% gene occupancy; 32 orthogroups), Matrix 2 (> 87.5% gene occupancy; 440 orthogroups), and Matrix 3 (>75% gene occupancy; 1464 orthogroups). To examine the effects of gene evolutionary rate on topological inference, we subdivided Matrix 3 into tertiles of 488 orthogroups by percent pairwise similarity (slowest bin: Matrix 4; fastest bin: Matrix 6). To examine the effects of matrix completeness (i.e., total cell occupancy of the matrix, rather than gene occupancy, which is a proxy for completeness), Matrix 3 was subdivided into tertiles of 488 orthogroups by cell occupancy (most complete bin: Matrix 7; least complete bin: Matrix 9). Matrix composition, length, and completeness are detailed in Table 3. The number of loci available for every terminal in each matrix is provided in Supplementary File 1.

Maximum likelihood (ML) analyses were conducted using RAxML v. 7.7.5 (Stamatakis, 2006), incorporating mixture models (CAT + LG4XF; Lartillot and Philippe, 2004; Le et al., 2012) for all matrices, and 100 independent searches for the best tree topology. Nodal support was inferred using 500 bootstrap replicates (Stamatakis et al., 2008).

2.3. Tests of monophyly

A Shimodaira–Hasegawa (SH) test of monophyly (Shimodaira and Hasegawa, 1999) was conducted using RAxML ver. 7.7.5 and Matrix 2, enforcing the constraint of a traditionally recognized clade (Bothriuridae + Scorpionoidea). To generate the null distribution, 500 resampling replicates were conducted.

Table 1

Collecting localities of newly sequenced Bothriuridae.

Species	Accession number	Locality	Latitude	Longitude	Elevation	Date	Collector
Bothriurus coriaceaus	MCZ IZ 147001	Captive bred	n/a	n/a	n/a	n/a	n/a
Centromachetes sp.	MCZ IZ 138054	BIOBÍO, CHILE: Reserva Nacional Nonguén, Estero Nonguén, waterfall and picnic area	36.87843°S	72.99435°W	131 m	11-Nov- 14	G. Giribet, G. Hormiga, A. Pérez-González
Cercophonius queenslandae	MCZ IZ 147002	QUEENSLAND, AUSTRALIA: Finch Hatton Gorge, 0.8 km along main trail, under leaf litter	21.13°S	148.49°E	257 m	24-Jul- 15	P.P. Sharma, S.L. Boyer, J.T. Oberski, J. Johnson,
Cercophonius sulcatus	MCZ IZ 147003	WESTERN AUSTRALIA: 3.8 km SSE. of Pemberton, under bark of <i>Eucalyptus diversicolor</i>	34.47750°S	116.05028°E	82 m	12-Jul- 15	M.S. Harvey, M.E. Blosfelds
Cercophonius squama	MCZ IZ 147004	TASMANIA: The Domain, Hobart	42.6983°S	147.3261°E	71 m	16-Feb- 15	R.J. Raven

2.4. Molecular dating

Bayesian inference (BI) analyses were conducted to estimate divergence times, using PhyloBayes MPI v. 1.4f (Lartillot et al., 2013) and implementing the site-heterogeneous CAT + GTR model (Tavaré, 1986; Lartillot and Philippe, 2004). We selected the densest and most complete matrix (Matrix 1) to infer divergence times, to mitigate the deleterious effects of missing data on branch length estimation. Divergence times were inferred under both an autocorrelated lognormal model and an uncorrelated gamma rates model.

The age of crown group scorpions has long proven elusive (Jeram, 1998; Dunlop, 2010). Scorpions have a rich Paleozoic fossil record, but the external morphological stasis and high incidence of convergent evolution exhibited by this group precludes unambiguous placement of fossil scorpions, especially with regard to putative stem group aquatic members of the group (Kjellesvig-Waering, 1986; Jeram 1998; Dunlop, 2010; Waddington et al., 2015; Sharma et al., 2015). Given the possibility of a long internal branch length subtending the diversification of extant scorpions (Rehm et al., 2012; Sharma and Wheeler, 2014), the use of Paleozoic fossils that are difficult to interpret and place along such a long branch may predispose crown group ages to appearing older than they actually are.

For this reason, we dated the scorpion tree of life with emphasis on outgroup fossils, using crown group scorpion fossils only to set minimum ages on the origins of certain families. The following calibration priors were used to date the scorpion tree: (1) The crown age of Opiliones was set to a lower bound of 411 Myr, based on the inferred placement of the extinct suborder Tetrophthalmi (Eophalangium sheari and Hastocularis argus) between Cyphophthalmi and Phalangida (Dunlop et al., 2004; Garwood et al., 2014; Sharma and Giribet, 2014). (2) The crown age of Palpatores was set to a lower bound of 305 Myr, based on the inferred placement of Macrogyion cronus (Eupnoi) and Ameticos scolos (Dyspnoi) (Garwood et al., 2011). (3) The crown age of Araneae was set to a lower bound of 305 Myr, based on the Carboniferous age of the earliest fossil Mesothelae (Selden, 1996). (4) The stem age of Amblypygi was set to a lower bound of 385 Myr, based on fossil limb fragments that include a patella with trichobothria, a character that occurs uniquely on legs 2-4 of modern amblypygids (Dunlop, 2010). (5) The stem age of Buthidae was constrained to a minimum of 120 Ma, based on the age of the extinct buthid-like scorpion

Table 2

Assembly statistics of new Illumina libraries.

