

# Out of the Neotropics: Late Cretaceous colonization of Australasia by American arthropods

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The origins of tropical southwest Pacific diversity are traditionally attributed to southeast Asia or Australia. Oceanic and fragment islands are typically colonized by lineages from adjacent continental margins, resulting in attrition of diversity with distance from the mainland. Here, we show that an exceptional tropical family of harvestmen with a trans-Pacific disjunct distribution has its origin in the Neotropics. We found in a multi-locus phylogenetic analysis that the opiloid family Zalmoxidae, which is distributed in tropical forests on both sides of the Pacific, is a monophyletic entity with basal lineages endemic to Amazonia and Mesoamerica. Indo-Pacific Zalmoxidae constitute a nested clade, indicating a single colonization event. Lineages endemic to putative source regions, including Australia and New Guinea, constitute derived groups. Divergence time estimates and probabilistic ancestral area reconstructions support a Neotropical origin of the group, and a Late Cretaceous (*ca* 82 Ma) colonization of Australasia out of the Fiji Islands and/or Borneo, which are consistent with a transoceanic dispersal event. Our results suggest that the endemic diversity within traditionally defined zoogeographic boundaries might have more complex evolutionary origins than previously envisioned.

**Keywords:** biogeography; dispersal; disjunction; Arthropods; islands

## 1. INTRODUCTION

A growing body of evidence supports a role for transoceanic dispersal in historical biogeography [1–4]. One compelling biogeographic theatre that demonstrates the importance of transoceanic dispersal is the tropical South Pacific. The juxtaposition of a plethora of continental terranes and oceanic islands with diverse geological histories in this region has resulted in unique assemblages of biota found nowhere else in the world [5,6], with nearly all landmasses of the South Pacific categorized as biodiversity hotspots for conservation priority [7]. Biogeographic studies based on molecular phylogenies have implicated multiple source areas of South Pacific diversity, principally the Indo-Malay Archipelago, Australia, New Zealand, New Caledonia and the Neotropics (often via the Hawaiian Archipelago [4,8,9]).

A curious phenomenon that is periodically encountered in the biogeography of tropical Pacific lineages is the case of trans-Pacific disjunctions. These distributions typically include the Neotropics, continental parts of Australasia (e.g. Australia, New Guinea, the Thai–Malay Peninsula), and oceanic islands in the tropical belt (e.g. Fiji, Polynesia, Micronesia). Trans-Pacific disjunct distributions are generally attributed to one of three phenomena: taxonomic oversight (distant unrelated lineages are erroneously assigned to the same taxon), relictualism (range contraction of a previously broadly distributed taxon) and transoceanic dispersal. The last of these is considered uncommon owing

to the dimensions of the barrier to dispersal: the breadth of the Pacific Ocean.

Most studied examples of trans-Pacific disjunct distributions resulting from oceanic dispersal consist of plant lineages [10–14]. However, trans-Pacific disjunctions are difficult to account for when the taxon of interest does not have adaptations for long distance oceanic dispersal, such as winged seeds [11] or floating fruit [12]. Apropos, fewer studied examples of trans-Pacific disjunct taxa are known among animals. These include the kagu of New Caledonia (*Rhynchoetos jubatus*) and its putative sister lineage, the Neotropical sunbittern *Eurypyga helias* [15]. The charismatic Fijian and Tongan iguanas (*Brachylophus* [16]) are postulated to have colonized Melanesia by transoceanic rafting from the Neotropics, but neither the mechanism nor the timing of the colonization is well understood. Similarly, the fossorial lizard family Dibamidae is represented by 22 species in southeast Asia and a single species that is endemic to Mexico, likely the result of transoceanic or overland dispersal across Beringia in the Eocene [17].

Among arthropods, lineages that colonize oceanic islands typically originate in the proximate continental regions, perhaps best exemplified by multiple, unrelated lineages of the spider family Thomisidae [8,18]. Thus, Melanesian and Micronesian islands are typically colonized by Australian or southeast Asian lineages, whereas the Galapagos and Polynesian islands are largely colonized by Neotropical lineages [4]. True trans-Pacific disjunct distributions are known (e.g. the ant genera *Adelomyrmex*, *Gnamptogenys* and *Rogeria* [19]), but infrequently examined in a phylogenetic framework that establishes the monophyly of the group and the direction of colonization.

