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# Natatanuran frogs used the Indian Plate to step-stone disperse and radiate across the Indian Ocean

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Investigating the evolutionary history of widespread higher taxa, subjected to multiple tectonic events, can provide evidence for or against various palaeogeographical models of early Earth history [1,2]. Contemporary biotic distributions have been strongly influenced by events associated with the breakup of Gondwana into present-day Africa, Antarctica, Australia, South America, Madagascar and India, during the Late Mesozoic and Early Palaeogene [2]. The fragmentation of Gondwana and subsequent tectonic drift ultimately allowed biotic exchanges between Laurasia and Gondwana [2,3], influencing the global distributions of many taxa.

The relative positions of the post-breakup Gondwanan landmasses during the Late Cretaceous, especially of the Indian and Australian plates around the Indian Ocean, are highly debated [1]. The plate reshuffling was probably accompanied by the formation of multiple temporary land bridges and involved biotic exchange among the plates. Although most models agree that the Indian Plate carried a biotic 'ferry' of taxa (both plants and animals) to Asia after it broke away from other Gondwanan landmasses from about 88 to 55 Ma [2,4], both geological and paleontological data also support land bridges or minor marine barriers that permitted biotic exchanges with other Gondwanan landmasses (e.g. Africa, Madagascar; Fig. 1) [3,5]. There is also debate about whether Antarctica–Australia–New Guinea was connected to: (i) the Indian Plate via the Kerguelen Plateau (KP) land bridge and (ii) Madagascar via the Gunnerus Ridge (GR) land bridge in the Late Cretaceous [6–9]. Previous studies addressed some of these issues from palaeobiogeographical and evolutionary perspectives, but were inconclusive due to the selection of taxa that did not include all landmasses or the limited recovery of evolutionary relationships due to the use of sequence data from only a few genes [10–13].

The neobatrachian clade Natatanura are an ideal group to infer Gondwanan geological and environmental history due to their ancient origins (divergence from Afrobatrachia at around 100 Ma), high species diversity (>1500 extant species), almost cosmopolitan distribution (absent only from Antarctica), general low terrestrial vagility and poor overwater dispersal capabilities [14]. Previous studies suggested the divergence of Natatanura was characterized by a historical association with the breakup of Gondwanan plates [12]. These frogs are thus an appropriate group of organisms to test hypotheses of Cretaceous–Palaeogene biotic exchanges between Laurasia and Gondwana around the Indian Ocean. However, prior studies that have included Natatanura failed to resolve the major nodes in its phylogeny or suffered from incomplete lineage sampling, which, until now, hampered conclusive testing of these hypotheses [12,15].

Here, we integrate phylogenetic, biogeographic and molecular dating methods to reconstruct the spatiotemporal diversification of Natatanura (see

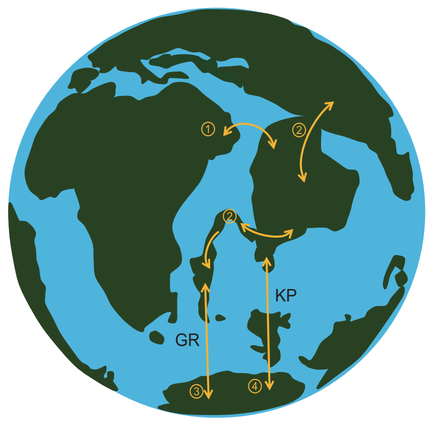


**Figure 1.** Schematic representation of different hypotheses regarding land connections and corridors for dispersal among the landmasses around the Indian Ocean from 88 to 55 Ma. (1) Africa and India were reconnected to each other directly [3,5]; (2) Asia and Madagascar were linked by India, with possible dispersal between Asia and Madagascar over India and the Seychelles plateau [5]; (3) Antarctic–Australia–New Guinea and Madagascar were connected by the Gunnerus Ridge (GR) [6,7]; (4) Antarctic–Australia–New Guinea and India were connected by the Kerguelen Plateau (KP) [13]. Paleo-reconstructions are modified from Briggs [5] and Bossuyt *et al.* [12].