Archaeobuthus estaphani (Lourenço, 2001; Baptista et al., 2006). All five of these calibration priors implemented open upper bounds. The crown age of Scorpiones was bounded between 120 and 430 Myr, to reflect the ages of the oldest scorpion that is indisputably nested within extant scorpions (*Archaeobuthus estaphani*) and the oldest Palaeozoic scorpion with modern appendage morphology (*Eramoscorpius brucensis*; Waddington et al., 2015), respectively. Finally, the root age prior, corresponding to the split between Xiphosura and Arachnida, corresponded to a gamma prior with a mean of 550 Myr and standard deviation of 20.

Four independent chains were run for 7078–15,657 cycles, and the initial 5000 cycles of each chain were discarded as burn-in, with convergence assessed using the maximum bipartition discrepancies across chains. Convergence and stationarity were assessed using inbuilt tools in PhyloBayes MPI v. 1.4f (bpcomp and tracecomp).

3. Results

3.1. Phylogenetic analyses and tests of monophyly

ML analyses of Matrices 1–3 uniformly recovered the monophyly of Scorpiones and a basal tree topology consisting of the mutual monophyly of Buthida and Iurida (*sensu* Sharma et al., 2015), with maximal nodal support in every analysis (Fig. 2). Monophyly of Bothriuridae and the superfamily Scorpionoidea were similarly recovered with maximal nodal support in all analyses, with no data partition supporting the sister group relationship of these two lineages. Bothriuridae was recovered as part of a grade with Iuridae at the base of parvorder Iurida, as in Sharma et al. (2015). A clade composed of ("Chactoidea" + Scorpionoidea), to the exclusion of Bothriuridae, was always recovered, and maximally supported except in the smallest matrix (Matrix 1; bootstrap support [BS] = 59).

We observed no effect of partitioning by evolutionary rate (Matrices 4–6) or matrix completeness (Matrices 7–9) on either tree topology or nodal support, in contrast to other phylogenomic studies (Borner et al., 2014; Telford et al., 2014; Sharma et al., 2014; Whelan et al., 2015). Matrices 4–9 uniformly supported the topology shown in Fig. 2, with maximal support for the seven nodes of interest.

The SH test of monophyly comparing the ML topology under Matrix 2 $(\ln L = -1466479.55)$ to the topology enforcing

Species	BioProject	BioSample	SRA Accession	Reads	Reads post trimming	Contigs	N50 (bp)	Longest peptide sequence (AA sites)	Estimated coverage
Bothriurus coriaceaus Centromachetes sp. Cercophonius queenslandae Cercophonius squama	PRJNA429236 PRJNA429234 PRJNA429232 PRJNA429229	SAMN08331723 SAMN08331856 SAMN08331814 SAMN08331791	SRR6467511 SRR6467879 SRR6466561 SRR6470446	23289767 5573242 18821400 28803697	22802826 5534430 18311139 28183895	196303 87052 250392 178147	596 589 746 809	11581 18906 17437 11240	35.52811239 19.21776509 20.44426012 41.19202914

Table 3

Data matrix assembly criteria, composition, size, and completeness.

Dataset	Criterion for assembly	Number of loci	Number of AA sites	Proportion missing (%)
Matrix 1	Minimum 42 loci per orthogroup	32	5,948	4.21
Matrix 2	Minimum 38 loci per orthogroup	440	97,522	12.13
Matrix 3	Minimum 33 loci per orthogroup	1464	330,974	19.55
Matrix 4	Slowest evolving tertile of Matrix 3	488	117,733	18.48
Matrix 5	Intermediate rate tertile of Matrix 3	488	117,483	19.44
Matrix 6	Fastest evolving tertile of Matrix 3	488	95,758	20.99
Matrix 7	Most complete tertile of Matrix 3	488	103,813	12.34
Matrix 8	Intermediate completeness tertile of Matrix 3	488	114,627	20.23
Matrix 9	Least complete tertile of Matrix 3	488	112,534	25.50

(Bothriuridae + Scorpionoidea) (lnL = -1467998.86) recovered a difference in log likelihood of 1519.31 (standard deviation of 123.11). The null hypothesis of equal likelihood of the two topologies was rejected at $\alpha = 0.01$.

family was estimated at 135.5 Ma (95% HPD interval: 86.0–193.0 Ma). The most recent common ancestor of *Cercophonius* was inferred to be 29.6 Ma (95% HPD interval: 14.3–43.9 Ma).

3.2. Molecular dating

Estimates of divergence times under an autocorrelated lognormal model placed the crown age of Scorpiones in the Devonian (mean age 381.3 Ma; 95% highest posterior density [HPD] interval: 333.6–423.1 Ma). The origin of Bothriuridae was estimated at 249.7 Ma (95% HPD interval: 193.2–305.2 Ma), whereas the diversification of the

As expected, comparatively younger ages were obtained under the uncorrelated gamma multipliers model (Lepage et al., 2007), with crown group Scorpiones diversifying in the Permian (mean age 285.2 Ma; 95% HPD interval: 209.1–380.6 Ma). The origin of Bothriuridae under this model was estimated at 124.2 Ma (95% HPD interval: 88.4–184.7 Ma), whereas the diversification of the family was estimated at 51.8 Ma (95% HPD interval: 31.1–90.9 Ma). The most recent common ancestor of *Cercophonius* was inferred to be 12.8 Ma (95% HPD interval: 6.8–23.6 Ma). The chronogram inferred under the



Fig. 2. Phylogenomic analysis of scorpion relationships based on 440 loci (Matrix 2; $\ln L = -1466479.55$). Boldface terminals correspond to newly sequenced libraries. Colors in tree topology correspond to higher level lineages. Numbers on nodes indicate bootstrap resampling frequencies. Navajo rugs correspond to seven nodes of interest; numbers in Navajo rug cells indicate bootstrap resampling frequencies.



