

## **Mesozoic origin and ‘out-of-India’ radiation of ricefishes (Adrianichthyidae)**

Kazunori Yamahira<sup>1</sup>, Satoshi Ansai<sup>2</sup>, Ryo Kakioka<sup>1</sup>, Hajime Yaguchi<sup>1,3</sup>, Takeshi Kon<sup>4</sup>, Javier Montenegro<sup>1</sup>, Hirozumi Kobayashi<sup>1</sup>, Shingo Fujimoto<sup>1</sup>, Ryosuke Kimura<sup>5</sup>, Yusuke Takehana<sup>6</sup>, Davin H. E. Setiamarga<sup>7</sup>, Yasuoki Takami<sup>8</sup>, Rieko Tanaka<sup>9</sup>, Ken Maeda<sup>10</sup>, Hau D. Tran<sup>11</sup>, Noriyuki Koizumi<sup>12</sup>, Shinsuke Morioka<sup>13</sup>, Vongvichith Bounsong<sup>14</sup>, Katsutoshi Watanabe<sup>15</sup>, Prachya Musikasinthorn<sup>16</sup>, Sein Tun<sup>17</sup>, L. K. C. Yun<sup>17</sup>, Kawilarang W. A. Masengi<sup>18</sup>, V. K. Anoop<sup>19</sup>, Rajeev Raghavan<sup>20</sup>, Jun Kitano<sup>21</sup>

<sup>1</sup>Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan

<sup>2</sup>Graduate School of Life Sciences, Tohoku University, Sendai, Japan

<sup>3</sup>School of Science and Technology, Kwansei Gakuin University, Sanda, Japan

<sup>4</sup>Center for Strategic Research Project, University of the Ryukyus, Okinawa, Japan

<sup>5</sup>Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan

<sup>6</sup>Faculty of Bio-Science, Nagahama Institute of Bio-Science and Technology, Japan

<sup>7</sup>Department of Applied Chemistry and Biochemistry, National Institute of Technology,  
Wakayama College, Wakayama, Japan

<sup>8</sup>Graduate School of Human Development and Environment, Kobe University, Kobe, Japan

<sup>9</sup>World Medaka Aquarium, Nagoya Higashiyama Zoo and Botanical Gardens, Nagoya, Japan

<sup>10</sup>Marine Eco-Evo-Devo Unit, Okinawa Institute of Science and Technology Graduate  
University, Okinawa, Japan

<sup>11</sup>Faculty of Biology, Hanoi National University of Education, Hanoi, Vietnam

<sup>12</sup>Institute for Rural Engineering, National Agriculture and Food Research Organization, Ibaraki,  
Japan

<sup>13</sup>Fisheries Division, Japan International Research Center for Agricultural Sciences, Ibaraki,  
Japan

<sup>14</sup>Living Aquatic Resources Research Center, Vientiane, Laos

<sup>15</sup>Division of Biological Sciences, Graduate School of Science, Kyoto University, Kyoto, Japan

<sup>16</sup>Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

<sup>17</sup>Inlay Lake Wildlife Sanctuary, Ministry of Natural Resources and Environmental Conservation,  
Inlay, Myanmar

<sup>18</sup>Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, Indonesia

<sup>19</sup>School of Ocean Science and Technology, Kerala University of Fisheries and Ocean Studies, Kochi, India

<sup>20</sup>Department of Fisheries Resource Management, Kerala University of Fisheries and Ocean Studies, Kochi, India

<sup>21</sup>Ecological Genetics Laboratory, National Institute of Genetics, Mishima, Japan

**Running title:**

Out-of-India radiation of ricefishes

**Author for correspondence:**

Kazunori Yamahira

Tel & Fax: +81-98-895-8937; e-mail: [yamahira@lab.u-ryukyu.ac.jp](mailto:yamahira@lab.u-ryukyu.ac.jp)