Supplementary Data). Results resolve the evolutionary history of Natatanuran frogs (Supplementary Figs 1–3), based on molecular data from 376 nuclear loci, representing by far the largest molecular data set assembled for this group. Samples include all major lineages, 85 Natatanura species and 20 outgroup taxa (Supplementary Data). The novel evidence reveals how Natatanuran frogs interchanged between Laurasia and Gondwana around the Indian Ocean during the Cretaceous–Palaeogene, challenging recent biogeographical assumptions and providing new insights into Indian Ocean biotic exchanges.

## STEPPING-STONE ROLE OF THE INDIAN PLATE FOR BIOTIC EXCHANGE BETWEEN AFRICA, ASIA AND MADAGASCAR

Using the traditional Gondwana and Laurasia model, it has been commonly assumed that the Indian Plate was an isolated island between ∼88–55 Ma, and the Indian landmass served as an 'ark' to transport lineages from various biotic groups 'Out-of-India' into Asia, after India broke away and drifted northward from Gondwana in the Late Cretaceous [4]. According to this model, there was no biotic exchange (which would have required crossing marine barriers) between India and its nearby landmass after it separated from Madagascar ∼88 Ma, and dispersal events only resumed after India's collision with Asia in the early Eocene (∼65–55 Ma, [16]). 'Rafting' of the flora and fauna on the Indian Plate enabled unidirectional migration of Gondwanan taxa into Asia. However, our phylogenomic results reject this model. We do not find any periods between 88 and 55 Ma, when there was no biotic exchange occurring between Africa and India, India and Asia, or India and Madagascar. In contrast, our ancestral reconstruction suggests Natatanura originated in Africa and then dispersed to Asia through India ∼75.6–72.8 Ma (Fig. 2 and Supplementary Fig. 3). It is unlikely that frogs could cross a large saltwater barrier, although a few extant species may have made more modest oceanic dispersals (e.g. *Ptychadena mascareniensis*, [17]). Briggs [5] and Chaterjee *et al.* [3] suggested a geographical model in which there were 'corridors' or 'landspans' that reconnected Africa and India from ∼75 to ∼60 Ma. Our topologies and divergence time estimates are consistent with this scenario. A terrestrial route possibly existed from Africa to Asia via India, which would have allowed frogs to disperse among these landmasses. Moreover, Malagasy mantellid frogs are phylogenetically deeply nested within the larger Asian clade and the ancestral reconstruction supports this clade originating from Asia and dispersing to Madagascar (Fig. 2). Taking into account that the oldest known rhacophorid fossils (*Indorana prasadi*) are from the Indian landmass [18] and that the Indian Plate would have been well placed to minimize oceanic dispersal distances between Asia and Madagascar, the Indian Plate could have served as a stepping stone for long-distance dispersal, as suggested previously [13,15].

Geological and paleontological evidence has previously challenged the 'Indian-biotic Ark' model. For example, Briggs [5] proposed that India was in close proximity to other landmasses during its journey northwards because a significant endemic biota did not evolve on the Indian Plate during this period. Ali and Aitchison [8] proposed the existence of a palaeogeographic connection between Madagascar and India in the Late Cretaceous, which may have been formed by the Seychelles–Mascarene Plateau. More recently, Chatterjee *et al.* [3] argued that biotic links were possibly re-established between India and Africa during the Late Cretaceous, during India's collision with the Kohistan–Ladakh Arc along the Indus Suture in the Late Cretaceous. Sister relationships and divergence dates for some vertebrate fossil groups (Supplementary Data) also support stepping-stone biotic exchange via the Indian Plate, consistently with our results. However, we did not find any studies of nonvolant extant groups that provide substantial evidence for using the Indian Plate as a stepping-stone route among these three plates from 88 to 55 Ma. Although several plant and animal groups exhibit sister relationships between India (or Asia) and Madagascar (or Africa) (e.g. [19,20]), their deep divergences (>88 Ma) attribute this relationship to ancient vicariance coinciding with the breakup of Gondwana. Nevertheless, other taxa with younger divergences, and distributions that thus cannot be attributed to ancient Gondwanan vicariance, will represent good candidates for testing 'Indian stepping-stone hypotheses' in the future (e.g. microhylid frogs, [21]). We provide the first case of extant taxa that appears to have taken advantage of the Indian Plate as a stepping-stone route between Africa, Asia and Madagascar, although the exact positions of land bridges or traversable ocean channels still remain unclear. And we predict that this geographic scenario will also be recovered for other nonvolant organisms with Indian Ocean distributions.