Fig. 3. Chronogram of Scorpiones inferred using most complete dataset (Matrix 1) under the autocorrelated lognormal model ($\ln L = -54392.6$). Bars on nodes correspond to 95% highest posterior density intervals. All outgroups have been removed for clarity.

lognormal model is depicted in Fig. 3; the chronogram inferred under the uncorrelated gamma multipliers model is provided in Supplementary File S2.

4. Discussion

190

4.1. Bothriuridae is not sister group to Scorpionoidea

Bothriuridae was once a member of Scorpionoidea, and cladistic analysis of morphological characters has supported a sister group relationship of the two lineages (Fig. 1). The basis for the widely held hypothesis that these groups must be closely related stemmed from the occurrence of a paraxial organ with a reflection of the internobasal sperm duct in both Scorpionoidea and Bothriuridae (Stockwell, 1989; Prendini, 2000; Soleglad and Fet, 2003; Peretti and Battán-Horenstein, 2003). Bothriurids were distinguished from Scorpionoidea in retaining the plesiomorphic state of apoikogenic development (i.e., large, yolky embryos with multiple extraembryonic membranes; Lourenço, 2000), and were thus held to be the sister group to the remaining Scorpionoidea (Prendini, 2000; Soleglad and Fet, 2003). A phylogenomic analysis of basal scorpion relationships rejected this placement, and furthermore, demonstrated the limited utility of numerous traditionally utilized morphological characters in predicting higher level relationships (Sharma et al., 2015). Specifically, large subsets of morphological characters were shown to be either uninformative or highly homoplastic at higher taxonomic levels, when contrasted with the molecular results.

A recent and rigorous reassessment of hemispermatophore morphology and evolution by Monod et al. reported that non-African bothriurids bear hemispermatophores more comparable to those of chactoids than to true Scorpionoidea; only in the African genus *Lisposoma* does the hemispermatophore bear a close resemblance to the scorpionoid counterpart (Monod et al., 2017). Historically, the distribution of these character states has been interpreted to mean that *Lisposoma* is the sister group to the remaining Bothriuridae (Prendini, 2000; Soleglad and Fet, 2003; Soleglad et al. 2005). But as Monod et al. (2017) point out, *Lisposoma* has never been included in a molecular phylogeny, and given documented discordance between morphology and molecular sequence data previously revealed by phylogenomic data (Sharma et al., 2015), it is imperative to examine historical assumptions of scorpion phylogeny in the light of an independent data class like molecular sequence data. In the critical evaluation offered by Monod et al. (2017), alternative possibilities raised by the authors are that *Lisposoma* is not a true bothriurid, or that the incidence of similar hemispermatophore morphology in *Lisposoma* and Scorpionoidea simply reflects convergence.

The only comprehensive phylogeny of bothriurids to date is the unpublished work of Mattoni et al. (2010), and a PDF of this presentation, delivered in 2008 at the annual meeting of the Willi Hennig Society, was generously provided to us by Camilo Mattoni. Mattoni et al. (2010) found that the South African genera formed a grade at the base of Bothriuridae, with Brandbergia as sister group to the remaining bothriurids. However, Brandbergia haringtoni was not available for sequencing in that study, and its placement was therefore determined only by morphological data. Furthermore, reflecting the zeitgeist of the forum wherein this presentation was delivered, the phylogenetic trees shown in that presentation were constructed either from morphological matrices only, or from combined analyses of both morphological and molecular sequence data, and always analyzed only with parsimony (equally weighted or under implied weighting); no trees based on molecular sequence data alone were presented. It is therefore unclear whether the placement of the African bothriurid genera in that analysis was driven by morphological data, missing sequence data, or some combination thereof.

All of these concerns weigh heavily upon the robust phylogenetic placement of Bothriuridae, which is essential for inferring morphological evolution and biogeographic history. Specifically, the representation of Bothriuridae with a single West Gondwanan exemplar in the phylogenomic study (*Bothriurus burmeisteri*) limited the scope of conclusions that could be drawn from this result (Sharma et al., 2015).

Propitiously, as shown herein, inclusion of both East and West Gondwanan bothriurid exemplars in the present study did not affect the placement of Bothriuridae within Scorpiones (Fig. 2), justifying the previous revalidation of its superfamilial rank (Bothriuroidea) (Sharma et al., 2015). A placement of Bothriuridae as part of a grade with Iuridae at the base of parvorder Iurida was recovered with support (\geq 95% BS) in every analysis, regardless of data matrix size, completeness, or evolutionary rate. No data matrix supported the traditionally defined Scorpionoidea (Bothriuridae + Scorpionoidea *sensu* Sharma et al., 2015), with this alternative hypothesis robustly rejected by a test of monophyly. These results suggest that depth of gene sampling may overcome low taxonomic sampling in relatively young clades that lack fast-evolving lineages, gene tree conflict, or otherwise problematic phenomena (e.g., Arachnida, Metazoa; Rokas and Carroll, 2005; Philippe et al., 2011; Sharma et al., 2014; Whelan et al., 2015).