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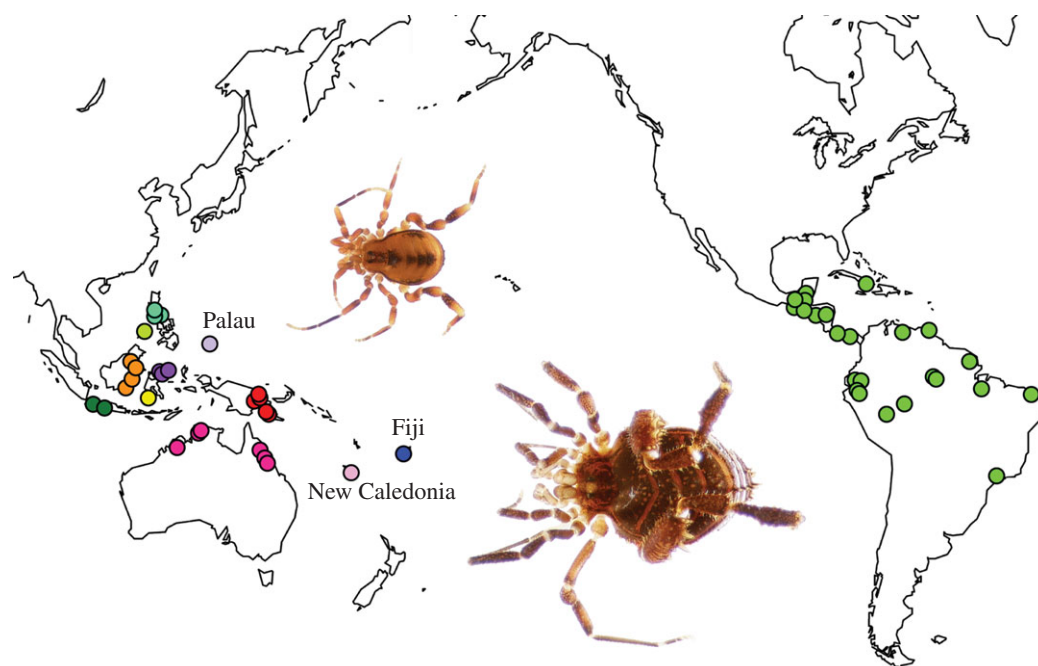


Figure 1. Distribution map of sampled Zalmoxoidea. Coloured circles indicate cluster(s) of collection localities; colours correspond to areas designated for biogeographic analysis (indicated in figure 2). Smaller islands and archipelagos are as indicated. Insets: Undescribed male *Zalmoxis*; top: *Zalmoxis* sp. DNA102528 from Papua New Guinea, bottom: *Zalmoxis* sp. DNA102487 from North Sulawesi.

Zalmoxidae is a constituent lineage within the suborder Laniatores—the largest subgroup of Opiliones—which includes much of the morphological, behavioural and ecological diversity within the order [20]. Several lineages of Laniatores are characterized by ornate, sexually dimorphic and striking armature. Multiple forms of paternal care, an unusual behaviour often associated with seahorses and sea spiders (pycnogonids), have evolved repeatedly in Laniatores, manifested as egg guarding or egg carrying behaviour [21]. Although zalmoxids do bear sexually dimorphic fourth legs, which are swollen and armed with spines, they are not particularly remarkable or superlative in most respects, save for one. Unlike the majority of arthropods, Zalmoxidae has a typical trans-Pacific disjunct distribution.

The *ca* 200 described species of Zalmoxidae are found throughout tropical leaf-litter habitats in Amazonia, Meso-America, parts of Melanesia and Micronesia, northern Australia, New Guinea, the Philippine Islands, Java and Borneo [22–25]. Two additional species are known from Mauritius and the Seychelle Islands [23]. Nearly all species exhibit micro-endemism and are known from a single locality. Zalmoxids do not occur in southern South America, southern Australia, New Zealand, Tasmania or Africa; species from some of these regions once postulated to belong to Zalmoxidae have since been transferred to distantly related lineages, based on evidence from morphology and molecular sequence data [20,23].

Intercontinental distributions do occur among harvestmen, but these have been attributed to ancient vicariant events—particularly the break-up of the supercontinent Gondwana—based on tree topology, analysis of ancestral areas and/or molecular dating [26–28]. The predominance of vicariance scenarios in the study of harvestman biogeography stems from the ancient age of the group, which is evidenced by Upper Devonian crown-group fossils [29]. However, recent phylogenetic analyses have

demonstrated that Zalmoxidae is derived among harvestmen [20,30], potentially challenging an explanation based on vicariance. Moreover, Zalmoxidae has only been represented in such studies by two or three terminals, which is inadequate for ruling out taxonomic oversights, e.g. the placement of unrelated Indo-Pacific and Neotropical lineages in the same family. Consequently, we assessed the phylogeny of Zalmoxidae in order to test the monophyly of the group and evaluate biogeographic scenarios for the trans-Pacific disjunction.

## 2. MATERIAL AND METHODS

Methods are described in greater detail with full references in the electronic supplementary material.

### (a) *Taxon and gene sampling*

We selected 147 specimens representing all families of the sister superfamilies Zalmoxoidea and Samooidea [20] (figure 1). To test the monophyly of Zalmoxidae, we included 116 zalmoxids sampled from throughout their known range. We used routine DNA extraction, amplification and sequencing protocols for partial fragments of two mitochondrial protein-encoding (cytochrome *b*, cytochrome *c* oxidase subunit I) and two nuclear protein-encoding (histone H3, histone H4) loci; and complete or nearly complete nuclear ribosomal genes (18S rRNA, 28S rRNA), yielding approximately 6500 bp of data.

