



India–Madagascar vicariance explains cascade beetle biogeography

EMMANUEL F. A. TOUSSAINT^{1*}, MARTIN FIKÁČEK^{2,3} and ANDREW E. Z. SHORT¹

¹Department of Ecology & Evolutionary Biology & Division of Entomology, Biodiversity Institute, University of Kansas, Lawrence, KS, USA

²Department of Entomology, National Museum, Cirkusova 1740, CZ-19800, Praha 9, Czech Republic

³Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-12843, Praha 2, Czech Republic

Received 19 November 2015; revised 22 January 2016; accepted for publication 23 January 2016

India and Madagascar drifted apart more than 80 Mya, yet few taxonomic groups currently found in these regions bear any signature of this split. When drifting in isolation, extensive volcanic activity covered almost half of India in lava flows, likely triggering widespread extinction on the island. Consequently, most of India's rich extant flora and fauna are considered to be the result of recent Cenozoic dispersal, and no lineages are conclusively a result of ancient vicariance. Many of Madagascar's lineages also stem from either Cenozoic diversification or dispersal events, with the latter being a result of the close proximity of the island with mainland Africa. In the present study, we focus on two remarkable lineages of cascade beetles in the genera *Scoliopsis* and *Tritonus* (Coleoptera, Hydrophilidae), respectively, dwelling in the mountains of south India and Sri Lanka, as well as in Madagascar. Based on a molecular phylogeny of the family Hydrophilidae dated with eight fossils, we show that these two lineages are sister taxa, and diverged when Madagascar and Greater India (India, Sri Lanka, Seychelles) separated, suggesting a pattern of Gondwanan vicariance. The results of the present study show that, despite geological upheaval, the present-day fauna of India still retains traces of its Gondwanan past. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 118, 982–991.

KEYWORDS: biogeography – Deccan Traps – East Gondwana – Hydrophilidae – volcanism.

INTRODUCTION

Vicariance associated with the breakup of the Gondwanan supercontinent into present day Africa, Australia, Antarctica, South America, Madagascar, and India is commonly invoked to explain the contemporary distribution of ancient lineages located in two or more of these regions (Lomolino *et al.*, 2006; Cox & Moore, 2010). The development and implementation of molecular clock calibrations in recent decades permitted the testing of numerous hypotheses of Gondwanan vicariance across the tree of life (Smith & Peterson, 2002). Many of these clades were shown to be too young to invoke vicariance, instead implicating long distance dispersal as the likely cause. As a result, substantiated examples of vicariant signatures in lineages with a so-called 'Gondwanan distribution'

are uncommon, although several examples have been documented, almost exclusively involving the fragments of West Gondwana. Geckoes (Squamata, Gekota) have been suggested as an example of such vicariance, with New World genera of the family Sphaerodactylidae being recovered as sister to the Old World genus *Saurodactylus* from Morocco (Gamble *et al.*, 2008). A dated phylogeny based on fossil and biogeographical calibrations recovers an age estimate for this split of approximately 96 Mya. The time frame of this cleavage corresponds with the opening of the Atlantic Ocean in the Cretaceous, therefore supporting a West Gondwana vicariance scenario (Gamble *et al.*, 2008). The Malagasy angiosperm genus *Takhtajania* (Canellales, Winteraceae) was reconstructed as the sister taxon of a large clade comprising species from the Australian region. Here also, a dated phylogeny of the family Winteraceae suggests that the split is sufficiently old (91.5 Mya)

*Corresponding author. E-mail: toussaint@ku.edu

to represent the signature of a Gondwanan vicariance (Thomas *et al.*, 2014). Among the plant taxa potentially supporting a Gondwana vicariance, the genus *Lomatia* (Proteales, Proteaceae) and the family Corsiaceae (Liliales) were suggested as examples of Australia–South America vicariance with somewhat congruent dating (49 and 53 Mya, respectively) (Mennes *et al.*, 2015; Milner *et al.*, 2015). In insects, stag beetles (Coleoptera, Lucanidae) have been introduced as likely candidates for repeated ancient Gondwana vicariance (Kim & Farrell, 2015). A robust dated phylogeny highlights several Gondwanan relationships in the family that present divergence times consistent with a vicariant scenario. This is the case for the charismatic subfamily Lampriminae (Australia–South America, 38 Mya), the tribe Chiasognathini (Australia–South America, 47 Mya), and the genus *Colophon* (South Africa–austral Gondwana, 87 Mya). These studies stand among a select few that have provided substantial evidence of Gondwanan vicariance through the identification of exclusive sister relationships, reconstruction of well-resolved phylogenetic relationships, inference of rigorous molecular dating, and support for synchronous time frames between continental drifting and lineage diversification.

Compelling examples of vicariance for taxa distributed on the East Gondwana fragments of Madagascar and the India/Seychelles block (Greater India) are much rarer. These fragments began rifting from each other approximately 100 Mya and became fully separated by 88 Mya (Storey *et al.*, 1995; Gibbons, Whittaker & Müller, 2013). Although sister relationships among clades that occur in India and Madagascar have been suggested previously, attempts to attribute this pattern to ancient vicariance have been unsuccessful because most of these lineages have a relatively young Cenozoic origin (Yoder & Nowak, 2006; Datta-Roy & Karanth, 2009; Warren *et al.*, 2010). Several amphibians occurring on the India/Seychelles block are sufficiently old to invoke such a pattern, although these clades also occur in Eurasia, and an incomplete sampling of these taxa has prevented any conclusive testing of this hypothesis (Datta-Roy & Karanth, 2009; Warren *et al.*, 2010; Pyron, 2014).