4.2. The age of scorpions is consistent with Permian origin and diversification

Fossil placement has proven straightforward in various arachnid orders, in part due to modern imaging methods that implement three dimensional reconstructions (e.g., Selden et al., 2008; Garwood et al., 2011, 2014, 2016). However, efforts to resolve the phylogenetic placement of the rich diversity of Paleozoic scorpions have been limited. Jeram (1998) produced a cladistic analysis of Paleozoic scorpions, which demonstrated a high incidence of convergence events, as well as numerous polytomies throughout the tree. Legg et al. (2013) included five fossil and two extant scorpions in a large dataset of 309 panarthropods (both fossil and extant) toward examining arthropod relationships based on morphological data alone, and recovered a "traditional" placement of scorpions as the sister group of Opiliones, a placement contradicted by phylogenomic studies (Borner et al., 2014; Sharma et al., 2014). Legg et al. (2013) also demonstrated that the extant scorpions (Orthosterni) form a clade nested within the Paleozoic scorpions, a result consistent with previous classification of scorpions into the lineages Palaeoscorpionina (extinct), Mesoscorpionina (extinct), and Neoscorpiones, with Orthosterni nested within the last of these. Given the difficulty of placing fossil taxa, as well as the demonstrable discordance in the phylogenetic signal of morphological and molecular datasets (Sharma et al., 2015), molecular dating approaches have not been applied across Scorpiones.

Previous efforts to date the crown age of scorpions have employed the hemocyanin gene family, including a method that leverages paralogy to enhance the precision of divergence times (Rehm et al., 2012; Sharma and Wheeler, 2014). However, these approaches used only a subset of outgroup taxa that bore hemocyanin sequences (nearly always absent in apulmonate arachnids), and were also limited to two scorpion exemplars. Other works have begun employing molecular dating in scorpion phylogenies, toward elucidating the biogeographic history of derived taxa like the bothriurid genus *Brachistosternus* (Ceccarelli et al. 2016) and New World buthid genera (Ojanguren-Affilastro et al., 2017). By the nature of their design, these works focused their taxonomic sampling on the focal genus or species group, rather than the entire order Scorpiones. The present study thus constitutes the first phylogenomic dating of the scorpion tree of life.

As with many other terrestrial panarthropod groups with a sparse fossil record, we found that the crown age of scorpions was contingent upon model implementation (e.g., Murienne et al., 2010, 2013; Sharma and Giribet, 2014). Under a lognormal model with autocorrelated rates, scorpions were inferred to be an ancient group, with initial diversification in the Devonian (Fig. 3). By contrast, an uncorrelated gamma multipliers models suggested diversification significantly later, in the Permian (Supplementary File 2). These dates are older than those inferred by analysis of the hemocyanin gene family alone (Rehm et al., 2012; Sharma and Wheeler, 2014) and indicate that most of the scorpion superfamilial lineages diversified in the mid- to late-Mesozoic. Either model supports the previous interpretation of scorpions as a Pangean group (i.e., diversification by the Permian or earlier; Sissom, 1990), and are consistent with the candidacy of scorpions as a lineage that diversified by vicariance in accordance with continental breakup (Sissom, 1990).

This estimated timeframe also suggests that the evolutionary history of Scorpiones is largely comparable to such groups as spiders (Garwood et al., 2016; Garrison et al., 2016), harvestmen (Garwood et al., 2014; Sharma and Giribet, 2014), or pseudoscorpions (Schawaller et al., 1991; Judson, 2012), with respect to a Paleozoic age of diversification. In contrast, extant species of the order Xiphosura (horseshoe crabs) have been shown to have a most recent common ancestor in the Cretaceous (Obst et al., 2012), in spite of a rich fossil record beginning in the Ordovician. Nevertheless, scorpions are distinguished from groups like spiders and harvestmen by the long, 141 Myr (lognormal model) to 235 Myr (uncorrelated gamma multipliers model) gap between the time of scorpion origin and diversification, consistent with estimates using hemocyanins (Sharma and Wheeler, 2014). These long branches are consistent with extinctions of a large paraphyletic group of scorpion lineages in the Paleozoic, as clearly shown by the fossil record (Jeram, 1998; Legg et al., 2013). Long branch lengths subtending extant clades incur limits to evolutionary inference and, when occurring in extreme ratios to extant terminal lengths, are regarded as evolutionary relicts, as exemplified by Xiphosura (Obst et al., 2012; Sharma and Wheeler, 2013, 2014). Such gaps, and the attendant limits of taxonomic sampling of extant lineages in molecular phylogenies, may partly account for the difficulty of resolving the phylogeny of Arachnida (Sharma et al., 2014).

4.3. Divergence of Bothriuridae is contemporaneous with other temperate Gondwanan groups

Under either the lognormal or the uncorrelated gamma multipliers model, we obtained a Mesozoic origin (stem group age) of bothriurids (249.7 Ma under the lognormal model; 124.2 Ma under the uncorrelated gamma multipliers model). The large HPD intervals on these estimates span the Late Jurassic. Furthermore, both age of origin and diversification of the family are approximately contemporaneous with two other well-characterized temperate Gondwanan panarthropod groups, the harvestman family Pettalidae (Boyer et al., 2007; Boyer and Giribet, 2007, 2009; Giribet et al., 2012; but see Giribet et al., 2016 for an alternative dating) and the velvet worm family Peripatopsidae (Allwood et al., 2010; Murienne et al., 2013; Daniels et al., 2016), but only under the lognormal model (Fig. 4). Under the uncorrelated rates model, the divergence of the six exemplars representing the family is estimated to be 51.8 Ma (95% HPD interval: 31.1-90.9 Ma)-too young for the distribution to be attributable to Gondwanan vicariance. We treat the results under the autocorrelated lognormal model as more plausible than under the uncorrelated rates model, based upon previous simulations and empirical studies that have shown the superiority of autocorrelated rate models over uncorrelated rate models in a range of datasets (Lepage et al., 2007; Paradis, 2013).

