



Exploring the evolution and terrestrialization of scorpions (Arachnida: Scorpiones) with rocks and clocks

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Abstract

Scorpions (Arachnida: Scorpiones Koch, 1837) are an ancient chelicerate arthropod lineage characterised by distinctive subdivision of the opisthosoma and venomous toxicity. The crown group is represented by over 2400 extant species, and unambiguous fossil representatives are known at least from the Cretaceous Period. However, a number of extinct scorpion lineages existed in the Palaeozoic Era, many of which are of a contentious marine (or at least semi-aquatic) lifestyle, and have long caused confusion regarding the nature of arachnid terrestrialization and arachnid phylogeny more broadly. To clarify the process of terrestrialization, there is a need to marry fossil and extant scorpions in a common evolutionary framework utilising modern advances in phylogenetics. Here, we review phylogenetic hypotheses of arachnid and scorpion interrelationships, relevant advances in phylogenetic divergence time estimation and the scorpion fossil record—especially with reference to terrestrialization. In addition, we provide a list of scorpion fossil calibrations for use in molecular dating and demonstrate their utility in deriving a novel scorpion time tree using Bayesian relaxed-clock methods. Our results reveal a window of divergence from 335 to 266 Mya for the scorpion crown group, consistent with a Pangean origin of crown scorpions inferred from the biogeographical distribution of the extant fauna.

Keywords Scorpions · Arachnids · Molecular clocks · Terrestrialization · Phylogenetics · Palaeobiology

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Introduction

Scorpions (Arachnida: Scorpiones Koch, 1837) are a relatively speciose (~2400 extant species, <https://www.ntnu.no/ub/scorpion-files/>) and medically significant (e.g. Isbister and Saluba Bawaskar 2014) group of chelicerates. The group has an almost cosmopolitan biogeographical distribution, being absent only in boreal environments, Antarctica and on some more isolated island land masses—though they have been translocated as anthropogenic introductions (e.g. Wanless 1977). Like most arachnids, with the exception of the more ecologically diverse mites, scorpions are predators, generally feeding on other arthropods and occasionally small vertebrates.

Extant scorpions are instantly recognisable in possessing a pair of chelate pincer-like pedipalps, a post-anal telson equipped with a stinger, and a pair of unique ventral comb-like sensory organs called pectines. The scorpion body plan is also unique among arachnids with respect to its tagmosis. Scorpions exhibit a clearly demarcated tripartite organisation

consisting of an anterior appendage-bearing prosoma (as in all chelicerates generally), a medial mesosoma that houses the reproductive and respiratory systems, and a posterior tail-like metasoma that terminates with the anus and precedes the telson—comprising a vesicle and aculeus via which venom is delivered.

Scorpions have an ancient evolutionary history, represented by a reasonably continuous fossil record stretching back as far as the Telychian Stage (Silurian, Llandovery) (Jeram 1998; Dunlop 2010; Dunlop and Selden 2013; Waddington et al. 2015). Scorpions therefore potentially constituted a component of the earliest faunas of complex terrestrial ecosystems, along with myriapods and, at least by the Early Devonian, hexapods (see Lozano-Fernandez et al. 2016). Little has been done however to constrain the radiation of the extant and fossil scorpion lineages together in a common evolutionary framework. This is unfortunate as the evolutionary radiation(s) of scorpions is of interest in the study of arthropod macroevolution and biogeography, terrestrialization (both from a physiological perspective and in the context of the evolution of the Earth system), and in resolving the problematic phylogenetic relationships between the arachnid orders.

As such, we aim to provide an interdisciplinary synthesis on scorpion evolution by reviewing modern and historical phylogenetic hypotheses of scorpion interrelationships, and scorpion palaeontology—framed within the context of arachnid terrestriality. We highlight the complementary nature of scorpions to critically evaluate molecular dating studies, and provide additional fossil calibrations for dating the scorpion Tree of Life. In addition, we apply these fossil calibrations in a Bayesian relaxed-clock analysis in order to constrain the age of the scorpion crown group.

Scorpions on the arachnid Tree of Life

Within the Chelicerata, scorpions belong to the familiar group Arachnida, the systematic origin of which can be traced back to the nineteenth century French naturalist Jean-Baptiste Lamarck (Lamarck 1801). In its modern iteration, Arachnida usually comprises 16 orders. These include the living Scorpiones (true scorpions), Pseudoscorpiones (pseudoscorpions or false scorpions), Araneae (spiders), Amblypygi (whip spiders or tailless whip scorpions), Thelyphonida (whip scorpions or vinegaroons), Schizomida (short-tailed whip scorpions), Acariformes (acariform mites), Parasitiformes (ticks and parasitiform mites), Opiliones (daddy long legs or harvestmen), Solifugae (sun spiders or camel spiders), Ricinulei (hooded tick spiders) and Palpigradi (micro whip scorpions); and the extinct Haptopoda, Phalangiotarbida, Trigonotarbita and Uraraneida.

Whilst the monophyly of the living arachnid groups (apart from the mites and ticks) is virtually undisputed (Dunlop et al.

2014), their interrelationships are generally poorly resolved (Fig. 1), though some reasonable higher level clades are emergent. Tetrapulmonata—comprising Araneae, Amblypygi, Thelyphonida, Schizomida, Uraraneida and Haptopoda; plus Trigonotarbita as Pantetrapulmonata (Shultz 2007)—is the most stable of these, with strong morphological support in the form of, among other characters, a common respiratory configuration with book lungs on the same two opisthosomal segments (Shultz 1990, 2007). Multiple molecular phylogenetic studies have also given weight to Tetrapulmonata (Wheeler and Hayashi 1998; Shultz and Regier 2000; Pepato et al. 2010; Regier et al. 2010; Rehm et al. 2012; Sharma et al. 2014), and the most recent and data-rich of these studies have allied this group to scorpions. Using different multilocus and phylogenomic-scale datasets Regier et al. (2010) and Sharma et al. (2014) each recovered a sister group relationship between Tetrapulmonata and Scorpiones. Sharma et al. (2014) renamed this clade Arachnopulmonata, replacing the earlier Pulmonata (Firstman 1973) to avoid confusion with the clade of terrestrial molluscs of the same name. Scorpions, like tetrapulmonates, possess book lungs, whereas all other arachnids have non-pulmonate respiratory systems (typically tracheae); and comparative study of scorpion and tetrapulmonate book lungs has revealed detailed structural similarities that are consistent with them being homologous (Scholtz and Kamenz 2006). Likewise, comparative work on the tetrapulmonate and scorpion vascular systems has implied homology (Klößmann-Fricke and Wirkner 2016). Furthermore, it has been identified that scorpions and spiders share a common ancestral whole genome duplication that is present in all arachnopulmonates, therefore being an additional line of evidence suggesting the monophyly of the group (Leite et al. 2018).

Previous phylogenetic hypotheses based on morphology have posited scorpions in a range of positions on the chelicerate tree including (I) sister group to the remaining Arachnida (Weygoldt and Paulus 1979); (II) not arachnids at all, but closer to eurypterids (sea scorpions) (Dunlop and Braddy 2001); and (III) distant from the tetrapulmonates but within Arachnida, allied with Opiliones (Shultz 1990, 2007; Wheeler and Hayashi 1998; Giribet et al. 2002). Hypothesis 1 (Fig. 2a) and hypothesis 3 (Fig. 2c) imply that the seemingly homologous book lungs are either homoplastic or are an arachnid symplesiomorphy that has been lost by all non-pulmonate lineages—which is not supported by any known fossils (but equally the lack of consensus in arachnid phylogeny complicates reconstructing the plesiomorphic condition for arachnids). Hypothesis 2 (Fig. 2b) again requires book lung homoplasy, and that scorpions share a marine origin with eurypterids. All of these morphological hypotheses are therefore less well-supported than Arachnopulmonata (hypothesis 4, Fig. 2d), which is corroborated by molecular and morphological evidence.