### (b) *Phylogenetic analysis and molecular dating*

We conducted analyses using four phylogenetic approaches: maximum likelihood (ML), Bayesian inference; weighted parsimony under dynamic homology; and simultaneous Bayesian inference of topology and divergence times. Divergence time estimation was conducted using relaxed clock methods [31]. We specified a unique general time-reversible (GTR) model of sequence evolution with corrections for a discrete gamma distribution and a proportion of invariant sites (GTR +  $\Gamma$  + I) for

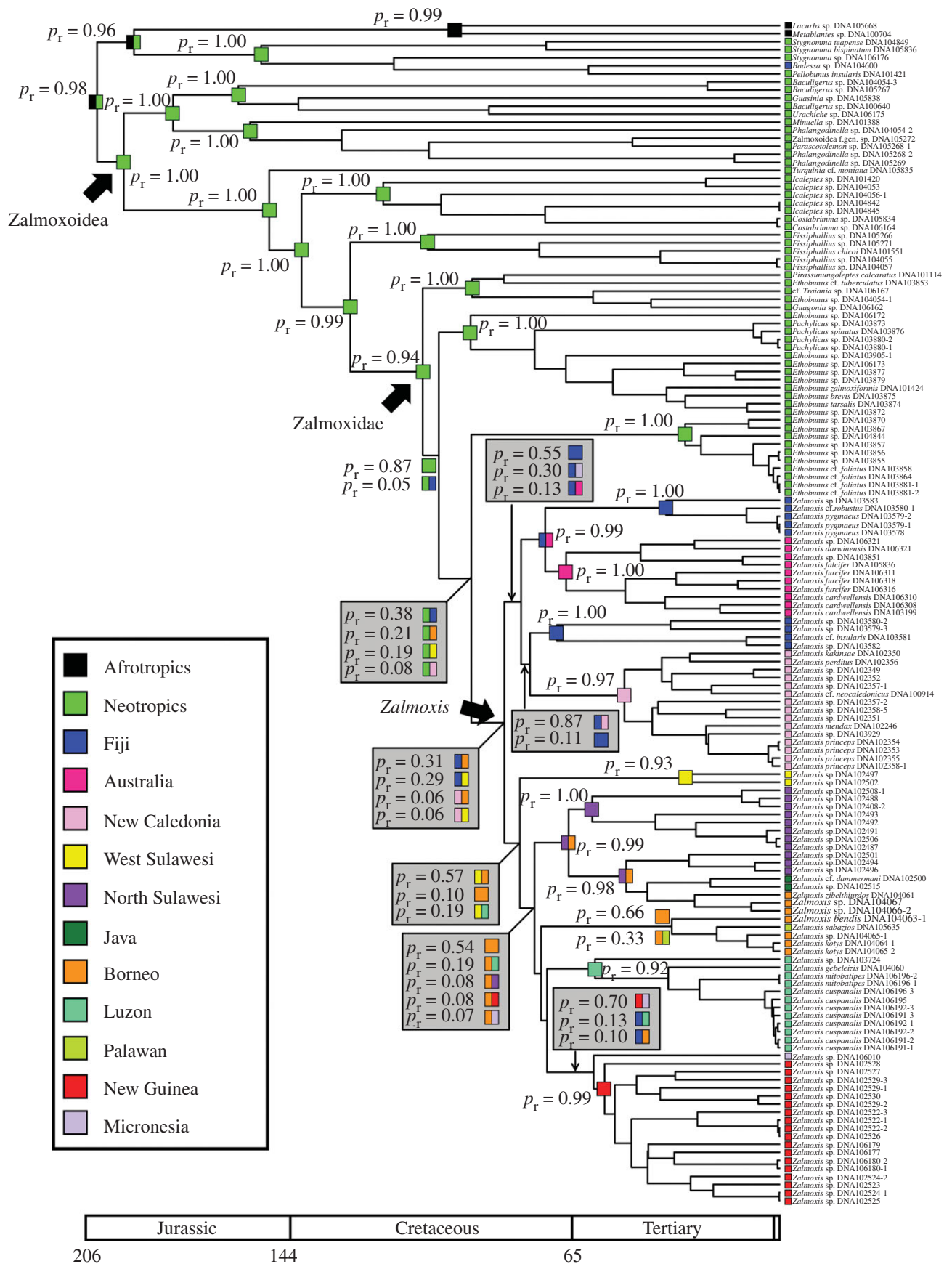


Figure 2. Ancestral area reconstruction based on Bayesian inference analysis (from BEAST ver. 1.6.1) of all molecular data using LAGRANGE. Coloured squares at terminals indicate ranges occupied by sampled species. Coloured squares at nodes indicate ranges reconstructed for hypothetical ancestors. Numbers on nodes indicate relative probability of ranges reconstructed.

each partition, as recommended by MODELTEST under the Akaike information criterion [32,33]. Calibration for the 147-taxon dataset was drawn from a larger phylogenetic study of

the suborder Laniatores [20]. To test the validity of normal distribution priors as secondary calibrations, we also constructed a 228-taxon dataset for the same six genes, combining the 147

focal taxa with exemplars of all known families of Laniatores, as well as representatives of the other three suborders of Opiliones. Calibration of the 228-taxon dataset was conducted using Palaeozoic fossils of crown-group Opiliones.