We further add the caveat that the limited taxonomic sampling in this study, and especially the exclusion of the South African genera, underestimates the age of crown group Bothriuridae; we also did not sample *Urophonius*, the putative sister genus of *Cercophonius* (Prendini, 2000; Mattoni et al. 2010). Additional sampling of bothriurid taxa can only increase the estimated crown age (or keep it the same, if the basalmost node has already been sampled), especially in light of the data on other temperate Gondwanan groups, such as Pettalidae and Peripatopsidae, where the basalmost lineages were endemic to South Africa (Murienne et al., 2013; Giribet et al., 2012, 2016). Because *Cercophonius* is the only representative of East Gondwanan bothriurids, the emphasis of the present work was to sample this lineage and infer the relative temporal context for the split between East and West Gondwanan clades, a reliable and replicated signal observed in tree



Fig. 4. (A) Comparison of divergence times in Peripatopsidae (Onychophora), Pettalidae (Opiliones), and Bothriuridae (Scorpiones), three candidate temperate Gondwanan clades. Bars indicate 95% HPD intervals of estimated divergence times from Murienne et al. (2013), Giribet et al. (2012), and this study. Colors of bars correspond to matched events across the three phylogenies. Note that multiple events can correspond to the same nodes. (B) Comparison of taxon area cladograms in the three candidate taxa.

topologies of multiple temperate Gondwanan panarthropod groups (Boyer and Giribet 2007, 2009; Giribet et al., 2012, 2016; Murienne et al., 2013). As indicated by Fig. 4, the age interval of the East-West Gondwanan split in Bothriuridae under the lognormal rate model overlaps the age of the corresponding split in other temperate Gondwanan taxa, albeit with a younger mean age than the geological timing of the East-West Gondwanan split, and affirms the candidacy of Bothriuridae as a temperate Gondwanan group.

A pattern of an early split between Africa (often with South America) and the remaining Gondwanan territories recurs in many panarthropod groups with limited dispersal ability (e.g., Boyer and Giribet, 2007, 2009; Giribet et al., 2012; Murienne et al., 2013; Vélez et al. 2014; Harvey et al., 2017; Fig. 4B). Future tests of this hypothesis should therefore emphasize the placement of the southern African bothriurids, with the predictions that the southern African bothriurids (1) are the sister group to the remaining members of the family, or alternatively form a grade with South American species at the base of the bothriurid tree; and (2) and diverged by the Late Jurassic.

4.4. Diversification of Cercophonius is younger than other Australian paleoendemic clades

In comparison to the age of diversification observed in endemic peripatopsid and pettalid clades, the crown age of *Cercophonius* as inferred from three species is much younger, postdating the Mesozoic even under the more conservative lognormal model (mean age of 29.6 Ma, 95% HPD interval of 14.3–43.9 Ma; Fig. 4A). As contrasting examples, the divergence of *Karripurcellia* (West Australian pettalids) and *Austropurcellia* (East Australian pettalids) is estimated at 129.8 Ma

(95% HPD interval: 113.1–146.4 Ma; Giribet et al., 2012). The divergence of (*Kumbadjena* + *Occiperipatoides*) (West Australian peripatopsids) and an East Australian clade composed of four genera is estimated at 111 Ma (95% HPD interval: 80–145 Ma; Murienne et al., 2013).

Furthermore, we obtained a placement of Cercophonius as nested within a grade of South American species (Fig. 2), consistent with morphological and total evidence phylogenies of Bothriuridae with denser (Prendini, 2000) or nearly complete (Mattoni et al., 2010) taxonomic sampling. Thus, the biogeographic history of bothriurids may not reflect the classic East-West Gondwanan mutual monophyly observed in Peripatopsidae and Pettalidae (Fig. 4b). The origin and diversification of Cercophonius may instead reflect subsequent vicariance across major trans-Antarctic corridors between southern South America and Australia that persisted through the Cenozoic until ca. 30 Ma (Sanmartín and Ronquist, 2004). One of the rare groups that may simultaneously reflect both the East-West Gondwanan split as well as the trans-Antarctic connections is the harvestman family Neopilionidae, wherein the genus Hesperopilio (distributed in Australia and Chile) is more closely related to Chilean Thrasychirus than to other Australian or New Zealand lineages (Vélez et al., 2014; Groh and Giribet, 2014). However, as molecular dating was not conducted by Vélez et al. (2014), it remains to be tested whether the diversification history of Hesperopilio is contemporaneous with that of Cercophonius.

5. Conclusion

This work constitutes the first phylogenomic dating of the arachnid order Scorpiones, toward establishing the timing of internal diversification of the temperate Gondwanan family Bothriuridae. Our results indicate that crown group scorpions are ancient, dating at least to the Permian or earlier, and thus diversified prior to the breakup of Pangea. Accordingly, the age of Bothriuridae and the timing of the East–West Gondwanan split within this group overlap age estimates of corresponding splits in other ancient panarthropod groups that exemplify temperate Gondwanan diversification. We establish the placement of Bothriuridae in the scorpion tree of life near the base of Iurida, which is anticipated to guide the selection of outgroup taxa in future studies of this family, and justifies the systematic distinction between the superfamilies Bothriuroidea and Scorpionoidea (*sensu* Sharma et al., 2015).

Acknowledgments

This work is dedicated to our colleague Camilo Mattoni, a scholar of bothriurid systematics, who provided helpful discussions and generously shared his wisdom during the preparation of this work. We are indebted to Associate Editor Miquel Arnedo, Andrés A. Ojanguren-Affilastro, and two anonymous referees for improving an earlier draft of this work. The *Urodacus planimanus* library used in this analysis was supplied by Mike Rix (now Queensland Museum) and was produced as part of an Australian Research Council grant number LP120200092 awarded to Andrew Austin (University of Adelaide), Mike Rix, Mark Harvey, and Steve Cooper (South Australian Museum). Fieldwork in Queensland was supported by NSF DEB-1020809 to SLB. PPS was supported by NSF IOS-1552610.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.01.003.