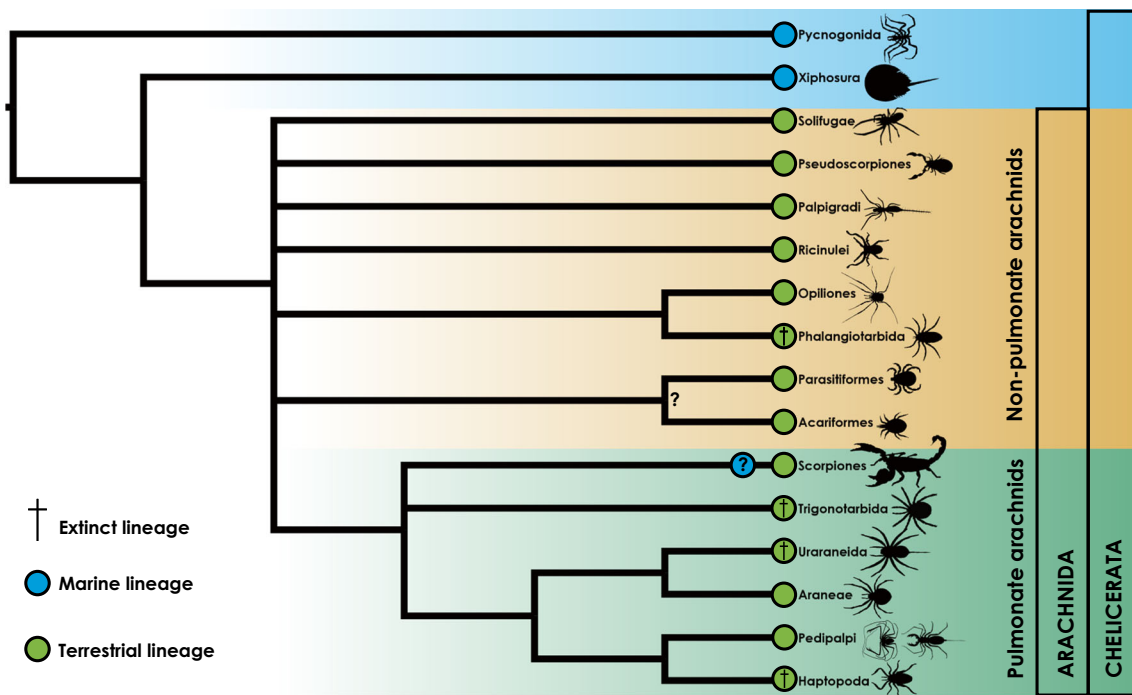


Fig. 1 General consensus of internal phylogenetic relationships of Chelicerata at present. Question marks represent uncertainty surrounding the monophyly of mites and ticks (Acari) and the unresolved marine life habit of early scorpions

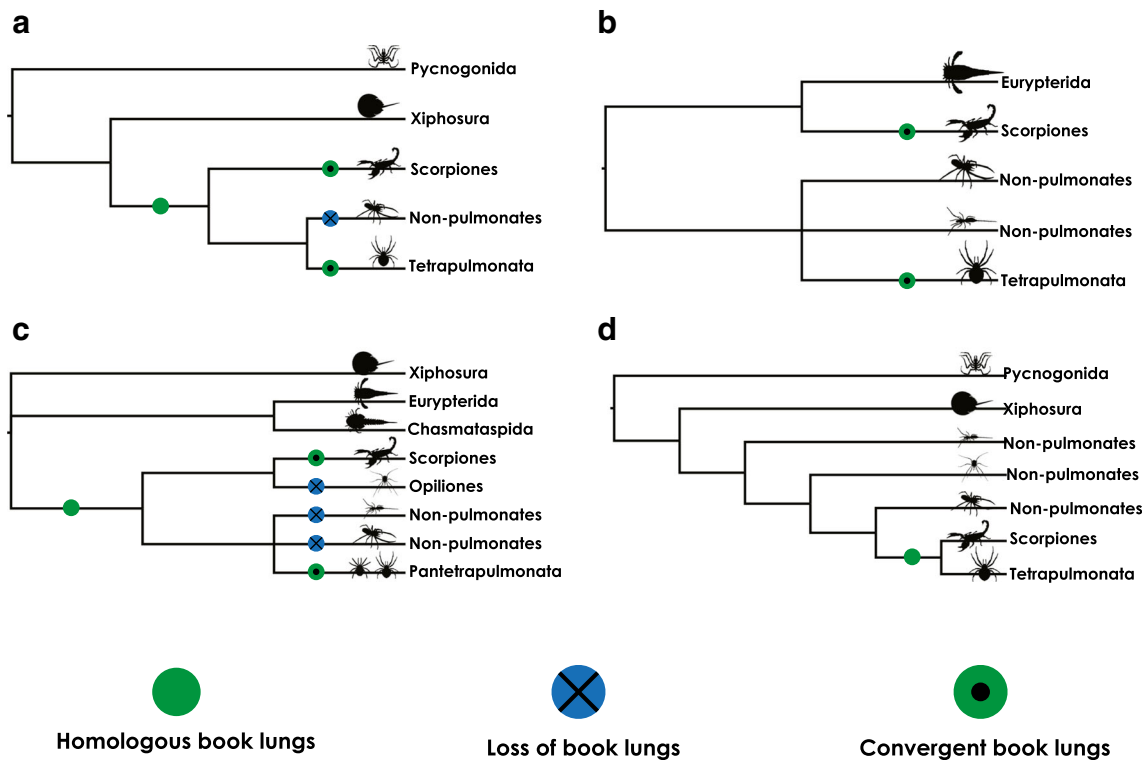


Fig. 2 Optimisation of book lung origin(s) on competing phylogenies of Chelicerata. **a** Scorpions as the sister group to other Arachnida (e.g. Weygoldt and Paulus 1979), implying either book lung loss in other Arachnida or book lung convergence between scorpions and tetrapulmonates. **b** Scorpions as sister group to Eurypterida (e.g. Dunlop and Braddy 2001), implying book lung convergence and

multiple terrestrialization events. **c** Scorpions as sister group to Opiliones (e.g. Shultz 1990, 2007; Wheeler and Hayashi 1998; Giribet et al. 2002), implying either book lung loss in other Arachnida or book lung convergence between scorpions and tetrapulmonates. **d** Arachnopulmonata—scorpions cluster with tetrapulmonates (e.g. Sharma et al. 2014), implying a single origin of book lungs

The internal phylogeny of Scorpiones, like that of Arachnida, is also of considerable contention, with new hope of consensus emerging with the rise of more sophisticated molecular phylogenetic methods. Earlier phylogenetic hypotheses, based almost exclusively on morphological characters (Lamoral 1980; Stockwell 1989; Sissom 1990; Soleglad and Fet 2003; Coddington 2004), conflict in some respects with the most recent hypotheses based on transcriptomes (Sharma et al. 2015, 2018) (see Fig. 3), and no morphological hypothesis has ever received widespread acceptance. Subsequently, this has led to conflict of opinion (Fet and Soleglad 2005; Prendini and Wheeler 2005) and a state of flux

in the taxonomic nomenclature of the group. Morphological tradition postulates a basal dichotomy between the family Buthidae (usually recognisable by the thin tweezer-like pedipalps and robust metasoma) and the non-buthid scorpions, with the positions of the ‘living fossil’ Pseudochactidae (Gromov 1998; Prendini et al. 2006) and the monogeneric Chaerilidae being subject to debate as they share characters with both buthid and non-buthid scorpions. The lack of morphological consensus may be a consequence of morphological stasis, which was suggested by Sharma et al. (2015), but no studies covering the breadth of scorpion diversity have attempted to quantify this in a morphometric context. The