The water scavenger beetles (Coleoptera, Hydrophilidae) comprise a cosmopolitan group of aquatic beetles with almost 3000 species. Members of the family occupy a diverse range of freshwater aquatic habitats, although terrestriality has evolved numerous times within the lineage (Bloom, Fikáček & Short, 2014). The hydrophilid genera *Scoliopsis* (one species) and *Tritonus* (seven species) are restricted to southern India/Sri Lanka and Madagascar, respectively (Spangler, Steiner & , 2005; Short,

2008). Both genera are extremely specialized, dwelling exclusively on the seeps and water films of rocky surfaces at the edge of cascades and waterfalls (Fig. 1). *Tritonus* and *Scoliopsis* species exhibit a highly compressed body form typical of other larger-bodied aquatic beetle lineages that are cascade specialists. No other closely-related taxa are known to exist. The phylogenetic relationships of these two genera were inferred using a multilocus dataset (Bloom *et al.*, 2014) and their divergence time was estimated using eight fossil calibrations across the family Hydrophilidae.

MATERIAL AND METHODS

We retrieved molecular alignments of the family Hydrophilidae (Coleoptera, Hydrophiloidea) from Short & Fikáček (2013) for six gene fragments (see Supporting information, Table S1): mitochondrial cytochrome *c* oxidase subunit 1, cytochrome *c* oxidase subunit 2, and ribosomal 16S, as well as nuclear arginine kinase (ARK), ribosomal 18S, and ribosomal 28S. The concatenated matrix comprised 5945 aligned nucleotide sites. We sequenced and added two species of the Malagasy genus *Tritonus* (Hydrophilidae, Laccobiini) to this matrix for a total of 259 taxa (Table 1). The DNA extraction was performed with a DNeasy kit (Qiagen) using whole specimens. The polymerase chain reaction protocols were the same as those reported by Short & Fikáček (2013). The sequences were edited and aligned in GENEIOUS, version 8 (Biomatters, <http://www.geneious.com>) using MUSCLE (Edgar, 2004). The reading frames of protein-coding genes were checked under MESQUITE, version 3.0.3 (<http://www.mesquiteproject.org>) and the concatenation of the different gene fragment alignments was conducted using the same software. The sequence data are archived on GenBank under the accession numbers KU711848–KU711853.

The phylogenetic relationships were reconstructed using Bayesian inference in MrBayes, version 3.2.5 (Ronquist *et al.*, 2012) and using maximum likelihood in IQ-TREE (Nguyen *et al.*, 2015). We searched the optimal partitioning scheme and best-fit models of substitution for each partition using PARTITION-FINDER, version 1.1.1 (Lanfear *et al.*, 2012). Partitions were specified a priori based on gene fragment boundaries and codon positions of protein-coding gene fragments for a total of 12 partitions. The theoretical scores used to select proper models of substitution for each subset were calculated using the corrected Akaike information criterion (AICc). We used the *greedy* algorithm and the *mrBayes* set of models to perform the partitioning analysis. The

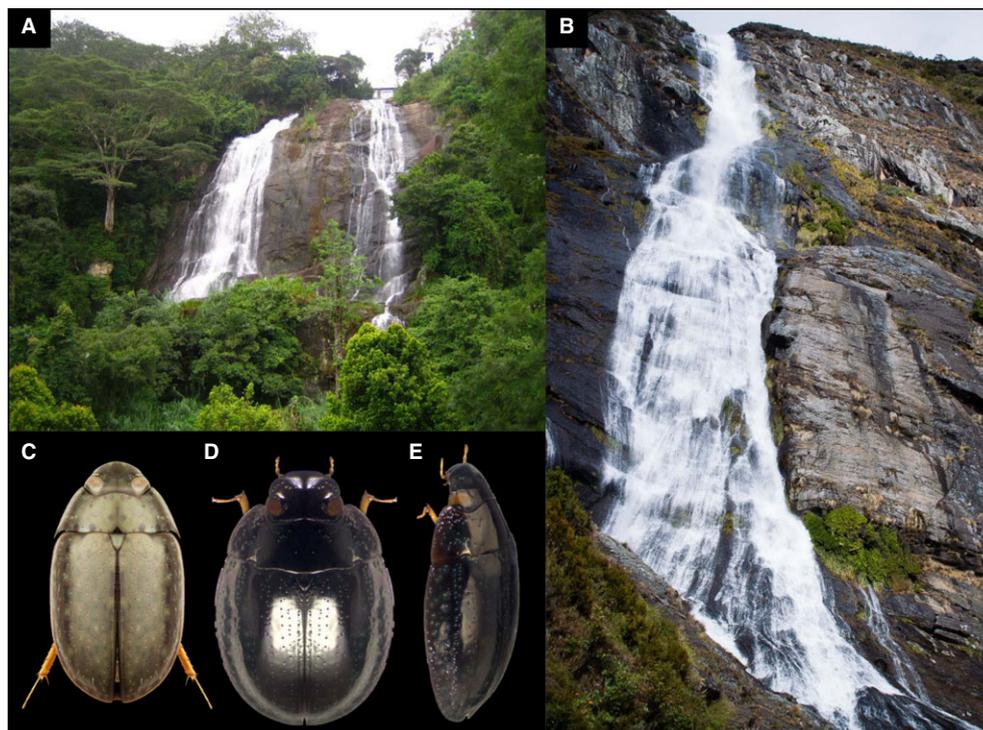


Figure 1. Habitat and morphology of the cascade beetle genera *Scoliopsis* and *Tritonus*. A, B, waterfall ecosystems in Sri Lanka (Hunas Falls, Knuckles mountain ranges) and Madagascar (Riandahry Falls, Andringitra national park), respectively, where *Scoliopsis* and *Tritonus* can be observed in associated seepage habitats. C, habitus of the *Scoliopsis* species used in the present study. D, E, habitus of one of the *Tritonus* species used in the present study in dorsal and lateral views. Beetle images not to scale. Picture credits: Aldan Jones (A); Francois Bouchet (B); Andrew Short (C, D, E).