References

- Allwood, J., Gleeson, D., Mayer, G., Daniels, S., Beggs, J.R., Buckley, T.R., 2010. Support for vicariant origins of the New Zealand Onychophora. J. Biogeogr. 37, 669–681. http://dx.doi.org/10.1111/j.1365-2699.2009.02233.x.
- Altenhoff, A.M., Schneider, A., Gonnet, G.H., Dessimoz, C., 2011. OMA 2011: Orthology inference among 1000 complete genomes. Nucleic Acids Res. 39, D289–D294.
- Altenhoff, A.M., Gil, M., Gonnet, G.H., Dessimoz, C., 2013. Inferring hierarchical orthologous groups from orthologous gene pairs. PLoS One 8, e53786.
- Baptista, C., Santiago-Blay, J.A., Soleglad, M.E., Fet, V., 2006. The Cretaceous scorpion genus, Archaeobuthus, revisited (Scorpiones: Archaeobuthidae). Euscorpius 35, 1–40.
- Borner, J., Rehm, P., Schill, R.O., Ebersberger, I., Burmester, T., 2014. A transcriptome approach to ecdysozoan phylogeny. Mol. Phylogenet. Evol. 80, 79–87. http://dx.doi. org/10.1016/j.ympev.2014.08.001.
- Boyer, S.L., Clouse, R.M., Benavides, L.R., Sharma, P., Schwendinger, P.J., Karunarathna, I., Giribet, G., 2007. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. J. Biogeogr. 34, 2070–2085. http://dx.doi.org/10.1111/jbi.2007.34.issue-12.
- Boyer, S.L., Giribet, G., 2007. A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. Cladistics 23, 337–361. http://dx.doi.org/10.1111/j.1096-0031.2007.00149.x.
- Boyer, S.L., Giribet, G., 2009. Welcome back New Zealand: regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). J. Biogeogr. 36, 1084–1099. http://dx.doi.org/10.1111/ j.1365-2699.2009.02092.x.
- Ceccarelli, F.S., Ojanguren-Affilastro, A.A., Ramírez, M.J., Ochoa, J.A., Mattoni, C.I., Prendini, L., 2016. Andean uplift drives diversification of the bothriurid scorpion genus *Brachistosternus*. J. Biogeogr. 43, 1942–1954.
- Coddington, J.A., Giribet, G., Harvey, M.S., Prendini, L., Walter, D.E., 2004. Arachnida. In: Cracraft, J., Donoghue, M.J. (Eds.), Assembling the Tree of Life. Oxford University Press, New York, NY, pp. 296–318.
- Daniels, S.R., Dambire, C., Klaus, S., Sharma, P.P., 2016. Unmasking alpha diversity, cladogenesis and biogeographical patterning in an ancient panarthropod lineage (Onychophora: Peripatopsidae: *Opisthopatus cinctipes*) with the description of five novel species. Cladistics 32, 506–537.
- Dunlop, J.A., Anderson, L.I., Kerp, H., Hass, H., 2004. A harvestman (Arachnida: Opiliones) from the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. Trans. R. Soc. Edinburgh Earth Sci. 94, 341–354.

Dunlop, J.A., 2010. Geological history and phylogeny of Chelicerata. Arthr. Struct. Dev. 39, 124–142. http://dx.doi.org/10.1016/j.asd.2010.01.003.
Fet, V., Soleglad, M.E., 2005. Contributions to scorpion systematics. I. On recent changes

in high-level taxonomy. Euscorpius 31, 1-13.