## DISPERSALS WITH AUSTRALIA–NEW GUINEA AND ASIA

The Antarctica–Australia–New Guinea Plate has been proposed to have been connected to the Indian Plate by the KP land bridge, or connected to Madagascar by the GR land bridge (Fig. 1) in the Late Cretaceous [6,7], although these land bridges have been disputed due to a lack of evidence that they were sub-aerial during this period [8,9]. Concerning Natatanura diversification, we find no sister relationships between frogs from Antarctica–Australia–New Guinea and either India or Madagascar (Fig. 2), and thus find no support for biota exchanges among these landmasses via a KP and GR land bridge. In addition, we date Natatanura dispersal into Australia–New Guinea to be much later than these hypothetical Late Cretaceous land bridges.

Bossuyt *et al.* [12] suggested that Australia–New Guinea acted as a raft, enabling Gondwanan Natatanura frogs to colonize Southeast Asia, although most of their basal relationships were not well resolved. Our genomic-based estimates of phylogeny, divergence times and biogeographic reconstruction cast doubt on this dispersal route. We found

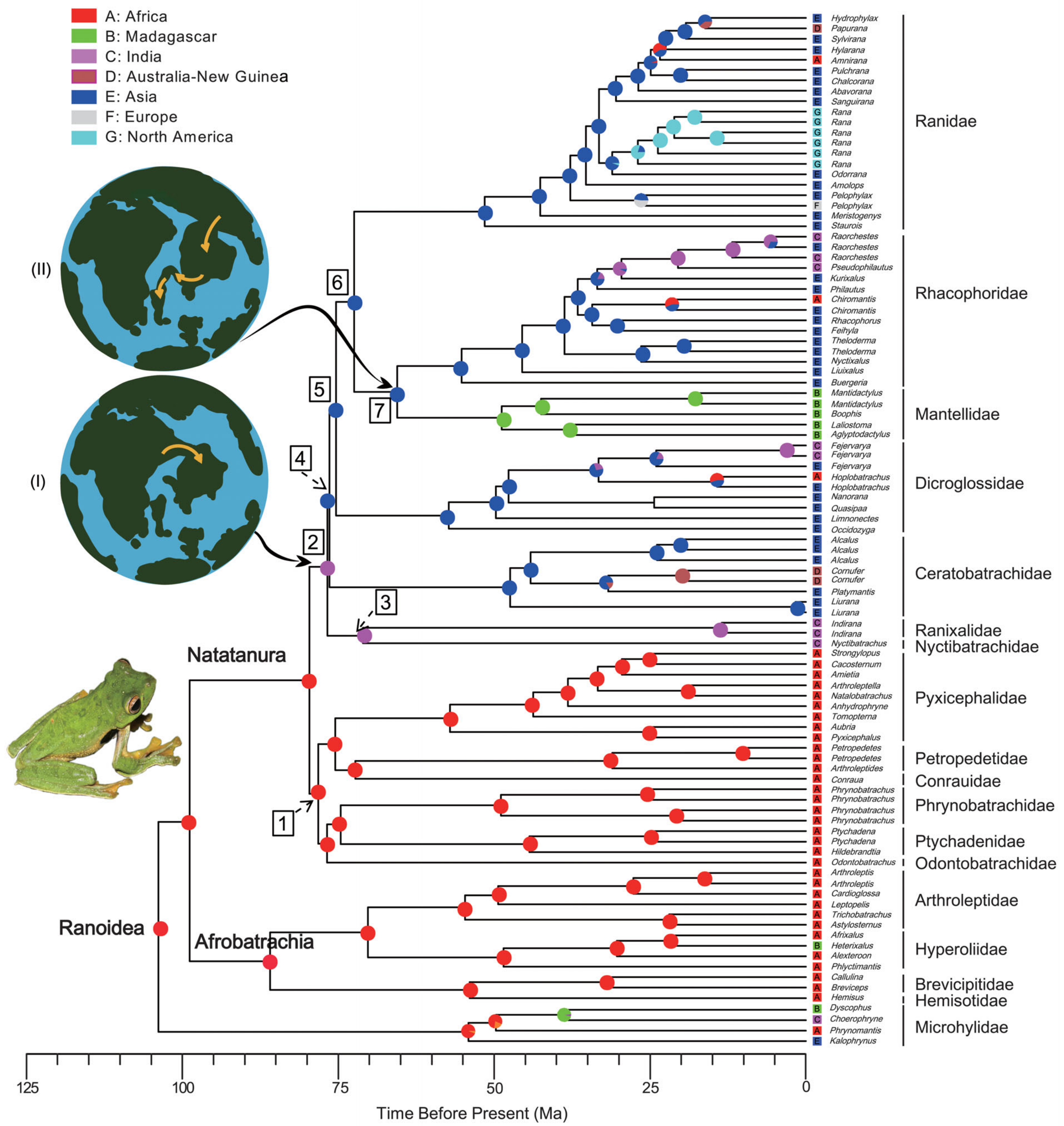


**Figure 2.** Ancestral-area estimations for the species of Ranoidea, using the DEC+J model in BioGeoBEARS. Circles at nodes represent the set of possible ancestral areas and the colours reflect biogeographic designations (see area code key). Clades of interest are numbered in boxes. Models show the stepping-stone role of the Indian Plate to biotic exchange between India and Africa (I) and among India, Asia and Madagascar (II). Paleo reconstructions are modified from Chatterjee *et al.* [3] and Briggs [5].

strong support that the two Australia–New Guinea clades, *Cornufer* and *Papurana*, were embedded within Asian clades of Ceratobatrachidae and Ranidae, respectively. Ancestral state analysis suggests Asian origins, followed by migration into Australia–New Guinea for *Cornufer* (30.2 Ma, 95% highest posterior density (HPD): 21.3–40.0 Ma) and *Papurana* (14.9 Ma, 95% HPD: 10.4–19.5 Ma) independently (Fig. 2 and Supplementary Fig. 3). These dispersals could have occurred after the Australia–New Guinea Plate first collided with Sundaland in the Early Miocene [22]. Tectonic collision and extensive island formations in Wallacea provided a direct colonization route between Asia and Australasia, and