Table 1. Fossil information used to calibrate the phylogeny of the family Hydrophilidae

Name	Age (Mya)	Placement	Offset	Mean
<i>Crenitulus paleodominicus</i> †	15–20	Stem Crenitulus*	13.13	73.70
<i>Baissalarva hydrobioides</i> †	135–146	Stem Hydrobiusini	134.00	40.93
<i>Cercyon</i> sp.†	44	Stem Megastenini	42.33	65.79
<i>Helophorus paleosibiricus</i> †	135–146	Stem <i>Helophorus</i>	134.00	40.93
<i>Helochares</i> sp.†	44	Stem <i>Helochares</i> + <i>Helobata</i>	42.33	65.79
<i>Hydrobius titan</i> †	33.9–37.2	Stem <i>Sperchopsis</i>	32.16	68.55
<i>Limnoxenus olenus</i> †	22.5	Stem <i>Limnoxenus</i>	20.69	71.64
<i>Protochares brevivalpis</i> †	145–161	Stem Hydrophilidae	144.00	38.21

†refers to extinct fossil taxa. Crenitulus*, refers to the clade comprising *Anacaena solstitialis* MSC1815, *Anacaena suturalis* MSC1816, *Anacaena hirsuta* SLE0369, and *Anacaena* sp SLE0346 (see Supporting information, Figures S1, S2). The Offset and Mean columns indicate the values for each parameter used in BEAUti for the dating analysis based on exponential distribution fossil priors.

MrBayes analyses consisted of two runs of eight Markov chain Monte Carlo simulations, each running for 50 million generations with tree sampling every 5000th generation to obtain 10 000 posterior trees. The convergence of the runs was checked in TRACER, version 1.6 (<http://tree.bio.ed.ac.uk/software/tracer>). Parameters for which effective sample

sizes (ESS) were equal or > 200 were acknowledged as sufficiently sampled. The convergence of the runs was also assessed by checking the mean SD of split frequencies as recovered from the *mcmc* file and by ensuring that all parameters have a potential scale reduction factor value close to 1.000 using the *sump* command in MrBayes. All trees that predated the

convergence of both runs were removed before assembling a 50% majority rule consensus tree using the command *sumt* in MrBayes. The IQ-TREE analyses were conducted via the webserver (<http://iqtree.cibiv.univie.ac.at>) with the same partitioning scheme as that used for the MrBayes analyses but with the optimal substitution model for each partition being searched using the Auto function based on the AICc. We performed 1000 ultrafast bootstrap replicates (Minh, Nguyen & von Haeseler, 2013) to investigate nodal support across the topology.

We inferred divergence times using BEAST, version 1.8.2 (Drummond *et al.*, 2012). We used the topology recovered in MrBayes as a fix input by modifying manually the *.xml* file and unchecking all *Tree* operators in BEAUti, version 1.8.2 (Drummond *et al.*, 2012). The dataset was partitioned by gene fragments as six partitions and the substitution model for each partition was selected in PARTITIONFINDER, version 1.1.1 (Lanfear *et al.*, 2012) with the *best* set of models. The clock models were linked for the mitochondrial genes (mtDNA) and unlinked for the nuclear genes. The molecular clock test was performed in MEGA6 (Tamura *et al.*, 2013) by comparing the maximum likelihood value of the MrBayes topology with and without the molecular clock constraints under the Tamura-Nei model. Based on the results of this test (see Results), we used a Bayesian relaxed clock approach as implemented in BEAST, version 1.8.2. We assigned a *log-normal relaxed clock* with uncorrelated rates to each clock model (18S, 28S, ARK and mtDNA). The *Tree Model* was set to *Speciation: Birth-Death Process*. The *ucl.d.mean* prior of each clock model was set to an uninformative interval (0.0001–1.0) with a uniform prior distribution. The analysis consisted of 50 million generations with a tree and parameter sampling every 5000 cycles. The convergence of the run and sufficient sampling of each parameter was checked in TRACER, version 1.6 (<http://tree.bio.ed.ac.uk/software/tracer>). For the MrBayes analyses, ESS \geq 200 were considered as good indicators of convergence.

To calibrate the topology, we used multiple fossil calibrations as carefully chosen and detailed in Bloom *et al.* (2014) and summarized in Table 1. Because these fossils have not been assigned to extent clades, we placed these at the stem in a conservative manner. Fossil information was enforced using uniform prior distribution running from the minimum age estimate of each fossil to 285 Mya, which is the age estimate for beetles (Hunt *et al.*, 2007). We also used exponential priors to cross-validate our age estimates and reduce the potential impact of a loose maximum prior at the root. The 95% confidence interval ranged from the age of the

fossil to 285 Mya. The parameters (Offset and Mean) used in these analyses are summarized in Table 1 for each fossil.

RESULTS

The results of our phylogenetic inference and dating conducted on a concatenated matrix of six gene fragments (see Supporting information, Table S1) are shown in Figure 2. The results of the model selection analyses are presented in the Supporting information (Table S2). The MrBayes and IQ-TREE analyses recover a very well-resolved topology with robust nodal support for most nodes (see Supporting information, Figs S1, S2). The two topologies are highly congruent with only a few unsupported inconsistencies in some derived parts of the tree (see Supporting information, Figs S1, S2). All tribes within the subfamily Hydrophilinae are recovered as monophyletic with strong support (Fig. 2). Amphiopini is recovered as sister to all other tribes of the subfamily. Berosini is found as sister to Hydrophilini + Hydrobiusini on the one hand and Laccobini on the other. Within the tribe Laccobini, we infer two major clades corresponding to the *Laccobius* species group and the *Paracymus* species group. Within the latter, *Tritonus* is recovered monophyletic and as sister to *Scoliopsis* with strong support. The sister group relationship is also supported by several adult morphological characters (e.g. the lack of hydrofuge hairs covering abdominal ventrites 3–5, very large body size, dorsoventrally compressed body form).