- Garrison, N.L., Rodriguez, J., Agnarsson, I., Coddington, J.A., Griswold, C.E., Hamilton, C.A., Hedin, M., Kocot, K.M., Ledford, J.M., Bond, J.E., 2016. Spider phylogenomics: untangling the spider tree of life. PeerJ 4, e1719. http://dx.doi.org/10.7717/peerj. 1719.
- Garwood, R.J., Dunlop, J.A., Giribet, G., Sutton, M.D., 2011. Anatomically modern Carboniferous harvestmen demonstrate early cladogenesis and stasis in Opiliones. Nat. Commun. 2, 444–447. http://dx.doi.org/10.1038/ncomms1458.
- Garwood, R.J., Dunlop, J.A., Selden, P.A., Spencer, A.R.T., Atwood, R.C., Vo, N.T., Drakopoulos, M., 2016. Almost a spider: a 305-million-year-old fossil arachnid and spider origins. Proc. R. Soc. B 283, 20160125–20160128. http://dx.doi.org/10.1098/ rspb.2016.0125.
- Garwood, R.J., Sharma, P.P., Dunlop, J.A., Giribet, G., 2014. A Paleozoic stem group to mite harvestmen revealed through integration of phylogenetics and development. Curr. Biol. 24, 1017–1023. http://dx.doi.org/10.1016/j.cub.2014.03.039.
- Giribet, G., Boyer, S.L., Baker, C., Fernández, R., Sharma, P.P., de Bivort, B.L., Daniels, S.R., Harvey, M.S., Griswold, C.E., 2016. A molecular phylogeny of the temperate Gondwanan family Pettalidae (Arachnida, Opiliones, Cyphophthalmi) with biogeographic and taxonomic implications. Zool. J. Linn. Soc. 178, 523–545.
- Giribet, G., Sharma, P.P., Benavides, L.R., Boyer, S.L., Clouse, R.M., de Bivort, B.L., Dimitrov, D., Kawauchi, G.Y., Murienne, J., Schwendinger, P.J., 2012. Evolutionary and biogeographical history of an ancient and global group of arachnids (Arachnida: Opiliones: Cyphophthalmi) with a new taxonomic arrangement. Biol. J. Linn. Soc. 105, 92–130.
- Groh, S., Giribet, G., 2014. Polyphyly of Caddoidea, reinstatement of the family Acropsopilionidae in Dyspnoi, and a revised classification system of Palpatores (Arachnida, Opiliones). Cladistics 31, 277–290. http://dx.doi.org/10.1111/cla. 12087.
- Harvey, M.S., Rix, M.G., Harms, D., Giribet, G., Vink, C.J., Walter, D.E., 2017. The biogeography of Australasian arachnids. In: Ebach, M. (Ed.), Handbook on Australasian Biogeography. CRC Press, Boca Raton.
- Jeram, A.J., 1998. Phylogeny, classifications and evolution of Silurian and Devonian scorpions. In: Selden, P.A. (Ed.), Proceedings of the 17th European Colloquium of Arachnology, Edinburgh 1997. The British Arachnological Society, Burnham Beeches, pp. 17–31.
- Kjellesvig-Waring, E.N., 1986. A restudy of the fossil Scorpionida of the world. Palaeontogr. Am. 55, 1–287.
- Lamoral, B.H., 1980. A reappraisal of the suprageneric classification of recent scorpions and their zoogeography. In: Gruber, J., (Ed.), Verhandlungen. 8. Internationaler Arachnologen-Kongress abgehalten ander Universität für Bodenkultur Wien, 7–12 Juli, 1980. Vienna (Austria): H. Egermann, pp. 439–444.
- Lartillot, N., Philippe, H., 2004. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. Mol. Biol. Evol. 21, 1095–1109.
- Lartillot, N., Rodrigue, N., Stubbs, D., Richer, J., 2013. PhyloBayes MPI: Phylogenetic reconstruction with infinite mixtures of profiles in a parallel environment. Syst. Biol. 62, 611–615.
- Le, S.Q., Dang, C.C., Gascuel, O., 2012. Modeling protein evolution with several amino acid replacement matrices depending on site rates. Mol. Biol. Evol. 29, 2921–2936.
- Legg, D.A., Sutton, M.D., Edgecombe, G.D., 2013. Arthropod fossil data increase congruence of morphological and molecular phylogenies. Nat. Commun. 4, 2485. http:// dx.doi.org/10.1038/ncomms3485.
- Lepage, T., Bryant, D., Philippe, H., Lartillot, N., 2007. A general comparison of relaxed molecular clock models. Mol. Biol. Evol. 24, 2669–2680. http://dx.doi.org/10.1093/ molbev/msm193.
- Lourenço, W.R., 2000. Reproduction in scorpions, with special reference to parthenogenesis. Eur. Arachnol. 2002, 71–85.
- Lourenço, W.R., 1996. Can a bothriurid scorpion be present in the Himalayas of India? Entomol. Mitteil. Zool. Mus. Hamburg 12 (154), 83–90.
- Lourenço, W.R., 2001. A remarkable fossil scorpion from the amber of Lebanon. Implications for the phylogeny of Buthoidea. Comptes Rendus des Séances de l'Académie des Science 332, 641–646.
- Mattoni, C., Ochoa, J., Ojanguren-Affilastro, A., Prendini, L., 2010. Towards an all-species phylogeny of the scorpion family Bothriuridae. Cladistics 26, 216–217. Monod, L., Cauwet, L., González-Santillán, E., Huber, S., 2017. The male sexual apparatus
- Monod, L., Cauwet, L., González-Santillán, E., Huber, S., 2017. The male sexual apparatus in the order Scorpiones (Arachnida): a comparative study of functional morphology as a tool to define hypotheses of homology. Front. Zool. 14, 51.
- Murienne, J., Daniels, S.R., Buckley, T.R., Mayer, G., Giribet, G., 2013. A living fossil tale of Pangaean biogeography. 20132648-20132648. Proc. R. Soc. B 281. http://dx.doi. org/10.1073/pnas.1102473108.
- Murienne, J., Edgecombe, G.D., Giribet, G., 2010. Including secondary structure, fossils and molecular dating in the centipede tree of life. Mol. Phylogenet. Evol. 57, 301–313. http://dx.doi.org/10.1016/j.ympev.2010.06.022.
- Obst, M., Faurby, S., Bussarawit, S., Funch, P., 2012. Molecular phylogeny of extant horseshoe crabs (Xiphosura, Limulidae) indicates Paleogene diversification of Asian species. Mol. Phylogenet. Evol. 62, 21–26. http://dx.doi.org/10.1016/j.ympev.2011. 08.025.
- Ojanguren-Affilastro, A.A., 2013. Bothriuridae, Cercophonius Peters, 1861. In: Kovarik, F., Ojanguren-Affilastro, A., (Eds.), Illustrated Catalog of Scorpions, Part II. Bothriuridae; Chaerilidae; Buthidae I., genera Compsobuthus, Hottentotta, Isometrus, Lychas and Sassanidotus.
- Ojanguren-Affilastro, A.A., Adilardi, R.S., Mattoni, C.I., Ramírez, M.J., Ceccarelli, F.S., 2017. Dated phylogenetic studies of the southernmost American buthids (Scorpiones; Buthidae). Mol. Phylogenet. Evol. 110, 39–49.
- Paradis, E., 2013. Molecular dating of phylogenies by likelihood methods: A comparison of models and a new information criterion. Mol. Phylogenet. Evol. 67, 436–444. http://dx.doi.org/10.1016/j.ympev.2013.02.008.