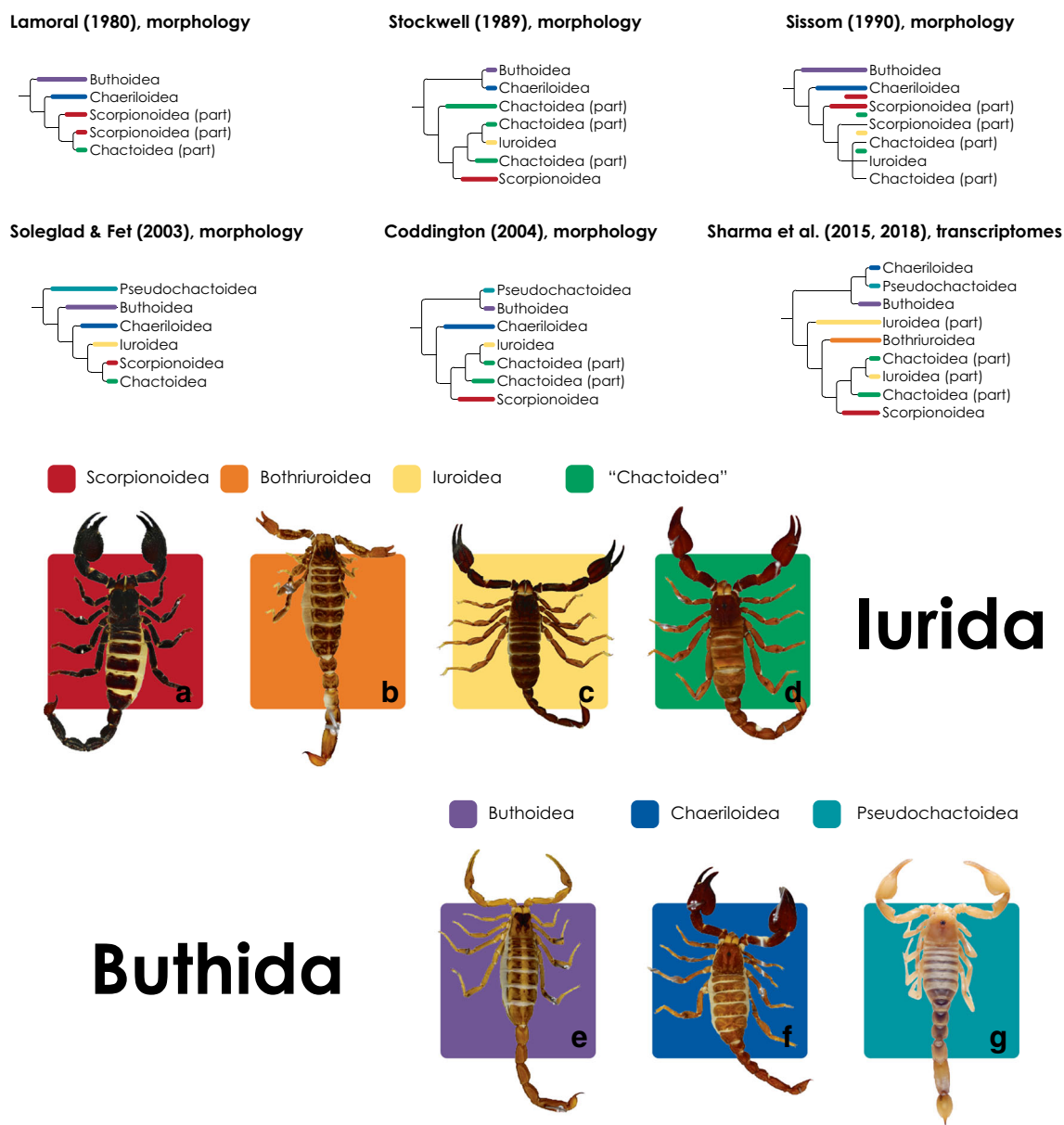


Fig. 3 Phylogenetic hypotheses of scorpion relationships, with representative taxa for each major group. (A) *Pandinus* (*Pandinopsis*) *dictator* Pocock, 1888; (B) *Cercophonius squama* Gervais, 1844; (C) *Iurus dufourei* Brullé, 1832; (D) *Brotheas* sp.; (E) *Centruroides*

vittatus Say, 1821; (F) *Chaerilus variegatus* Simon, 1877; (G) *Pseudochactas mischi* Soleglad et al. 2012 (image courtesy of Frantisek Kovarik). All other scorpion images by the authors

transcriptome-based study of Sharma et al. (2015) controverted morphological hypotheses, refuting the monophyly of a number of groups at various taxonomic levels, and places Buthidae, Pseudochactidae and Chaerilidae together in a clade (Buthida) which in turn is the sister group to all remaining extant scorpions (Iurida).

New frontiers in total evidence phylogeny

Morphological and molecular studies of scorpion phylogeny have so far failed to converge upon a common answer, and this is obfuscated further by a lack of fossil record integration beyond the work of Stockwell (1989) and subsequently Jeram (1994, 1998)—each limited to parsimony analyses of morphological characters. The rich scorpion fossil record is informative of stem group diversity, character evolution, and provides temporal constraints for molecular dating. Resolving the relative timings of the evolutionary divergences between species and clades in the geological past yields crucial information for interpreting evolutionary phenomena. Therefore, accurately dating the phylogenetic divergences of wholly terrestrial arthropod clades is of paramount importance in understanding the evolution of the terrestrial biosphere. Reconstructing such ‘time trees’, or phylograms, is becoming increasingly methodologically sophisticated and has become prominent as the backbone for comparative studies in evolutionary biology and palaeontology.

Molecular phylogenies were initially dated by assuming a constant clocklike rate of molecular evolution (known as the strict molecular clock), and calibrated with reference to the fossil record (Zuckerlandl and Pauling 1962a, b). However, it has long been known that the rate of molecular evolution changes across sites, genes and lineages. To address these problems, a variety of models have been developed to relax the assumptions of the molecular clock (e.g. Sanderson 1996; Rambaut and Bromham 1998; Thorne et al. 1998; Thorne and Kishino 2002; Drummond et al. 2006; Lepage et al. 2007; Linder et al. 2011; Ronquist et al. 2012). Accordingly, current software to estimate divergence times integrate fossil evidence and genomic information in a Bayesian framework (Heled and Drummond 2011) using ‘relaxed’ molecular clock models (Drummond and Rambaut 2007; Yang 2007; Höhna et al. 2016). Two alternative approaches have been developed to integrate fossil information in molecular clock analyses. The most commonly used is ‘node-dating’, where stratigraphic range data based on the occurrence of fossil taxa are assigned probability distributions (Yang and Rannala 2006) and used to describe prior knowledge on the age of a set of nodes in the phylogeny (see Dos Reis et al. 2015). The second is ‘tip-dating’ where the fossils are directly integrated into the analysis through the generation of a ‘total evidence’ (i.e. molecular and morphological) dataset (Ronquist et al. 2012). Total evidence

dating differs over node-calibration methodologies in that it incorporates fossils into the analysis without prior assumption of their phylogenetic position, and can therefore directly integrate phylogenetic uncertainty in the placement of fossils.

However, there are still considerable obstacles to overcome for total evidence dating to become ‘the industry standard’, with studies often recovering demonstrably incorrect ages (e.g. Ronquist et al. 2012). O’Reilly et al. (2015) identified a number of key issues facing total evidence dating that seem to contribute to the frequent recovery of unrealistic divergence time estimates, including a lack of realistic models to describe morphological evolution, the non-random nature of missing character information in fossils and how to accommodate uncertainty in fossil ages. A significant development to overcoming these issues has come from the development of the fossilised birth-death process (Heath et al. 2014; Zhang et al. 2016), which takes advantage of the Bayesian approach to incorporate additional information concerning fossilisation and the sampling process, with the aim of uniting extinct and extant species with a single evolutionary model.