The null hypothesis of equal evolutionary rate throughout the tree was rejected at a significance level of 0.05. The median ages derived from our dating analyses based on eight fossil calibrations (Table 1) are presented in Figure 2 along with the 95% height posterior distributions (HPD). The use of an exponential or uniform prior distribution to model the fossil information only had a slight impact on the divergence time estimates (Table 2). The analyses conducted with uniform prior distributions delivered slightly older ages than those using exponential prior distributions. These differences are shallow for the *Tritonus*–*Scoliopsis* split recovered at 91.56 Mya (95% HPD = 63.7–126.82 Mya) and 83.95 Ma (95% HPD = 61.04–111.50 Mya), respectively, for the uniform and the exponential prior distributions.

DISCUSSION

According to our Bayesian inference and maximum likelihood phylogenetic reconstructions, the Malagasy genus *Tritonus* is recovered as sister to the

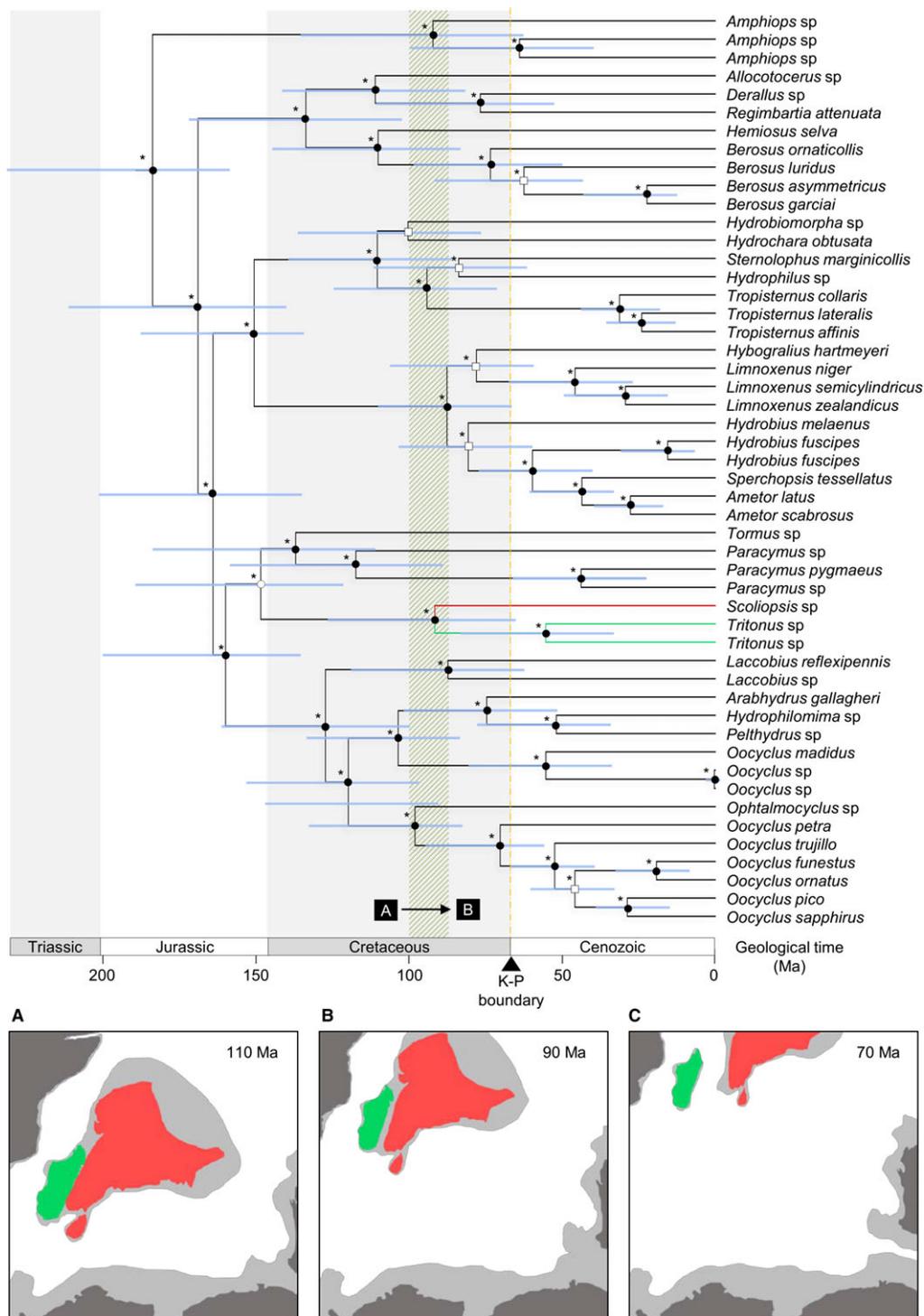


Figure 2. Evolution of *Scoliopsis* and *Tritonus* cascade beetles in a paleogeological framework. Time-calibrated phylogeny of the Hydrophilidae in the context of the break-up of East Gondwana, pruned to show only the subfamily Hydrophilinae. Bars at nodes indicate 95% confidence intervals of estimated ages. Closed circles at nodes indicate a posterior probability (PP) ≥ 0.95 ; open circles PP ≥ 0.90 , and open squares PP < 0.90 from the MrBayes analysis. Asterisks indicate a bootstrap support ≥ 80 from the IQ-TREE analysis. A hatched bar indicates the period during which India and Madagascar were still in contact; B, ~ 90 Ma Madagascar already separated from India but remained on the same plate; C, ~ 70 Ma India and Madagascar were fully separated and on different plates.