- Peretti, A.V., Battán-Horenstein, M., 2003. Comparative aspects of the male reproductive system in Bothriuridae scorpions: struc- ture associated with the paraxial organs and the presence of sperm packages (Chelicerata, Scorpiones). Zool. Anz. 242, 21–31.
- Philippe, H., Brinkmann, H., Lavrov, D.V., Littlewood, D.T.J., Manuel, M., Wörheide, G., Baurain, D., 2011. Resolving difficult phylogenetic questions: why more sequences are not enough. PLoS Biol. 9, e1000602. http://dx.doi.org/10.1371/ journal.pbio. 10006602.
- Prendini, L., 2000. Phylogeny and classification of the superfamily Scorpionoidea Latreille 1802 (Chelicerata, Scorpiones): an exemplar approach. Cladistics 16, 1–78. http://dx.doi.org/10.1006/clad.1999.0127.
- Rehm, P., Pick, C., Borner, J., Markl, J., Burmester, T., 2012. The diversity and evolution of chelicerate hemocyanins. BMC Evol. Biol. 12, 19. http://dx.doi.org/10.1186/ 1471-2148-12-19.
- Rokas, A., Carroll, S.B., 2005. More genes or more taxa? The relative contribution of gene number and taxon number to phylogenetic accuracy. Mol. Biol. Evol. 22, 1337–1344. http://dx.doi.org/10.1093/molbev/msi121.

Selden, P.A., 1996. First fossil mesothele spider, from the Carboniferous of France. Rev. Suisse Zool. hors série 585–596.

- Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by eventbased models: plant versus animal Patterns. Syst. Biol. 53, 216–243. http://dx.doi. org/10.1080/10635150490423430.
- Selden, P., Shear, W., Sutton, M., 2008. Fossil evidence for the origin of spider spinnerets, and a proposed arachnid order. Proc. Natl. Acad. Sci. 105, 20781–20785.
- Sharma, P., Giribet, G., 2014. A revised dated phylogeny of the arachnid order opiliones. Front. Genet 5, 255. http://dx.doi.org/10.3389/fgene.2014.00255/abstract.
- Sharma, P.P., Fernández, R., Esposito, L.A., Gonzalez-Santillan, E., Monod, L., 2015. Phylogenomic resolution of scorpions reveals multilevel discordance with morphological phylogenetic signal. Proc. R. Soc. B 282http://dx.doi.org/10.1093/molbev/ mss208. 20142953-20142953.
- Sharma, P.P., Kaluziak, S.T., Perez-Porro, A.R., Gonzalez, V.L., Hormiga, G., Wheeler, W.C., Giribet, G., 2014. Phylogenomic interrogation of Arachnida reveals systemic conflicts in phylogenetic signal. Mol. Biol. Evol. 31, 2963–2984. http://dx.doi.org/ 10.1093/molbev/msu235.
- Sharma, P.P., Wheeler, W.C., 2014. Cross-bracing uncalibrated nodes in molecular dating improves congruence of fossil and molecular age estimates. Front. Zool. http://dx.

doi.org/10.1186/s12983-014-0057-x.

- Sharma, P.P., Wheeler, W.C., 2013. Revenant clades in historical biogeography: the geology of New Zealand predisposes endemic clades to root age shifts. J. Biogeogr. 40, 1609–1618. http://dx.doi.org/10.1111/jbi.12112.
- Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.
- Sissom, W.D., 1990. Systematics, biogeography, and paleontology. In: Polis, G.A. (Ed.), The Biology of Scorpions. Stanford University Press, Stanford (CA), pp. 64–160. Soleglad, M.E., Fet, V., 2003. High-level systematics and phylogeny of the extant scor-
- pions (Scorpiones: Orthosterni). Euscorpius 11, 1–56. Soleglad, M.E., Fet, V., Kovarik, F., 2005. The systematic position of the scorpion genera
- Heterscorpton Birula, 1903 and Urodacus Peters, 1861 (Scorptones: Scorptonidea). Euscorptius 20, 1–38.
- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML Web servers. Syst. Biol. 57, 758–771.
- Stockwell, S.A., 1989. Revision of the Phylogeny and Higher Classification of Scorpions (Chelicerata). Ph.D. Thesis, University of Berkeley, Berkeley, California. University Microfilms International, Ann Arbor, Michigan. 319 pp.
- Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lect. Math. Life Sci. 17, 57–86.
- Telford, M.J., Lowe, C.J., Cameron, C.B., Ortega-Martinez, O., Aronowicz, J., Oliveri, P., Copley, R.R., 2014. Phylogenomic analysis of echinoderm class relationships supports Asterozoa. Proc. Roy. Soc. B 281http://dx.doi.org/10.1007/s00427-009-0277-9. 20140479-20140479.
- Vélez, S.N., Fernández, R., Giribet, G., 2014. A molecular phylogenetic approach to the New Zealand species of Enantiobuninae (Opiliones : Eupnoi : Neopilionidae). Invertebr. Syst. 28, 565. http://dx.doi.org/10.1071/IS14030.
- Waddington, J., Rudkin, D.M., Dunlop, J.A., 2015. A new mid-Silurian aquatic scorpion – one step closer to land? Biol. Lett. 11http://dx.doi.org/10.1016/j.palaeo.2008. 05.008. 20140815-20140815.
- Whelan, N.V., Kocot, K.M., Moroz, L.L., Halanych, K.M., 2015. Error, signal, and the placement of Ctenophora sister to all other animals. Proc. Natl. Acad. Sci. 201503453. http://dx.doi.org/10.1073/pnas.1503453112.