Dating the scorpion Tree of Life

Scorpions are the oldest arachnids in the fossil record (Dunlop 2010; Dunlop and Selden 2013), with *Dolichophonus loudonensis* Laurie, 1899 from the Pentland Hills, Scotland, being dated to the Telychian Stage of the Silurian Period (438.5–433.4 Mya). *D. loudonensis* remains the most ancient record of both scorpions and arachnids, and is therefore a critical fossil calibration point in node-calibrated divergence time estimations (Rota-Stabelli et al. 2013a; Wolfe et al. 2016; Sharma et al. 2018). To date, the most comprehensive attempt at dating the scorpion tree was that of Sharma et al. (2018), wherein a tree inferred from a large phylogenomic dataset was dated under relaxed-clock models using five node-calibrations based on arachnid fossils. The resultant age estimates recovered by Sharma et al. (2018) were significantly influenced by model selection and present wide distributions. The study used the autocorrelated lognormal and uncorrelated gamma multiplier clock models and recovered crown group divergence time estimates in the Silurian–Carboniferous (95% HPD 423.1–333.6 Mya) and Devonian–Triassic (95% HPD 380.6–209.1 Mya), respectively. Whilst model selection and data partitioning have a great effect on the precision of molecular dating, the results of Sharma et al. (2018) are also reflective of a paucity of candidate calibration fossils in node-dating the scorpion tree. Sharma et al. (2018) used two scorpion fossil calibrations, the oldest total group scorpion and the oldest crown group scorpion. Little has been done to clarify the phylogenetic relationships of fossil scorpions, particularly with

respect to extant diversity. As such, node-dating falls short when suitable calibrations are scarce, as it is ultimately reliant on prior interpretations of where fossils are located on the tree. The scorpions present an ideal scenario wherein total evidence dating could overcome the limitations of node-dating, but is itself limited by the challenges of interpreting the highly homoplastic nature of scorpion morphology through time. Therefore, at present, total evidence dating is unlikely to be possible without a new and bespoke morphological dataset for fossil and extant scorpion.

We therefore present a more comprehensive node-calibrated molecular dating analysis to accompany this review. Our results show that the origin of the scorpion crown group can be relatively precisely constrained using this method, provided more substantial (and systematically justified) fossil calibrations are applied (see Table 2 for additional scorpion calibration descriptions and Table 3 for full list of calibrations used in this study).

Phylogenomic matrix generation

Transcriptomes of 18 scorpions and five outgroups (two spiders, an amblypygid, a thelyphonid and a pseudoscorpion) were downloaded from NCBI (see Table 1), and mRNA transcripts were subsequently reconstructed using the Trinity assembler (Grabherr et al. 2011; Haas et al. 2013), and then translated into proteins using TransDecoder (<https://transdecoder.github.io/>). To then compile a phylogenomic matrix of protein-coding genes, we predicted the orthologs of a set of 290 conserved ecdysozoan protein-coding sequences (from the flour beetle *Tribolium castaneum* Herbst, 1797) gathered mostly from a previous study (Rota-Stabelli et al. 2013a) using a custom BLAST (Altschul et al. 1990) based pipeline (https://github.com/jairly/MoSuMa_tools). Selected hits of all taxa clustered in orthologous groups were then aligned using MUSCLE (Edgar 2004) with default settings, and the outputted gene alignments were concatenated

Table 1 Transcriptomes used to generate the multiple sequence alignment for phylogenetic analyses and divergence time estimation (see ‘Dating the scorpion Tree of Life’). AMNH, American Museum of Natural History

Group	Species	Source	Predicted peptides	Reference	Source institution
Scorpiones					
	<i>Androctonus australis</i>	Illumina	19,170	Sharma et al. (2015)	AMNH
	<i>Anuroctonus phaiodactylus</i>	Illumina	23,533	Sharma et al. (2015)	AMNH
	<i>Brotheas granulatus</i>	Illumina	19,513	Sharma et al. (2015)	AMNH
	<i>Centruroides vittatus</i>	Illumina	5024	NCBI SRR1146578	AMNH
	<i>Chaerilus celebensis</i>	Illumina	24,310	Sharma et al. (2015)	AMNH
	<i>Diplocentrus diablo</i>	Illumina	26,245	Sharma et al. (2015)	AMNH
	<i>Euscorpius italicus</i>	Illumina	20,096	Sharma et al. (2015)	AMNH
	<i>Hadogenes troglodytes</i>	Illumina	21,651	Sharma et al. (2015)	AMNH
	<i>Hadrurus arizonensis</i>	Illumina	19,266	Sharma et al. (2015)	AMNH
	<i>Iurus dekanum</i>	Illumina	17,619	Sharma et al. (2015)	AMNH
	<i>Liocheles australasiae</i>	Illumina	22,581	Sharma et al. (2015)	AMNH
	<i>Pandinus imperator</i>	Illumina	20,279	Sharma et al. (2015)	AMNH
	<i>Parabuthus transvaalicus</i>	Illumina	20,217	Sharma et al. (2015)	AMNH
	<i>Scorpiops</i> sp.	Illumina	24,941	Sharma et al. (2015)	AMNH
	<i>Tityus serrulatus</i>	Illumina	23,167	SR1575611	Butantan Institute
	<i>Troglokhammouanus steineri</i>	Illumina	19,657	Sharma et al. (2015)	AMNH
	<i>Urodacus yaschenkoi</i>	Illumina	30,675	NCBI SRR1557168	University of Melbourne
	<i>Vietbocap lao</i>	Illumina	20,007	Sharma et al. (2015)	AMNH
Araneae					
	<i>Liphistius malayanus</i> (Mesothelae)	Illumina	11,221	Fernández et al. (2014)	AMNH
	<i>Dysdera crocata</i> (Araneomorphae)	Illumina	30,336	Fernández et al. (2014)	Harvard
Amblypygi					
	<i>Damon variegatus</i>	Illumina GAI	11,823	Sharma et al. (2014)	AMNH
Thelyphonida					
	<i>Mastigoproctus giganteus</i>	Illumina GAI	17,674	Sharma et al. (2014)	AMNH
Pseudoscorpiones					
	<i>Synsphyronus apimelus</i>	Illumina	17,820	Sharma et al. (2014)	AMNH

using FASconCAT (Kück and Meusemann 2010) to generate a final super-alignment of 53,634 amino acid sites.

Phylogenetic analysis

From the super-alignment, we inferred a phylogenetic tree under maximum likelihood (ML) using IQ-TREE (Nguyen et al. 2015). We implemented the ProtTest option (Darriba et al. 2011) to select the best fitting substitution model (LG+F+I+G4, Le and Gascuel 2008) and used the ultrafast bootstrap approximation method (UFBoot, Minh et al. 2013; Hoang et al. 2018) to run 1000 bootstrap replicates. The resultant tree topology (see Fig. 4) was then used to run molecular dating analyses. We include supplementary data files containing our phylogenomic matrix and our tree file and dating results.

Molecular dating

Node-calibrated Bayesian relaxed-clock molecular dating analyses were performed in the program MCMCTree in the PAML 4 package (Yang 2007). First, we implemented the CODEML program (also in the PAML 4 package) to generate a Hessian matrix for the super-alignment under the LG model with gamma rates among sites. We then used the approximate likelihood method to estimate divergence times. Time priors were constructed from 9 soft upper and hard lower bounded calibration points with uniform distributions and a uniform birth-death process (Yang and Rannala 2006). Fossil calibrations were either revised from Wolfe et al. (2016), or newly described for this study (see Table 2 and Fig. 5). We used the MCMCTreeR

program (<https://github.com/PuttickMacroevolution/MCMCTreeR>) to generate calibration inputs for analyses in MCMCTree (Table 3). Analyses were repeated using both the independent and correlated rate relaxed-clock models in MCMCTree, and both iterations were again repeated to ensure convergence of the MCMC chains.