Table 2. Divergence time estimates of major lineages among the family Hydrophilidae

Clade	Median ages with uniform priors	Median ages with exponential priors
Acidocerinae	147.41 (119.27–187.17)	131.33 (112.34–155.34)
Amphiopini	92.19 (61.65–134.08)	85.75 (57.74–117.67)
Berosini	133.60 (104.45–171.62)	118.50 (95.53–140.79)
Chaetarthriinae	174.22 (143.67–215.98)	155.63 (135.40–183.39)
Enochrinae	140.76 (108.57–178.38)	139.30 (105.01–155.06)
Hydrobiusini	87.60 (68.45–114.14)	74.27 (57.37–94.51)
Hydrophilidae	196.46 (165.36–243.73)	176.43 (173.23–228.11)
Hydrophilinae	183.61 (155.88–228.92)	165.71 (148.00–189.85)
Hydrophilini	110.31 (86.42–143.32)	99.49 (74.48–121.53)
Laccobiini	159.80 (136.42–199.89)	144.69 (131.98–166.11)
Megasternini	97.46 (79.79–125.61)	86.94 (72.73–102.41)
Root	240.29 (198.97–296.04)	217.06 (178.96–264.88)
Rygmodynae	111.37 (84.97–150.05)	97.88 (77.63–122.78)
Sphaeridiinae	158.24 (129.87–196.01)	141.43 (121.25–162.90)
<i>Tritonus</i> + <i>Scoliopsis</i>	91.56 (63.7–126.82)	83.95 (61.04–111.50)

The median ages are given in million years followed by the 95% height posterior distribution.

monotypic south Indian–Sri Lanka genus *Scoliopsis* with strong support (Fig. 2); this close relationship has also been suggested based on adult morphology (Hansen, 1991). The two genera are on a long stem with no close relatives, and resolved as sister to a clade with a cosmopolitan distribution. Our Bayesian relaxed clock analyses using uniform or exponential prior distributions generally agree with the age estimates of Bloom *et al.* (2014). We recover a split between both genera in the late Cretaceous coinciding with the breakup of East Gondwana.

Our results support the hypothesis suggesting that the biogeography of this clade is the result of India–Madagascar vicariance. The common ancestor of the *Tritonus* + *Scoliopsis* likely occurred on both Greater India and Madagascar. The rifting of East Gondwana created a barrier to gene flow between the two landmasses, leading to the vicariant pattern observed today as a result of *Scoliopsis* surviving on the drifting Indian block.

As India drifted northwards, the Deccan Traps erupted at the end of the Cretaceous, and covered large parts of emerged India in thick lava flows (Courtillot *et al.*, 1986; Chenet *et al.*, 2008). This unprecedented volcanism is considered to have triggered mass extinctions on the drifting island (Schoene *et al.*, 2015), although refugia might have existed (Joshi & Karanth, 2013). Post-eruption fossil deposits in western India have shown little evidence of an endemic fauna; instead, they reveal a fauna comprised primarily of widespread or Eurasian-derived lineages (Rust *et al.*, 2010). *Scoliopsis* likely survived because of its unusual association with rocky habitats. The existence of freshwater resources and swampy areas on some parts of India was

suggested to explain the survival of Mantellidae frogs in this inhospitable basaltic landscape (Bossuyt & Milinkovitch, 2001). If such ephemeral habitats did exist throughout the drifting of India toward Eurasia, they might have provided sufficient patchy habitats for *Scoliopsis* to survive when most other lineages went extinct. Waterfall habitats are also known to serve as reservoirs of other relictual water beetle taxa around the world, including South Africa and the Guiana Shield region of South America, adding support to this hypothesis (Ribera *et al.*, 2002).

We consider that a vicariant scenario best explains our data; however, distribution artefacts and dispersal cannot be definitively ruled out as alternative hypotheses. It is possible that we now only see the extant representatives of a previously taxon-rich or more broadly distributed clade, although invoking an unsupported ad hoc extinction hypothesis is not a parsimonious explanation of the data, nor would it change the age estimate supporting the ancient vicariance signature. The fauna of waterfalls is more poorly known than common lotic and lentic habitats, and there have been a number of focused collecting efforts in such habitats around the world, including mainland Africa and Asia. Consequently, it is unlikely the currently observed distribution is a collecting artefact.

Although there is no way to disprove ancient dispersal as the mechanism for any observed Gondwanan biogeographical pattern, we consider that invoking it in this example requires more assumptions and is less parsimonious than vicariance. Such a scenario would imply that the highly derived and specialized *Tritonus*–*Scoliopsis* lineage either (1) occurred initially only on one side of East Gondwana,

and then dispersed to the other fragment soon after it rifted, or (2) that the lineage dispersed to the fragments of East Gondwana from a mainland stock.