are suggested to have triggered much of the biotic interchange between the regions. For example, Miocene dispersals between these two regions are known in plants [23], birds [24] and mammals [25]. However, few dated phylogenies of herpetofauna exist that directly shed light on this issue, although those that do show similar patterns with evidence of immigrations [26]. Additional studies with increased taxon sampling should help to identify the common time periods and directions of dispersals taken by these species.

## MULTIPLE DISPERSALS FROM ASIA TO AFRICA

Reconnections between Gondwana and Laurasia-origin landmasses in the Neogene allowed extensive biotic interchanges between Africa and Eurasia [3]. These biotas could have migrated across the western margin of the Mediterranean Sea or through the Afro-Arabian to Eurasian land bridge [27]. Our results suggest three groups of ranoid frogs dispersed independently from Asia to Africa. The dispersals of Ranidae (*Amnirana*) and Rhacophoridae (*Chiromantis*) appear to have occurred in a similar time period: ∼21.6 and ∼20.6 Ma, respectively (Fig. 2 and Supplementary Fig. 3). This period is consistent with collision of the Afro-Arabian Plate with Eurasia during the mid-Burdigalian (∼19–21 Ma) causing the emergence of a terrestrial corridor, called 'the Gomphotherium Land Bridge' [28]. This land bridge later became disconnected intermittently, but it appears to have been continuously present since ∼15 Ma ago, triggering mammals [29], reptiles [30], invertebrate [31] and possibly also frogs (our results) to exchange between Africa and Eurasia. Interestingly, our results also support another colonization from Asia to Africa by a lineage of Dicroglossidae (*Hoplobatrachus*), which occurred much later (∼12.7 Ma). This implies that habitats in the North Africa and Afro-Arabian plates were suitable for amphibian dispersal during the middle Miocene. As a consequence of shrinkage of the Tethys Sea, desert conditions expanded across North Africa in the late Miocene (∼7 Ma), marking the origin of the Sahara Desert, and also the deserts of the Middle East and the Arabian Peninsula [32], which subsequently hindered the migration of most mesic-adapted species between Africa and Eurasia.

## SUPPLEMENTARY DATA

Supplementary data are available at *NSR* online.

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