Results

Our results (summarised in Fig. 6) indicate that the scorpion crown group originated (i.e. the divergence between total group Buthida + Iurida) during an interval spanning the Carboniferous-Permian, possibly as early as the Viséan (Carboniferous, Mississippian) and possibly as late as the Wordian (Permian, Cisuralian). Both the correlated rate (CR) and independent rate (IR) models yielded similar estimates (CR = 287.28–335.03 Mya; IR = 266.27–324 Mya), indicating that these results are robust to model selection. This interval is comfortably within the stratigraphic range of the super-continent Pangaea, which had started to form earlier during the Devonian, and had largely assembled via the closure of the Rheic Ocean by the beginning of the Carboniferous (Nance and Linnemann 2008). Our estimate is therefore concordant with a hypothesis of Pangaeic vicariance to explain the global distribution of crown scorpions.

Deep nodes within the scorpion crown group are younger in our tree than those inferred by Sharma et al. (2018). Our estimation of the Buthida-Iurida divergence as Carboniferous-Permian contrasts with theirs as Silurian-Carboniferous, and the same applies to the deepest splits within Buthida

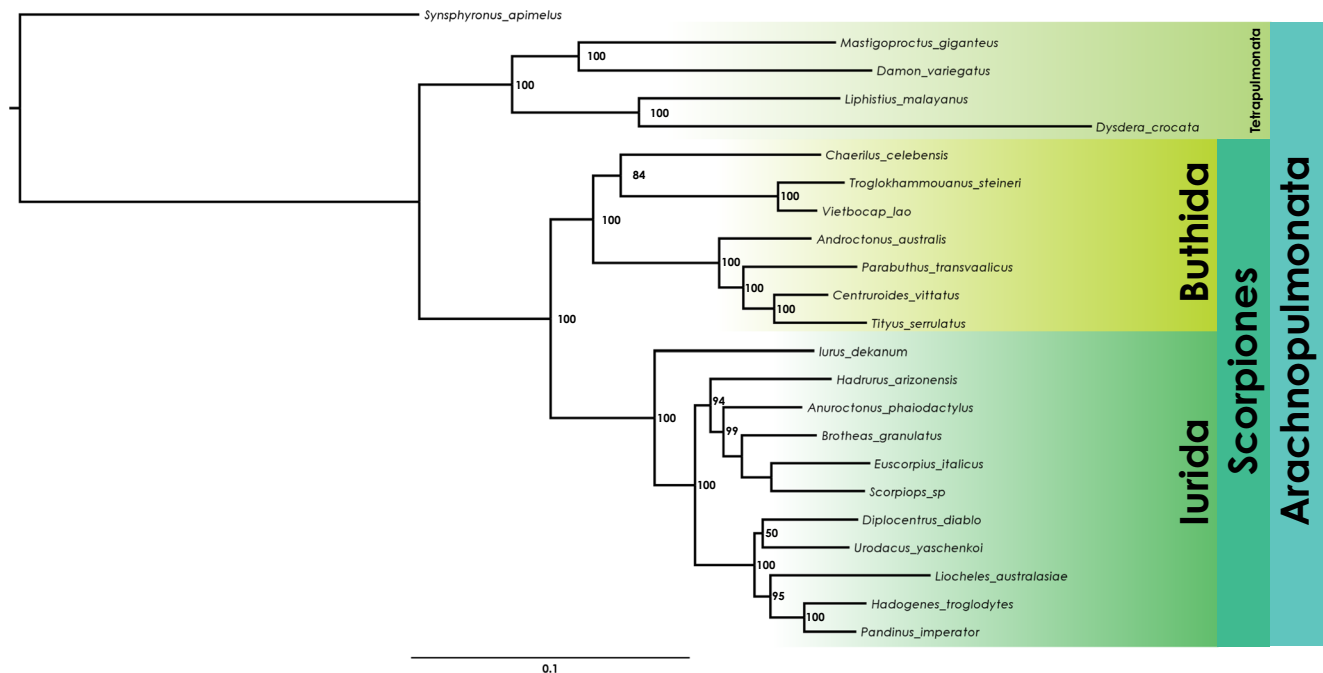


Fig. 4 Maximum likelihood tree inferred in IQ-TREE under LG+F+I+G4 model (Le and Gascuel 2008). Nodal support values determined from 1000 ultrafast bootstrap replicates (Minh et al. 2013; Hoang et al. 2018). Scale bar = branch length

Table 2 Scorpion fossil node-calibration points for molecular dating. NMS – National Museum of Scotland, MN – Museu Nacional, Rio de Janeiro, DMNH – Denver Museum of Natural History.

Node calibrated	Candidate fossil	Phylogenetic justification	Locality & minimum age	Soft maximum age	Comments
Total group Scorpiones	<i>Dolichophonus loudenensis</i> (Laurie 1899). NMS 1897.122.196 (holotype), figured in Wolfe et al. (2016) (Fig. 5H).	Total group scorpion.	“Eurypterid Bed”, Reservoir Formation, Pentland Hills, Scotland, UK. 435.15 Ma (Llandovery, Telychian), constrained by upper boundary of <i>Oktavites spiralis</i> Biozone (Melchin et al. 2012).	514 Ma (Cambrian Series 2, Stage 4), constrained by oldest known chelicerate <i>Wisangocaris barbarahydae</i> of the Emu Bay Shale, Kangaroo Island, Australia (Jago et al. 2016).	Oldest fossil scorpion, uncontroversial.
Crown Orthosterni	<i>Protoischnurus axelrodurum</i> (de Carvalho and Lourenço 2001). MN-7601-I (holotype), see (Menon 2007) for additional material.	Iurid scorpion, placed in extant family Hemiscorpiidae (Iurida, Scorpionoidea) by Menon (2007) based on a suite of morphological characters of the pedipalps.	Nova Olinda Member, Crato Formation, Araripe Basin, northeastern Brazil. 112.6 Ma (Lower Cretaceous, Aptian), constrained by Albian/Aptian boundary (Martill et al. 2007).	313.7 Ma (Lower Pennsylvanian, Bashkirian), constrained by the scorpion fauna of the British Middle Coal Measures. In a recent systematic revision, Legg et al. (2012) recover 8 valid terrestrially adapted scorpion species, none of which show all of the derived characters typical of crown group Orthosterni (i.e. dispersed lateral ocelli). The British Middle Coal measures are constrained by U-Pb dating of west European Carboniferous tuffs (Pointon et al. 2012). Example taxon <i>Compsoscorpius buthiformis</i> in Fig. 3.	Other scorpions identifiable to extant groups are known from the Crato Formation such as the chactid <i>Araripescorpis ligabuei</i> (Campos 1986). <i>P. axelrodurum</i> is the preferred taxon by Wolfe et al. (2016) in a review of arthropod fossil calibrations, based on preservation quality – and we retain it here. Older scorpions have been described as buthids, but have been rejected as such in cladistic studies (Soleglad and Fet 2001; Baptista et al. 2006). However, the studies cited are problematic, relying exclusively on trichobothrial characters, and treating predefined orthobothriotaxic “types” as terminals. Regardless, the position of taxa such as <i>Archaeobuthus</i> is poorly resolved, and therefore they are not suitable to constrain Orthosterni.
Crown Buthida	<i>Electrochaerilus buckleyi</i> (Santiago-Blay et al. 2004a). Private collection, figs 1–9 in Santiago-Blay et al. (2004b).	Chaerilid scorpion (Buthida, Chaeriloidea), identifiable as such from characters relating to trichobothrial pattern, sternum, pectines etc. (Santiago-Blay et al. 2004a).	Burmese amber. 98.17 Ma (Upper Cretaceous, Cenomanian), Burmese amber constrained by U-Pb dating of zircons from surrounding volcaniclastic matrix (Shi et al. 2012).	As for Crown Orthosterni.	Uncontroversial chaerilid scorpion, but known only from one specimen in a private collection.
Crown Buthoidea	<i>Uintascorpio halandrasi</i> (Perry 1995), and see (Santiago-Blay et al. 2004b). DMNH 6004 (holotype), figured in	Referred to Buthidae by Santiago-Blay et al. (2004b) based on the presence of numerous diagnostic characters including pedipalp and metasomal carination, and elongation of leg coxae IV.	Parachute Creek Member, Green River Formation, Uinta Basin, Colorado, USA. 49.26 Ma (Eocene, Ypresian), Parachute Creek Member constrained by U-Pb	As for Crown Orthosterni.	Uncontroversial buthoid scorpion. As discussed for Crown Orthosterni, older putative buthoids are known, but cannot be referred with confidence.