The first scenario implies that a lineage initially unable to disperse across East Gondwana when it was a continuous landmass was subsequently able to

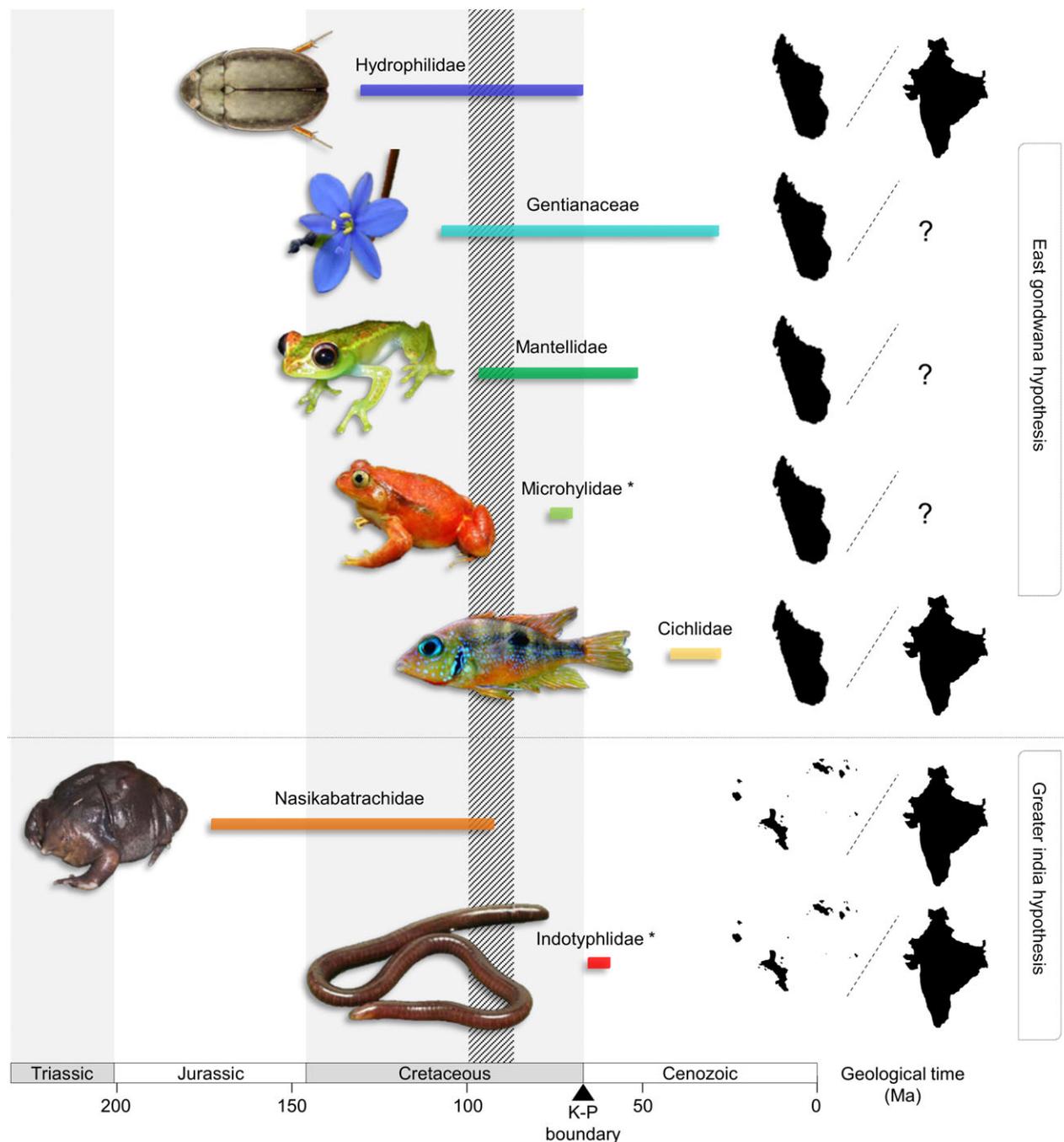


Figure 3. Summary of groups that have been suggested as evidence of India–Madagascar or India/Seychelles vicariance. Schematic view of divergence time credibility intervals inferred for groups candidate to an East Gondwana vicariance scenario. Coloured bars indicate 95% confidence interval of divergence age (values derived from the uniform priors are shown for *Scoliopsis/Tritonus*). Taxa marked by an asterisk indicate studies in which only absolute ages were provided. A hatched bar indicates the period during which India and Madagascar were rifting. Question marks indicate that the lineage also occurs outside of India and complicates the pattern of vicariance. Picture credits, from top to bottom: Andrew Short, Cherubino, Axel Strauss, Franco Andreone, Cedric Loury, Karthickbala, Nirmal Kulkarni.

disperse between its fragments after it separated. The second scenario requires at least two unidirectional dispersal events coupled with mainland extinction. We consider both these scenarios to be unlikely.

The Ninety-East Ridge (NER), a linear chain of now-submerged small islands in the middle of the Indian Ocean, has also been suggested to have served as 'stepping stones' for some taxa that show patterns of Gondwanan vicariance (Renner, 2010), particularly as a route out of Australia. However, any small exposed islands of the NER would have been thousands of kilometres to the east of East Gondwana as it rifted. Invoking the NER in biogeographical scenarios involving East Gondwana would thus have require multiple long distance dispersal events in opposite directions. This also appears unlikely because the chain initially formed in the north of the Indian Ocean with the age of those sites closest to Madagascar not forming until 43.2 Mya, and the islands would have had to possess permanent running freshwater for aquatic beetles populations to persist. Although the species of *Tritonus* and *Scolioipsis* are winged, their flight ability and dispersal dynamics are unknown.