### (c) *Ancestral areas and diversification through time*

We inferred ancestral areas using a Bayesian approach as implemented in RASP [34], and using a dispersal–cladogenesis–extinction model, implemented in LAGRANGE [35,36]. We used a series of dispersal constraint models in LAGRANGE, including unconstrained, stepping-stone and a stratified model with seven matrices incorporating geological history (electronic supplementary material, table S3). Tests of diversification rate constancy were conducted using the R package LASER [37].

## 3. RESULTS

### (a) *Phylogenetic analysis*

Analysis of the six-gene, 147-taxon dataset using BEAST v. 1.6.1 reached stationarity after *ca*  $10^7$  generations;  $2 \times 10^7$  generations (20%) were discarded as burn-in. In the resulting tree topology (figure 2; electronic supplementary material, figure S1), the superfamilies Samooidea and Zalmoxoidea (the latter includes Zalmoxidae) form mutually monophyletic groups (both with posterior probabilities ( $PP_{\text{BEAST}}$ ) of 1.00). Within Zalmoxoidea, early-diverging lineages are divided between two groups: the families Escadabiidae, Guasiniidae and Kimulidae form one group ( $PP_{\text{BEAST}} = 0.99$ ); and Icaleptidae, Fissiphalliidae, Zalmoxidae and the Cuban genus *Turquinia* form another ( $PP_{\text{BEAST}} = 1.00$ ). Zalmoxidae *sensu stricto* is monophyletic ( $PP_{\text{BEAST}} = 1.00$ ), and early-diverging lineages within Zalmoxidae that are endemic to the Neotropics (e.g. *Ethobunus*, *Pachylicus*) form a grade with respect to Indo-Pacific zalmoxids. By contrast, the Indo-Pacific Zalmoxidae constitute a derived, monophyletic group ( $PP_{\text{BEAST}} = 1.00$ ), i.e. the genus *Zalmoxis*. Two clades of *Zalmoxis* are recovered: a southwest Pacific clade, with constituent species endemic to Australia, Fiji and New Caledonia; and a southeast Asian clade, with constituent species endemic to Borneo, Java, New Guinea, the Philippines, Palau and Sulawesi. Relationships at the base of these clades are poorly supported.

ML analysis using RAxML v. 7.2.7 resulted in a tree topology with  $\ln L = -57789.507$  (electronic supplementary material, figure S2) with major similarities to the BEAST tree topology, with respect to the monophyly of Zalmoxoidea (bootstrap resampling frequency (BS) of 76%) and the recovery of a basal grade that is endemic to the Neotropics. Early-diverging lineages of *Zalmoxis* are endemic to three terranes: the West Sulawesi plate, Fiji and New Caledonia. As with the BEAST topology, relationships among the constituent lineages of *Zalmoxis* are unstable and poorly supported.

Three of the four runs of MRBAYES v. 3.1.2 reached stationarity in  $5 \times 10^6$  generations, but the fourth did not converge with the other three runs until *ca*  $1.75 \times 10^7$  generations;  $2 \times 10^7$  generations (50%) were hence discarded as burn-in. The Bayesian inference (BI) analysis resulted in a topology largely similar to the ML tree (electronic supplementary material, figure S3). Both the monophyly of Zalmoxidae and *Zalmoxis* are supported in the BI analysis (posterior probability (PP) of 0.95

and 1.00, respectively). Some differences exist with respect to supported nodes within Zalmoxidae.

Iterative rounds of tree-fusing and driven searches using direct optimization in POY v. 4.1.2 resulted in two most parsimonious trees (MPTs) with a length of 18 137 weighted steps. A strict consensus of the MPTs (electronic supplementary material, figure S4) indicates that the difference between the two is restricted to internal relationships of a derived group of *Ethobunus* from southern Mexico. In general, the parsimony direct optimization (DO) tree topology is very similar to the topologies based on static alignments, particularly with respect to relationships among outgroups and the derived clades of Zalmoxidae. As with ML and BI analyses, the monophyly of Zalmoxidae and *Zalmoxis* are supported (jack-knife resampling frequencies (JF) of 85% and 97%, respectively), but internal relationships of *Zalmoxis* are unstable and poorly supported.