Table 2 (continued)

Node calibrated	Candidate fossil	Phylogenetic justification	Locality & minimum age	Soft maximum age	Comments
Crown Chaeriloidea	above references. As for Crown Buthida.	As for Crown Buthida.	dating (Skyline ash tuff, Smith and Carroll 2015) As for Crown Buthida.	As for Crown Buthida.	As for Crown Buthida.
Crown Iurida	As for Crown Orthosterni	As for Crown Orthosterni	As for Crown Orthosterni	As for Crown Orthosterni	As for Crown Orthosterni
Crown Scorpionoidea	As for Crown Orthosterni	As for Crown Orthosterni	As for Crown Orthosterni	As for Crown Orthosterni	As for Crown Orthosterni

(Permian-Triassic herein versus Devonian-Permian) and Iurida (Triassic-Jurassic herein versus Carboniferous-Triassic). These younger dates and shorter 95% highest probability density are likely attributable to the inclusion of more scorpion calibration fossils. In particular, setting a soft maximum for crown group Orthosterni based on Carboniferous fossils (see Table 2) contributes to younger crown group divergences.

Scorpion terrestrialization

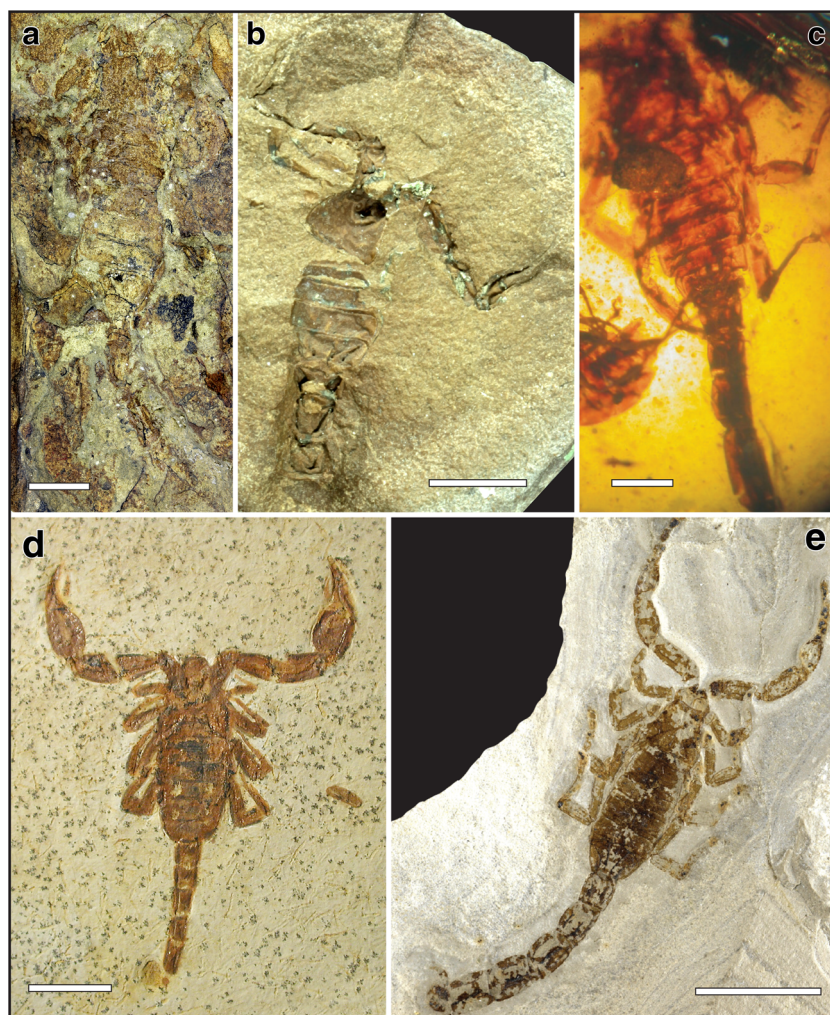
Among terrestrial animal clades, only the unparalleled insects outnumber arachnids by number of described species. Arachnids are thoroughly widespread in the continental realm and have managed to adapt to live permanently in some of the most hostile environments imaginable, including the Arctic tundra (e.g. the Arctic wolf spider *Pardosa glacialis* Thorell, 1872) and at extreme high altitudes (e.g. the Himalayan jumping spider *Euophrys omnisuperstes* Wanless, 1975). The overwhelming majority of extant arachnids are wholly terrestrial, and those adapted to a semi-aquatic or aquatic mode of life, such as the raft spiders, the diving bell spiders, several groups of aquatic mites and the troglobitic scorpions *Alacran* (Santibáñez-López et al. 2014), are thought to have returned to the water secondarily.

As scorpions appear so early in the terrestrial arthropod fossil record, there has been much interest in the palaeobiology of the ancient Siluro-Devonian scorpions (Dunlop et al. 2008b; Poschmann et al. 2008; Kühl et al. 2012; Waddington et al. 2015), which surely hold crucial insight into the dynamics of arachnid evolution and terrestrialization. Central to this interest is the debate over whether or not there was a single terrestrial common ancestor to all living arachnids. Terrestrialization is fundamental in arachnid evolutionary history. Whether terrestrialization occurred in a piecemeal fashion across various lineages, or just once, is hugely significant as it greatly influences how we

perceive the evolution of an array of morphological characters that relate to a terrestrial mode of life (e.g. respiratory systems, sensory systems, reproductive systems, locomotory appendages, feeding appendages). The physiological demands of life on land require major modification to such anatomical features, and this is probably best illustrated by the respiratory organs—a great range of which are exhibited by extant chelicerates (book gills, book lungs, sieve tracheae, tube tracheae, etc.).

Some authors through the 1980s and 1990s inferred that various chelicerate groups made the transition to land independently of each other (e.g. Selden and Jeram 1989; Dunlop and Webster 1999), or that the monophyly of Arachnida may be questionable (Dunlop 1998; Dunlop and Braddy 2001). The phylogeny and palaeobiology of early scorpions are critical to this line of reasoning, with some of these authors suggesting that scorpion adaptations to terrestrial life were potentially convergent with other arachnids (Dunlop 1998; Dunlop and Webster 1999). This was further supported by the hypothesis of a close relationship between scorpions and eurypterids (Braddy et al. 1999; Dunlop and Webster 1999; Dunlop and Braddy 2001), and interpretations of the earliest fossil scorpions as marine in life habit (Rolfe and Beckett 1984; Kjellesvig-Waering 1986; Jeram 1998; Dunlop and Webster 1999). Taken together, these two lines of evidence suggest terrestrialization occurred within the scorpion lineage independently of other terrestrial chelicerates. In this scenario, a monophyletic arachnid ancestor (which is contradicted implicitly by the eurypterid hypothesis) need not have been a terrestrial organism, and terrestrial adaptations shared by extant scorpions and tetrapulmonates, chiefly book lungs, are homoplastic. However, this has been much contested. Eurypterids have successively failed to be recovered in phylogenetic analyses of morphological characters as the sister group to scorpions despite superficial similarity (Shultz 1990, 2007; Garwood and Dunlop 2014), and doubt has been cast on the marine habit of early scorpions (Kühl et al. 2012). Whilst the case for Scorpiones derived within Arachnida is

Fig. 5 Scorpion calibration fossils, as specified in Table 1. **a** *Dolichophonus loudonensis*, scale 1 cm. **b** *Compsoscorpius buthiformis*, scale 1 cm. **c** *Electrochaerilus buckleyi*, scale 1 mm. **d** *Protoischnurus axelrodurum*, scale 1 cm. **e** *Uintascorpio halandrasi*, scale 1 cm. Images **a** and **b** belong to the authors. Image **c** reproduced with permission from Santiago-Blay et al. (2004a). Image **e** reproduced with permission from Santiago-Blay et al. (2004b). Image **d** courtesy of Wilson Lourenço



strong (see ‘Introduction’), the marine Siluro-Devonian scorpion debate remains a point of contention.