Our findings provide one of the strongest cases of ancient Madagascar/India vicariance (Fig. 3). Mantellidae and Microhylidae frogs (Anura) were first assumed to be good candidates for such a scenario (Bossuyt & Milinkovitch, 2001; Bocxlaer *et al.*, 2006; Bossuyt *et al.*, 2006; van der Meijden *et al.*, 2007). However, in both cases, the Malagasy taxa are found as sister to large clades of more broadly distributed Eurasia taxa, some of which occur in India. Additional taxon sampling in these frog families is necessary before their distribution can be unambiguously attributed to India–Madagascar vicariance. Furthermore, the dating of the split between Malagasy and Eurasian clades is to some extent incompatible with the hypothesis of ancient vicariance (Lomolino *et al.*, 2006) and the biogeographical pattern of these two families is the result of a combination of vicariant and dispersal events (Pyron, 2014). Recently flowering plants of the genus *Exacum* (Gentianales, Gentianaceae) were suggested as a candidate group for India–Madagascar vicariance based on extremely wide age credibility intervals (Pirie *et al.*, 2015). In addition to the unclear dating, the presence of Arabian Peninsula species in the Indian/Sri Lanka sister group and the mode of dispersal of the seeds by the wind both cast serious doubts upon this hypothesis. Some lineages of cichlid fishes (Perciformes, Cichlidae) were long considered to be of very ancient origin and their disjunct distribution in Madagascar and India to be the result of Gondwanan vicariance. However, there is now a growing body of evidence

indicating that these cichlids are of much more recent origin (Friedman *et al.*, 2013). A few other groups exhibit a pattern of Greater India vicariance with distribution of taxa between India and Seychelles. This is the case for the recently described frog family Nasikabatrachidae (Anura) (Biju & Bossuyt, 2003) and the caecilian family Indotyphlidae (Apoda) (Kamei *et al.*, 2012; Pyron, 2014). In both cases, age estimates and phylogenetic relationships support a vicariant origin, although the full biogeographical history of these lineages is obscured by the lack of fossils or an extant close representative in Madagascar (Fig. 3). These lineages could have dispersed from other Gondwana fragments to Greater India between the split from Madagascar and the separation of the Seychelles from India.

Our phylogenetic and dating analyses in the present study substantiate one of the first examples of ancient India–Madagascar vicariance. This result significantly broadens our understanding not only of early aquatic beetle early evolution, but also of Gondwanan biogeography.

ACKNOWLEDGEMENTS

Grey Gustafson is warmly thanked for providing the *Tritonus* specimens used in the present study. We thank Michael Balke, Christopher Beard, Gael Kergoat, Praveen Karanth, and two anonymous reviewers for providing helpful comments on earlier versions of the manuscript. This research was supported by the University of Kansas General Research Fund Award #2301754 and NSF-DEB Award #1453452, and by the Ministry of Culture of the Czech Republic (DKRVO 2016/14, National Museum, 00023272) to M. Fikáček.

REFERENCES

- Biju SD, Bossuyt F. 2003. New frog family from India reveals an ancient biogeographical link with the Seychelles. *Nature* **425**: 711–714.
- Bloom DD, Fikáček M, Short AEZ. 2014. Clade age and diversification rate variation explain disparity in species richness among water scavenger beetle (Hydrophilidae) lineages. *PLoS ONE* **9**: e98430.
- Bocxlaer IV, Roelants K, Biju SD, Nagaraju J, Bossuyt F. 2006. Late Cretaceous vicariance in Gondwanan amphibians. *PLoS ONE* **1**: e74.
- Bossuyt F, Milinkovitch MC. 2001. Amphibians as indicators of early tertiary 'out-of-India' dispersal of vertebrates. *Science* **292**: 93–95.
- Bossuyt F, Brown RM, Hillis DM, Cannatella DC, Milinkovitch MC. 2006. Phylogeny and biogeography of a cosmopolitan frog radiation: late Cretaceous diversification