### (b) *Estimation of divergence times*

Diversification of relevant lineages using BEAST for the 147-taxon dataset is estimated as follows: origin of Zalmoxidae *sensu stricto*, 127.4 Ma (95% HPD: 106.6–148.1 Ma); diversification of Zalmoxidae *sensu stricto*, 105.9 Ma (95% highest posterior density interval (HPD): 87.3–124.5 Ma); origin of *Zalmoxis*, 91.5 Ma (95% HPD: 75.0–107.8 Ma); diversification of *Zalmoxis*, 81.7 Ma (95% HPD: 67.8–94.8 Ma).

Subsequent to alignment and culling ambiguously aligned positions, the 228-taxon dataset constructed to test the validity of the secondary calibrations was smaller than the 147-taxon dataset (6172 versus 6563 nucleotide positions), owing to sequence variability in the nuclear ribosomal markers across Opiliones. Divergence time estimates were effectively identical to those obtained using the 147-taxon dataset (electronic supplementary material, figure S5). Median ages obtained for the three nodes used as secondary calibrations differed from the calibration medians by 7.7–12.7 Myr, and standard deviations by 2.7–3.93 Myr. Ages of relevant nodes in the 228-taxon dataset were as follows: origin of Zalmoxidae *sensu stricto*, 124.0 Ma (95% HPD: 104.1–143.0 Ma); diversification of Zalmoxidae *sensu stricto*, 101.9 Ma (95% HPD: 84.8–117.7 Ma); origin of *Zalmoxis*, 93.3 Ma (95% HPD: 75.4–108.2 Ma); diversification of *Zalmoxis*, 81.0 Ma (95% HPD: 65.7–94.4 Ma).

### (c) *Biogeographic analysis*

To maintain comparability between the BEAST, ML, BI and DO topologies, and given the similar results of the two calibrations, we used the dated tree topology of the 147-taxon dataset for biogeographic analyses. All probabilistic approaches for inferring ancestral areas reconstruct the origin of Zalmoxidae (i.e. the split between Zalmoxidae and its sister family Fissiphalliidae) as unambiguously Neotropical (figure 2, table 1). The split between Neotropical and Indo-Pacific zalmoxids is reconstructed as the union of the Neotropics and the Fijian Islands under the unconstrained and stepping-stone models in LAGRANGE analysis, or alternatively the union of the Neotropics and either Borneo, West Sulawesi or the Philippine Islands (excluding Palawan). Results from the Bayesian reconstruction of ancestral areas across all four topologies are largely congruent, favouring

an ancestral distribution of *Zalmoxis* that united the Neotropics and either Fiji, Borneo or Sulawesi.

As the geology of some candidate ancestral areas (e.g. Fiji) is inconsistent with the timing of the Zalmoxidae radiation, a stratified model replete with geological scenarios of landmass movements, fragmentations, appearance and subsidence was implemented to reconcile the biogeographic history of Zalmoxidae with geology and estimated divergence times. The stratified model reconstructs the split between Neotropical and Indo-Pacific zalmoxids as the union of the Neotropics and Borneo ( $p_r = 0.32$ ) or alternatively, the Neotropics alone ( $p_r = 0.30$ ). Diversification of *Zalmoxis* in the Indo-Pacific is reconstructed as the union of Borneo and Australia ( $p_r = 0.29$ ), the latter being the only terrane of the South Pacific clade (in the BEAST topology) that has consistently maintained suitable habitat for Opiliones since *ca* 81.7 Ma (while Gondwanan in origin, New Caledonia did not emerge in its present form until *ca* 37 Ma; [6,38]). Alternative reconstructions are a pan-Pacific diversification uniting Borneo and the Neotropics ( $p_r = 0.20$ ) or diversification out of Borneo alone ( $p_r = 0.19$ ).

#### 4. DISCUSSION

Like many arthropod lineages, nearly all families of Opiliones are restricted to specific biogeographic provinces, such as the Neotropics or southeast Asia (Wallacea/Sundaland), or to constituents of ancient supercontinents, such as Gondwana and Laurasia [20,28]. The fidelity of opilionid distributions to particular regions, in conjunction with the ancient age of the group, has engendered the prevalence of vicariance scenarios to account for the evolution of most divisions of Opiliones—especially within the suborder Cyphophthalmi—and the concomitant conformity of systematics with putative vicariance scenarios [28,39]. Trans-Pacific disjunct distributions strongly discord with this pattern, and could potentially indicate either the remnant of a formerly pantropical clade or a dispersing taxon. We investigated the biogeography of an exceptional tropical opilionid family presenting a classic trans-Pacific disjunction, testing three hypotheses for its present distribution: taxonomic oversight, ancient vicariance followed by range extinction and colonization by dispersal. Each hypothesis engenders a set of phylogenetic and/or temporal predictions, which we evaluated in turn.