A marine lifestyle for early scorpions has often been inferred primarily on depositional environment, often without

firm morphological support. Waddington et al. (2015) described limb morphology in a Silurian scorpion as consistent with terrestrial or at least semi-aquatic locomotion. It seems many early scorpion fossils are known from marginal marine

Table 3 Full list of fossil calibrations used in our molecular dating analyses

Node on tree	Hard minima	Soft maxima	Source	MCMCTree input
Root	435.15 Ma	514 Ma	Revised from ‘Arachnida’ in Wolfe et al. (2016).	B(4.3515,5.14,1e-300,0.025)
Crown Arachnoplumonata	435.15 Ma	514 Ma	Revised from ‘Arachnida’ in Wolfe et al. (2016).	B(4.3515,5.14,1e-300,0.025)
Crown Tetrapulmonata	319.9 Ma	514 Ma	Revised from ‘Tetrapulmonata’ in Wolfe et al. (2016)	B(3.199,5.14,1e-300,0.025)
Crown Orthosterni	112.6 Ma	313.7 Ma	This study	B(1.126,3.137,1e-300,0.025)
Crown Buthida	98.17 Ma	313.7 Ma	This study	B(0.9817,3.137,1e-300,0.025)
Chaeriloidea-Pseudochactoidea divergence	98.17 Ma	313.7 Ma	This study	B(0.9817,3.137,1e-300,0.025)
Crown Buthoidea	49.26 Ma	313.7 Ma	This study	B(0.4926,3.137,1e-300,0.025)
Crown Iurida	112.6 Ma	313.7 Ma	This study	B(1.126,3.137,1e-300,0.025)
Crown Scorpionoidea	112.6 Ma	313.7 Ma	This study	B(1.126,3.137,1e-300,0.025)

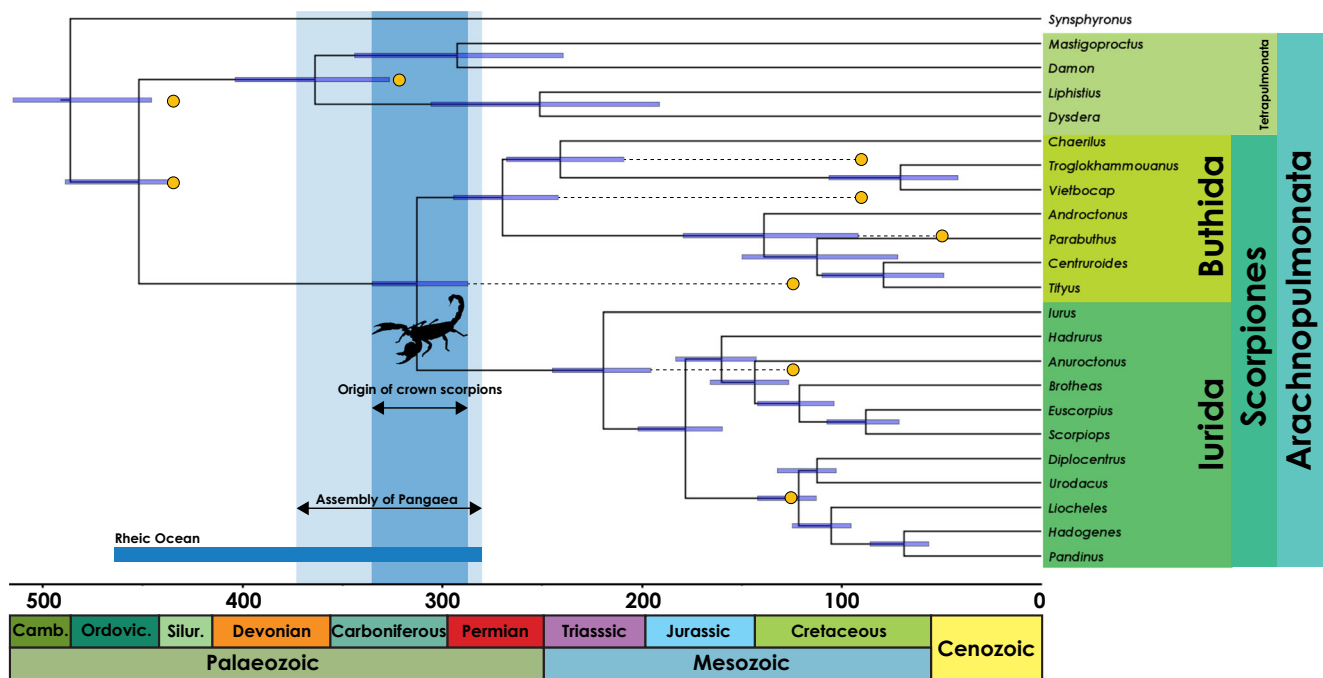


Fig. 6 Time tree for scorpions inferred in MCMCTree under the correlated rate model (Yang 2007), using 9 soft upper and hard lower bounded calibrations derived from the scorpion fossil record (see

Tables 2 and 3). Node bars = 95% HPD intervals. Gold circles represent fossil node-calibration minima

depositional environments, as part of an assemblage that includes certain allochthonous components such as land plants (e.g. Kühl et al. 2012; Waddington et al. 2015). Therefore, it is difficult to determine with certainty whether a fossil scorpion was aquatic in life or was transported post-mortem, or perhaps was even semi-aquatic (as suggested by Waddington et al. 2015). Gills are yet another point of contention in scorpion evolution. Poschmann et al. (2008) described putative gills in a Devonian scorpion, *Waeringoscorpia* Størmer, 1970, but also hypothesised a secondary derivation of the external filamentous structures from book lungs, stressing their uniqueness and similarity to the tracheal gills of secondarily aquatic freshwater insects. Poschmann et al. (2008) therefore postulated a secondarily aquatic mode of life, and that the gills were an autapomorphy of *Waeringoscorpia*, rather than being evidence of a gill-to-lung water-to-land transition in scorpions. Similarly, a gill to lung transition also cannot simply be inferred from modern aquatic chelicerates. Comparative studies in ultrastructure and embryology are inconclusive with regard to homology between scorpion book lungs and the book gills of horseshoe crabs (Farley 2010, 2011). This is complicated further by the origin of chelicerate opisthosomal appendages, which is likely to be telopodal rather than from epipod gills (Di et al. 2018). An extant homeotic mutant scorpion was described by Di et al. (2018) exhibiting stunted walking legs in the place of various opisthosomal appendages, including genital opercula and pectines, and an appendicular extension of a book lung. This suggests that the diversity of opisthosomal appendages exhibited by chelicerates is serially

homologous with walking legs, rather than derived from epipod gills, and therefore book lungs need not implicitly be part of the same transformation series as book gills based on a common original function.