## **Abstract**

The Indian subcontinent has an origin geologically different from Eurasia, but many terrestrial animal and plant species on it have congeneric or sister species in other parts of Asia, especially in the Southeast. This faunal and floral similarity between India and Southeast Asia is explained by either of the two biogeographic scenarios, 'into-India' or 'out-of-India.' Phylogenies based on complete mitochondrial genome and five nuclear genes were undertaken for ricefishes (Adrianichthyidae) to examine which of these two biogeographic scenarios fits better. We found that *Oryzias setnai*, the only adrianichthyid distributed in and endemic to the Western Ghats, a mountain range running parallel to the western coast of the Indian subcontinent, is sister to all other adrianichthyids from eastern India and Southeast–East Asia. Divergence time estimates and ancestral area reconstructions reveal that this western Indian species diverged in the late Mesozoic during the northward drift of the Indian subcontinent. These findings indicate that adrianichthyids dispersed eastward 'out of India' after the collision of the Indian subcontinent with Eurasia, and subsequently diversified in Southeast–East Asia. A review of geographic distributions of 'out-of-India' taxa reveals that they may have largely fuelled or modified the biodiversity of Eurasia.

## **Keywords:**

biodiversity, Eurasia, Indian subcontinent, *Oryzias setnai*, Western Ghats

## 1. Introduction

The Indian subcontinent geologically originated from Gondwana, a Neoproterozoic supercontinent composed of the present-day Africa, South America, Australia, Antarctica, Arabian Peninsula, Madagascar, and the Indian subcontinent [1]. During the breakup of Gondwana, the Indian subcontinent became isolated from Africa around 130–160 Mya, drifted northwards, and eventually collided with Eurasia, which originated from Laurasia, around 35–55 Mya (figure 1a) [2–7]. Though the origin of the Indian subcontinent differs geologically from that of Eurasia, many terrestrial animal and plant species on it have congeneric or sister species in other part of Asia, especially in the Southeast (e.g., [8–15]).

The faunal and floral similarity between India and Southeast Asia has been attributed to dispersals from Eurasia to the Indian subcontinent [16,17]. One hypothesis on which this ‘into-India’ biogeographic scenario is based is the ‘Satpura hypothesis’ by S. L. Hora, an Indian ichthyologist [16,18], which considers that the westward dispersals of Southeast Asian fauna occurred through the central Indian Satpura hill ranges in the Pleistocene. In contrast, it is also theoretically possible that the Indian taxa originated on the Indian subcontinent, and that the dispersals occurred ‘out-of-India,’ from India to Southeast Asia, after the collision of the Indian plate with Eurasia [19,20]. It is essential to evaluate the ‘into-India’ versus ‘out-of-India’ scenarios to understand how the biodiversity in these regions of different geological origin was formed [e.g., 21–25].

Fauna and flora in the Western Ghats, a mountain range running parallel to the western coast of the Indian subcontinent, hold the key for the test of these two biogeographic scenarios. The Western Ghats harbours unique, evolutionarily distinct lineages of many taxa (e.g., [26–30]). The unique fauna and flora in the Western Ghats suggest that they have long been isolated from other regions of the Indian subcontinent. If this isolation predates the collision of the Indian subcontinent, and if the Western Ghats clade is sister to all other clades, then the presence of their common ancestor in the Indian subcontinent is supported, consistent with the ‘out-of-India’ scenario (figure 1b). In contrast, if the Western Ghats taxa are nested within a larger phylogeny consisting of species from outside the region, and if the isolation of the Western Ghats

postdates the collision of the Indian subcontinent, then the ‘into-India’ scenario is rather supported.

We examine which of these two biogeographic scenarios better fits a group of small-sized ricefishes (family Adrianichthyidae) comprising 37 species (figure 1c) distributed throughout Southeast and East Asia, the Indian subcontinent [31], and with one species, the Malabar ricefish *Oryzias setnai* (formerly *Horaichthys setnai* named after S. L. Hora), endemic to the Western Ghats lowlands [32,33]. Though endemism of *O. setnai* suggests long-term isolation, no study has investigated its phylogenetic position or evolutionary history. Using sequences of complete mitochondrial genome and five nuclear genes, we reconstruct a comprehensive phylogeny of the family Adrianichthyidae including this Western Ghats endemic species, and estimate divergence times and ancestral areas of major adrianichthyid lineages. We demonstrate that this family originated in India and subsequently dispersed east as far as Wallacea, the biogeographical transition zone between Indomalaya and Australasia, where it there became one of the most important elements of the region’s current freshwater ichthyofauna.

## **2. Materials and methods**

### **(a) Field collections**

Twenty-two adrianichthyid species were collected from throughout the geographic range of this family (table S1). Full details of field collections are provided in the electronic supplementary file.