The recovery of zalmoxid monophyly across all topologies obtained (figure 2; electronic supplementary material, figures S1–S5), with nodal support (BS = 76%; PP = 0.95; JF = 85%; PP<sub>BEAST</sub> = 1.00), rules out dismissal of zalmoxid distribution based on taxonomic error. Although we included genera of uncertain monophyly and lineages of unknown familial placement, these did not contradict the monophyly of Zalmoxidae, nor did they confound biogeographic inference, insofar as all these lineages are endemic to the Neotropics and form a paraphyletic group with respect to the Indo-Pacific zalmoxids (the genus *Zalmoxis*; figure 2; electronic supplementary material, figures S1–S4). *Zalmoxis* constitutes a derived and monophyletic, yet comparatively diverse, group containing *ca* 33 per cent of described zalmoxid species.

A hypothesis of ancient Gondwanan diversification engenders two predictions. First, the origin of *Zalmoxis*

has to coincide with the age of the geological event that precipitated the vicariance between the Neotropics and the Indo-Pacific landmasses. Second, as a corollary, Zalmoxidae must be sufficiently old to predate the vicariance, i.e. the family must have been present before the fragmentation of Tropical Gondwana. Both predictions are falsified by the divergence time estimates. The molecular dating of the radiation indicates that the split between the Indo-Pacific and the Neotropical Zalmoxidae occurred *ca* 92 Ma (95% HPD: 75.0–107.8 Ma), or approximately 80 Myr after the fragmentation of Tropical Gondwana (figure 2; electronic supplementary material figure S1 and S5). Even if the upper bound of the 95% HPD interval is taken as the estimate of this node's age, the origin of the Indo-Pacific lineage occurred over 60 million years after the geological event that precipitated the separation of the Neotropics from the Palaeotropics. In fact, the age of this node postdates the timing of the isolation of the Neotropics from other tropical landmasses (110 Ma). Secondly, both the paraphyletic group of Neotropical lineages at the base of Zalmoxidae and their age estimates corroborate diversification in the Neotropics after it became isolated from the rest of Tropical Gondwana. Diversification of the family is estimated to have occurred *ca* 106 Ma (95% HPD: 87.3–124.5 Ma). Again taking the estimate's upper limit, Tropical Gondwana had fragmented long before the family began to diversify in the Neotropics—too young for the disjunction to be characterized as the remnant of a former pantropical lineage (figure 2).

The establishment of zalmoxid diversity in the Indo-Pacific is therefore consistent with a scenario of colonization by a New World lineage, given an unambiguous origin in the Neotropics (figure 2; table 1). As with other taxa with trans-Pacific disjunct distributions, colonization of the Indo-Pacific could have followed multiple routes, such as (i) overland dispersal via Beringia; (ii) overland dispersal via trans-Antarctic connections between the Australian plate and South America; and (iii) transoceanic dispersal. However, overland dispersal hypotheses are inconsistent *a priori* with zalmoxid distribution and tree topology. Distributions for which Beringian migration has been invoked include Nearctic and Palaeartic elements, such as parts of North America, Japan and/or southeast Eurasia [40–42], none of which is inhabited by Zalmoxidae [23,25]. Similarly, no Zalmoxidae occur in the former constituents of the trans-Antarctic connection, such as southern Australia, Tasmania, New Zealand or southern South America.

Reconciling overland dispersal scenarios with the present restriction of zalmoxid range to the tropical belt on both sides of the Pacific requires invocation of significant extinction events, due to the micro-endemicity exhibited by almost all known Zalmoxoidea. To investigate the possibility of cryptic extinction, we investigated temporal shifts in diversification rates within the dated zalmoxid phylogeny. We postulated that cryptic extinction in the evolutionary history of Zalmoxidae would engender a log-lineage through time plot with an anti-sigmoidal shape, characteristic of cryptic extinction events [43]. If a hypothetical extinction event is not sufficiently drastic, the curve may not be anti-sigmoidal, but could still exhibit a recent increase in diversification rate—a documented manifestation of a birth–death process [44].

Table 1. Biogeographic analysis of ancestral areas, with associated probabilities, across all topologies recovered using RASP or LAGRANGE models. AUS, Australia; BRN, Borneo; FJI, Fiji; LZN, Philippines (excluding Palawan); NCA, New Caledonia; NGA, New Guinea; NSL, North Sulawesi; NTR, Neotropics; WSL, West Sulawesi.