- resulted in continent-scale endemism in the family Raniidae. *Systematic Biology* **55**: 579–594.
- Chenet AL, Fluteau F, Courtillot V, Gérard M, Subbarao KV. 2008.** Determination of rapid Deccan eruptions across the Cretaceous–Tertiary boundary using paleomagnetic secular variation: results from a 1200-m-thick section in the Mahabaleshwar escarpment. *Journal of Geophysical Research: Solid Earth* **113**: B04101.
- Courtillot V, Besse J, Vandamme D, Montigny R, Jaeger J-J, Cappeta H. 1986.** Deccan flood basalts at the Cretaceous/Tertiary boundary? *Earth and Planetary Science Letters* **80**: 361–374.
- Cox CB, Moore PD. 2010.** *Biogeography: an Ecological and Evolutionary Approach*, 8th edn. Hoboken, NJ: Wiley.
- Datta-Roy A, Karanth KP. 2009.** The Out-of-India hypothesis: what do molecules suggest? *Journal of Biosciences* **34**: 687–697.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012.** Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.
- Edgar RC. 2004.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.
- Friedman M, Keck BP, Dornburg A, Eytan RI, Martin CH, Hulseley CD, Wainwright P, Near TJ. 2013.** Molecular and fossil evidence place the origin of cichlid fishes long after Gondwanan rifting. *Proceedings of the Royal Society of London B* **280**: 20131733.
- Gamble T, Bauer AM, Greenbaum E, Jackman TR. 2008.** Evidence for Gondwanan vicariance in an ancient clade of gecko lizards. *Journal of Biogeography* **35**: 88–104.
- Gibbons AD, Whittaker JM, Müller RD. 2013.** The breakup of East Gondwana: assimilating constraints from Cretaceous ocean basins around India into a best-fit tectonic model. *Journal of Geophysical Research: Solid Earth* **118**: 808–822.
- Hansen M. 1991.** The hydrophiloid beetles. Phylogeny, classification and a revision of the genera (Coleoptera, Hydrophiloidea). *Biologiske Skrifter* **40**: 1–367.
- Hunt T, Bergsten J, Levkanicova Z, Papadopoulou A, St John O, Wild R, Hammond PM, Ahrens D, Balke M, Caterino MS, Gomez-Zurita J, Ribera I, Barraclough TG, Bocakova M, Bocak L, Vogler AP. 2007.** A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science* **318**: 1913–1916.
- Joshi J, Karanth P. 2013.** Did southern Western Ghats of peninsular India serve as refugia for its endemic biota during the Cretaceous volcanism? *Ecology and Evolution* **3**: 3275–3282.
- Kamei RG, San Mauro D, Gower DJ, Bocxlaer IV, Sherratt E, Thomas A, Babu S, Bossuyt F, Wilkinson M, Biju SD. 2012.** Discovery of a new family of amphibians from northeast India with ancient links to Africa. *Proceedings of the Royal Society of London B* **279**: 2396–2401.
- Kim SI, Farrell BD. 2015.** Phylogeny of world stag beetles (Coleoptera: Lucanidae) reveals a Gondwanan origin of Darwin's stag beetle. *Molecular Phylogenetics and Evolution* **86**: 35–48.
- Lanfear R, Calcott B, Ho SY, Guindon S. 2012.** PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695–1701.
- Lomolino MV, Riddle BR, Whittaker RJ, Brown JH. 2006.** *Biogeography*, 3rd edn. Sunderland, MA: Sinauer Associated Inc.
- van der Meijden A, Vences M, Hoegg S, Boistel R, Channing A, Meyer A. 2007.** Nuclear gene phylogeny of narrow-mouthed toads (Family: Microhylidae) and a discussion of competing hypotheses concerning their biogeographical origins. *Molecular Phylogenetics and Evolution* **44**: 1017–1030.
- Mennes CB, Lam VKY, Rudall PJ, Lyon SP, Graham SW, Smets EF, Merckx SFT. 2015.** Ancient Gondwana break-up explains the distribution of the mycoheterotrophic family Corsiaceae (Liliales). *Journal of Biogeography* **42**: 1123–1136.
- Milner ML, Weston PH, Rossetto M, Crisp MD. 2015.** Biogeography of the Gondwanan genus *Lomatia* (Proteaceae): vicariance at continental and intercontinental scales. *Journal of Biogeography* **42**: 2440–2451.
- Minh BQ, Nguyen MAT, von Haeseler A. 2013.** Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* **30**: 1188–1195.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015.** IQ-tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274.
- Pirie MD, Litsios G, Bellstedt DU, Salamin N, Kissling J. 2015.** Back to Gondwanaland: can ancient vicariance explain (some) Indian Ocean disjunct plant distributions? *Biology Letters* **11**: 20150086.
- Pyron RA. 2014.** Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. *Systematic Biology* **63**: 779–797.
- Renner SS. 2010.** Biogeographic insights from a short-lived Palaeocene island in the Ninetyeast Ridge. *Journal of Biogeography* **37**: 1177–1178.
- Ribera I, Beutel RG, Balke M, Vogler AP. 2002.** Discovery of Aspidytidae, a new family of aquatic Coleoptera. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 2351–2356.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard M, Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Rust J, Singh H, Rana RS, McCann T, Singh L, Anderson K, Sarkar N, Nascimbene PC, Stebner F, Thomas JC, Solorzano Kraemer M, Williams CJ, Engel MS, Sahni A, Grimaldi D. 2010.** Biogeographic and evolutionary implications of a diverse paleobiota in amber from the early Eocene of India. *Proceedings of the National Academy of Science of the United States of America* **107**: 18360–18365.
- Schoene B, Samperton KM, Eddy MP, Keller G, Adatte T, Bowring SA, Khadri SFR, Gertsch B. 2015.** U-Pb

- geochronology of the Deccan Traps and relation to the end-Cretaceous mass extinction. *Science* **347**: 182–184.
- Short AEZ. 2008.** Revision of the Afrotropical genus *Tritonus* Mulsant (Coleoptera: Hydrophilidae). *Zootaxa* **1855**: 41–55.
- Short AEZ, Fikáček M. 2013.** Molecular phylogeny, evolution and classification of the Hydrophilidae (Coleoptera). *Systematic Entomology* **38**: 723–752.
- Smith AB, Peterson KJ. 2002.** Dating the time of origin of major clades: molecular clocks and the fossil record. *Annual Review of Ecology Evolution and Systematics* **30**: 65–88.
- Spangler PJ, Steiner WEA. 2005.** A new aquatic beetle family, Meruidae, from Venezuela (Coleoptera: Adephaga). *Systematic Entomology* **30**: 339–357.
- Storey M, Mahoney JJ, Saunders AD, Duncan RA, Kelley SP, Coffin MF. 1995.** Timing of hot spot-related volcanism and the breakup of Madagascar and India. *Science* **267**: 852–855.
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. 2013.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–2729.
- Thomas N, Bruhl JJ, Ford A, Weston PH. 2014.** Molecular dating of Winteraceae reveals a complex biogeographical history involving both ancient Gondwanan vicariance and long-distance dispersal. *Journal of Biogeography* **41**: 894–904.
- Warren BH, Strasberg D, Bruggemann JH, Prys-Jones RP, Thébaud C. 2010.** Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics* **26**: 526–538.
- Yoder AD, Nowak MD. 2006.** Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annual Review of Ecology Evolution and Systematics* **37**: 405–431.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. MrBayes consensus topology with nodal posterior probabilities.

Figure S2. IQ-TREE consensus topology with nodal ultrafast bootstrap values.

Table S1. Taxon sampling, gene fragment coverage information, and GenBank accession numbers.

Table S2. PartitionFinder and IQ-TREE models of substitutions selected for the MrBayes and IQ-TREE analyses.