Two other important interrelated hypotheses inform the terrestrialization debate: book lung homology across scorpions and tetrapulmonates, and the clustering of scorpions and tetrapulmonates in phylogenomic studies (Arachnopulmonata). There is strong evidence for homology of book lungs derived from rigorous comparative study. Scholtz and Kamenz (2006) described a number of detailed similarities in the book lungs of scorpions, amblypygids, uropygids and spiders, and concluded that the structures are homologous despite differences in their segmental position, although conceding small differences such as the orientation of the trabeculae relative to the parallel lamellae. Scholtz and Kamenz (2006) therefore ascribed book lung homology between scorpions and tetrapulmonates as evidence in favour of a single terrestrialization event and a monophyletic Arachnida. Only tetrapulmonates and scorpions possess book lungs, and so their homology (given that phylogenetic hypotheses at the time of Scholtz and Kamenz (2006) publication placed scorpions distant from tetrapulmonates within Arachnida) strongly suggested they represented a plesiomorphy for the ancestral Arachnida. However, in contrast to this, independent molecular studies utilising different sources of data have recovered scorpions as the sister group to tetrapulmonates (Regier et al. 2010; Rota-Stabelli et al. 2013b; Sharma et al. 2014; Sharma and Wheeler 2014; Leite et al.

2018)—Arachnoplumonata, as discussed in ‘Introduction’. The Arachnoplumonata hypothesis therefore suggests book lungs are synapomorphic for scorpions and tetrapulmonates, but limits how they can inform the sequence of terrestrialization.

The scorpion fossil record

Chelicerates are well represented in the fossil record, almost 2000 valid arachnid species were documented a decade ago (Dunlop et al. 2008a). Much of this palaeodiversity is concentrated into Konservat-Lagerstätten, sites that exhibit fossils with exceptional preservation, and unusually more Palaeozoic scorpions are known than Mesozoic or Cenozoic ones. The most comprehensive account of fossil scorpions is the posthumous monograph of Erik N. Kjellesvig-Waering (Kjellesvig-Waering 1986), which comprises the sum of his work on fossil scorpions of the world throughout the 1950s, 1960s and 1970s. Few scorpions younger than Palaeozoic in age were known at the time the work was undertaken, but this has changed considerably with new discoveries in Cretaceous and Cenozoic ambers over the past decade or so in particular (Lourenço 2016). Kjellesvig-Waering’s work is often resorted to despite its flaws, owing to a lack of alternatives. Kjellesvig-Waering recorded a number of dubious morphological observations (such as gills and gill opercula) in some fossils, which were frequently inferred to encompass entire higher groups. As a result, Kjellesvig-Waering’s systematic classification is basally divided into the Branchioscorpionina, which includes the majority of Palaeozoic scorpions and is presumed to be aquatic, and the terrestrial Neoscorpionina, which originates in the Carboniferous. ‘Branchioscorpionina’ was conceived as unequivocally paraphyletic. The classification erects a number of cumbersome monotypic higher groups, as well as many families, genera and species based on trivial morphology, and characters that have subsequently been reinterpreted as developmental or taphonomic in nature (e.g. Dunlop et al. 2008b; Legg et al. 2012) or based on erroneous interpretations of morphology (Dunlop et al. 2007). Unsurprisingly, the scheme has failed to be supported by subsequent cladistic studies (Stockwell 1989; Jeram 1993, 1994, 1998). Jeram (1998) recognised the significance of the terrestrialization process in a cladistic analysis of fossil scorpions, noting that most morphological characters available in fossils are in some way linked to adaptations for a terrestrial life. As such, if there were multiple parallel terrestrialization events within the scorpion lineage, we would expect homoplasy in the dataset due to similar selection pressures, obscuring the true phylogenetic signal. Characters independent of terrestrial adaptations are required to test this, but this is limited by fossil preservation and must be addressed in future studies.

Whilst a workable systematic classification is still desired, some recent accounts do recognise an outline developing in scorpion evolutionary history (Dunlop 2010). An early diverging group seems to be recognised, the Palaeoscorpionina, alongside a more derived lineage containing Mesoscorpionina and Neoscorpionina as sister groups. The monophyly of these groups are untested, but at the least a broad picture of scorpion evolution seems to be encapsulated by them. The oldest group, the palaeoscorpions, are known from the Silurian of Europe and North America (Thorell and Lindström 1885; Whitfield 1885; Laurie 1899; Kjellesvig-Waering 1954; Dunlop and Selden 2013; Waddington et al. 2015). The palaeoscorpions exhibit a coxo-sternal region (the conjunction of the walking leg coxae on the ventral surface of the prosoma) that is interpreted as less derived, and the informal group persists into the Carboniferous (Leary 1980). In palaeoscorpions, the sternum itself is broad, unlike the reduced pentagonal sterna of modern scorpions, and there are no coxapophyses—which are proximal extensions of first and second pairs of walking leg coxae that together form part of the stomotheca (the feeding chamber). The mesoscorpions first appear in the Devonian and were recognised as distinct by Stockwell (1989), showing a more derived coxo-sternal region with coxapophyses as exhibited by extant scorpions. Mesoscorpions show the first direct evidence for book lungs (Jeram 1990) and seem to persist into the Mesozoic (Wills 1947; Dunlop et al. 2007). Mesoscorpions were often large (300–700 mm in length) and were probably important predators in the Late Devonian and Carboniferous (Jeram 1998). The neoscorpions have reduced lateral eyes and are divided into two groups. These are Orthosterni, which appears in the Carboniferous and contains the scorpion crown group (Jeram 1994), and Palaeosterni, which is restricted to the Carboniferous only. The Orthosterni are characterised by their spiracles being located within their sternites (the ventral plates of the mesosoma) rather than at the sternite margins. The oldest fossil material potentially assignable to a modern taxon (the superfamily Buthoidea) is Early Triassic in age (Lourenço and Gall 2004), and the oldest unequivocal members of living families (Chactidae and Hemiscorpionidae) are Early Cretaceous (Menon 2007).

Conclusions

Scorpions have confounded our understanding of animal terrestrialization for several decades. Arachnids are one of the most successful terrestrial animal groups, but at present, the details of their journey out of the water are unclear. It is therefore fundamental in resolving arachnid evolutionary history to constrain the phylogeny of the scorpion total group using the fossil record and implement advances in phylogenetic divergence time estimation in synergy. This is challenging, as their conservative (or cryptic) morphology seems to

have given us little consensus on the interrelationships both within their lineage, and among the other arachnid groups. Fossil scorpions are also problematic in that they have suffered from tenuous systematic interpretations and a lack of consensus on their general palaeobiology, most notably whether key species were aquatic or terrestrial. Although a comprehensive scorpion time tree that is up to date with the most recent phylogenetic methods and hypotheses is currently not available, we demonstrate that established methods (i.e. node-dating) can place a reasonable temporal constraint on the origination of the crown group, at least. However, recent advances in dating phylogenies, particularly total evidence dating methods using relaxed molecular clocks and the recently described fossilised birth-death model for calibrating divergence time estimates (see ‘[Scorpions on the arachnid Tree of Life](#)’), could prove extremely fruitful. With such recent advancements in phylogenetics, coupled with the rapid accumulation of molecular sequence data, the stage is set for a potential revolution in our understanding of scorpion evolution that would reverberate to arachnids more broadly. A well-constrained time tree combining extant and fossil taxa would allow researchers to address arachnid evolution accurately in a more holistic, geobiological context (scorpions have survived at least three mass extinctions, for example). Unification of fossil and extant organisms in a common phylogenetic and macroevolutionary framework elucidates otherwise untenable deep evolutionary relationships by circumventing biases specific to certain types of data, such as long branch attraction in molecular data (Bergsten 2005; Lartillot et al. 2007; Rota-Stabelli et al. 2011) and biases introduced by decay in fossil data (Sansom and Wills 2013). Therefore, it is critical to continue to describe and interpret new fossils, with care taken to focus on the acquisition of reliably homologous characters. Fossils are the only direct record of cladogenesis, and their integration into rapidly advancing and computationally intense phylogenetic methodologies is of paramount relevance. Molecular sequence data are only informative in dating evolutionary (i.e. geological) timescales as long as their veracity can be ground-truthed by fossils.

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