	RASP analyses				LAGRANGE analyses		
	ML-RASP	BI-RASP	DO-RASP	BEAST-RASP	unconstrained	stepping-stone	stratified
origin of <i>Zalmoxidae</i>	NTR: 1.00	NTR: 1.00	NTR: 1.00	NTR: 1.00	NTR: 0.94	NTR: 0.99	NTR: 0.95
origin of <i>Zalmoxis</i>	NTR: 0.98	NTR: 0.99	NTR: 0.99	NTR: 0.99	NTR + FJI: 0.38 NTR + BRN: 0.21 NTR + WSL: 0.19 NTR + NCA: 0.08	NTR + FJI: 0.34 NTR + LZN: 0.24 NTR + WSL: 0.13 NTR + NGA: 0.13 NTR: 0.10	NTR + BRN: 0.32 NTR: 0.30 NTR + AUS: 0.20 NTR + NGA: 0.07
diversification of <i>Zalmoxis</i>	NTR: 0.57 FJI: 0.31 NTR + FJI: 0.05	NTR: 0.65 FJI: 0.22	NTR: 0.86	NTR: 0.81	FJI + BRN: 0.31 FJI + WSL: 0.29 NCA + BRN: 0.06 NCA + WSL: 0.06	FJI + LZN: 0.48 FJI + NGA: 0.20 WSL + AUS: 0.14 NTR + FJI: 0.07	BRN + AUS: 0.29 NTR + BRN: 0.20 BRN: 0.19

Of the eight competing models we examined, the optimal model was a Yule-three-rate model, which indicates that the diversification rate of Zalmoxidae has decreased steadily over time (figure 3 and table 2). Other suboptimal models examined similarly indicate diversification rate slowdowns. These data are inconsistent with the extinction episodes required to engender the trans-Pacific disjunct distribution of Zalmoxidae, assuming a scenario of overland dispersal. Diversification rate slowdowns must be interpreted cautiously, as they may be artefactual manifestations of inadequate lineage sampling [45], or a result of variable rates of cladogenesis and extinction over time [46]. Nevertheless, that neither an anti-sigmoidal curve nor recent recovery of diversification rate is observed for Zalmoxidae disfavors scenarios requiring lineage extinctions. This also applies, and in greater measure, to the hypothesis of ancient Gondwanan diversification followed by drastic range extinction.

The biogeographic history of Zalmoxidae is in accordance with a rare phenomenon: an amphitropical distribution achieved by trans-Pacific dispersal. Ancestral area analyses across all topologies corroborate the Neotropical origin of the family and an initial colonization of the Indo-Pacific that began in the Fijian Islands, Borneo, West Sulawesi (a terrane that was connected to Borneo *ca* 55 Myr [47]) or some combination of these (table 1). The reconstruction of any of these areas at the base of *Zalmoxis* is consistent with the hypothesis of transoceanic dispersal. In the case of the Fijian Archipelago, these islands are oceanic in origin [6]. In the case of Borneo and Sulawesi, as mentioned previously, the last recent connection of the southeast Asian tropics to the Neotropics predates the origin of Zalmoxidae by at least 60 million years, based on the upper limit of the age estimate [47,48]. Although the ages of the Fijian Islands postdate the diversification of *Zalmoxis*, the stratified model incorporating the geological histories of all 14 areas circumscribed for analysis reconstructs Borneo as the most likely target of colonization by the hypothetical ancestor of *Zalmoxis* (table 1; electronic supplementary material, table S3). The size, age and geological stability of Borneo are consistent with the availability of this landmass for colonization during the Late Cretaceous [47].

Consistent with this scenario, Zalmoxidae have demonstrable transoceanic dispersal capability, with locality records including remote islands, such as the Moluccas, Palau, Pohnpei and the Jaluit Atoll [23]. Furthermore, the ancient continental fragments of the Indo-Pacific—areas that are traditionally held to be the source regions of Australasian diversity—are either not inhabited by Zalmoxidae (e.g. southern Australia, Tasmania and New Zealand) or are inhabited by a single, derived clade (e.g. New Guinea, New Caledonia, northern Australia). New Guinea is an especially diverse island with complex geological history, and the putative source region of many diverse metazoan radiations, some of which extend worldwide [5,49,50]. The recovery of all New Guinean species, which constitute the most morphologically diverse *Zalmoxis*, within a single derived clade (that may include a Palauan species) is therefore an unexpected result. The biogeography of this region is already famous for its complexity, owing to Wallace's and Huxley's Lines and the interface of southeast Asian and Australian biotas; to our knowledge, Neotropical origins have never been demonstrated for diverse, endemic lineages in this biogeographic theatre.

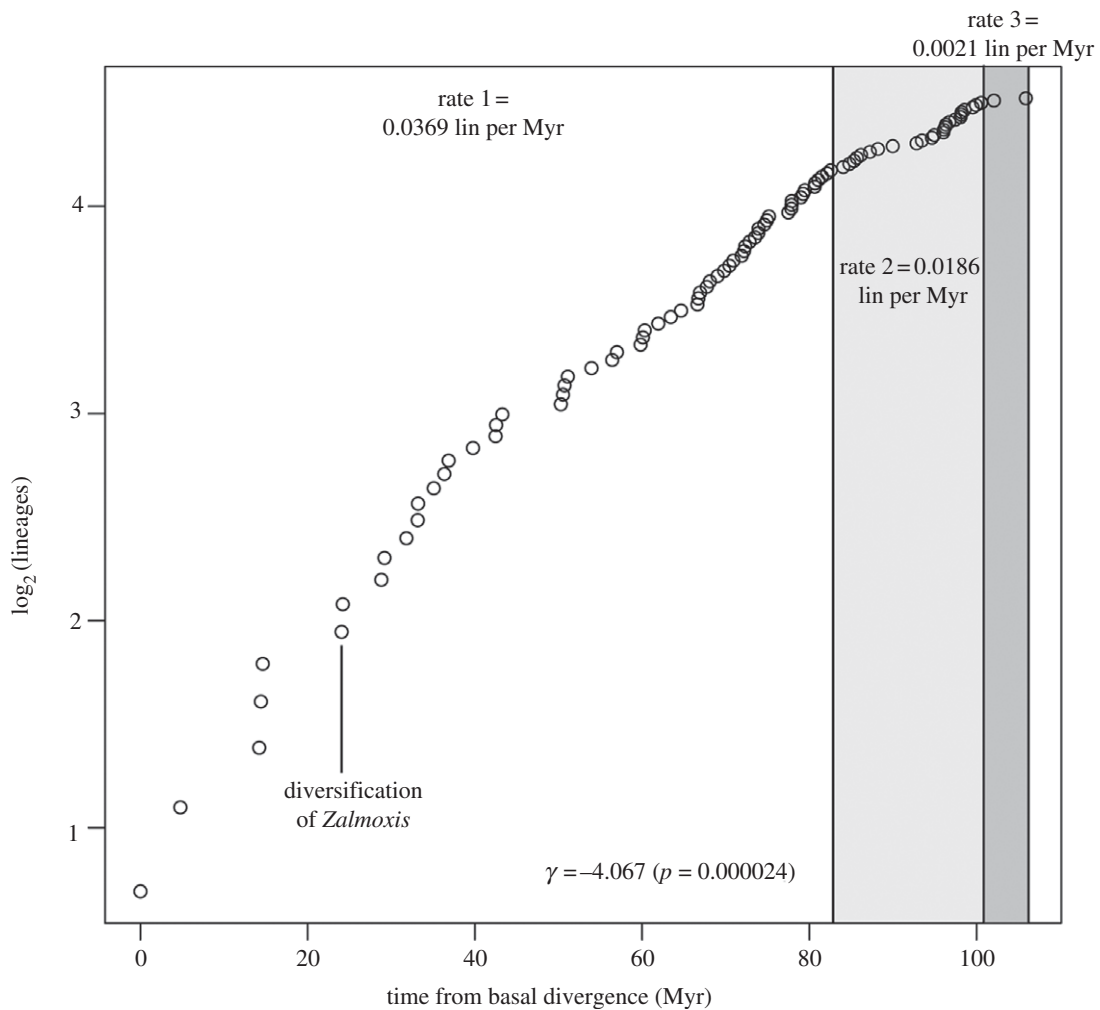


Figure 3. Log-lineage through time plot of Zalmoxidae, indicating diversification rates and timing of rate shifts inferred by the optimal, Yule-three-rate model. Note that the second best model, a Yule-two-rate, infers the same timing of the first rate shift (ca 82.6 Myr from basal divergence) as the optimal model.

Table 2. Fit of models to the zalmoxid log-lineage through time curve. DDL and DDX refer to density-dependent logistic and exponential models, respectively. Boldface text indicates optimal model.

model	no. parameters	ln L	AIC	ΔAIC
pure birth	1	-98.558	199.117	19.983
birth-death	2	-98.558	201.117	21.983
DDL	2	-92.282	188.565	9.431
DDX	2	-91.748	187.495	8.361
Yule-two-rate	3	-89.341	184.683	5.549
<b>Yule-three-rate</b>	<b>5</b>	<b>-84.567</b>	<b>179.134</b>	<b>0</b>
variable speciation	3	-91.711	189.421	10.287
variable extinction	3	-98.942	203.884	24.75

5. CONCLUSION

The family Zalmoxidae, similar to the Pacific iguanas, constitutes the unusual case of a lineage of Neotropical origin that colonized the Indo-Pacific, likely by ancient transoceanic dispersal during the Late Cretaceous.

While the mechanism of ancient dispersals is always a matter of speculation in the absence of a detailed fossil record, it is possible that Indo-Pacific colonization was achieved by rafting on floating vegetation propelled westwards by the Tethyan Seaway and/or southern equatorial currents, the directions of which are consistent with the direction of colonization. The timing here is well estimated and consistent with a Late Cretaceous dispersal event, and unlike other trans-Pacific taxa (e.g. iguanas, amphi-Pacific plant lineages) considerable diversity is retained on both sides of the Pacific Ocean. Zalmoxidae therefore presents a biogeographic conundrum: a radiation that has colonized the Indo-Pacific by ancient dispersal, engendering potentially hundreds of lineages endemic to Australasian terranes, but of Neotropical origins.

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