### **(b) Mitochondrial and nuclear sequencing of *O. setnai***

Total DNA was extracted from one *O. setnai* individual from an aquarium strain. The entire length of the mitogenome was *de novo* assembled using long PCR (table S2) [34,35] and shotgun-sequencing (figure S1). We also Sanger-sequenced five nuclear genes (table S3). Full details of sequencing are provided in the electronic supplementary file.

### **(c) Whole-genome sequencing of other adrianichthyids**

Whole-genome sequencing was performed for wild or laboratory individuals of 10 adrianichthyid species (table S1). Reads were mapped to a reference genome assembly of *O. latipes* (ASM223467v1) or *O. javanicus* (OJAV\_1.1), and bases were called across each reference mitochondrial genome and across the five nuclear genes of each reference. Each mitogenome sequence was annotated using MitoAnnotator [36,37]. Full details of sequencing are provided in the electronic supplementary file.

#### (d) Phylogenetic analysis

Sequences of mitogenome and the five nuclear genes were obtained for an additional 20 adrianichthyid taxa, using short read sequences of the whole genome retrieved from DDBJ-DRA (table S1); reads were mapped to a reference genome assembly of *O. celebensis* (OryCel\_1.0) or *O. latipes*, and bases were called. For two species (*O. javanicus* and *O. dancena*), mitogenome sequences retrieved from DDBJ were used (table S1). Sequences of mitogenome and nuclear genes of five beloniform, seven cyprinodontiform, five atheriniform, and two cichlid (perciform) species were also retrieved from DDBJ (table S1).

Alignments were performed separately for each gene. For mitochondrial genes, we excluded ambiguously aligned regions in the rRNA and tRNA genes, the third codon positions from the protein-coding genes, and the whole NADH dehydrogenase subunit 6, resulting in sequences of a total 11,233 bp. Alignments of nuclear genes resulted in sequences of a total 4,204 bp. A maximum likelihood phylogeny was estimated separately for the mitochondrial, nuclear, and concatenated sequences using raxmlGUI version 1.31 [38]. Full details of phylogenetic analyses are provided in the electronic supplementary material.

#### (e) Divergence time estimation

Lognormal relaxed clock analyses were performed separately on the 11,233-bp mitochondrial and 4,204-bp nuclear sequences using BEAST version 2.5.2 [39]. We employed three fossil records, (1) †*Mahengechromis* (the minimum age 45.46 Mya) [40] for the node between the two cichlids [41], (2) †*Rhamphexocoetus volans* (the minimum age 49.11 Mya) [42] for the branch leading to the flyingfish [41], and (3) †*Lithopoecilus brouweri* (the minimum age 5.33 Mya) [43] for the node between

*Oryzias sarasinorum* and *O. eversi* [44], and the opening of the Makassar Strait, ca 45 Mya [45–47], to time-calibrate the phylogenetic tree (see also figure 2). Appropriate substitution models were selected for each gene (for rRNA and tRNA genes) and codon (for coding genes). Full details on the divergence time estimation are provided in electronic supplementary material.

#### (f) Ancestral area reconstruction

The geographic range of Adrianichthyidae was divided into five geological areas: (A) Western Ghats, (B) Indian subcontinent (excluding Western Ghats), (C) Southeast Asia (excluding Wallacea and New Guinea), (D) East Asia, and (E) Wallacea and New Guinea. Using the tree obtained from the BEAST analysis above, ancestral areas at each node of the tree were reconstructed under different biogeographical models with RASP version 4.2 [48]. Likelihood under each model was estimated, and the fit of each model to the data was compared by consulting Akaike information criteria corrected for small sample size. Full details on the ancestral area reconstruction are provided in electronic supplementary material.

### 3. Results

#### (a) Phylogeny of Adrianichthyidae

All phylogenies revealed *O. setnai* to be a sister to all other members of the family Adrianichthyidae (figures 2 and S2). The branch of *O. setnai* in these phylogenies was disproportionately longer compared with other adrianichthyids. The latter were composed of three main clades—the ‘*latipes*,’ ‘*javanicus*,’ and ‘*celebensis*’ species groups. The *latipes* species group comprises species distributed mainly in the inland areas of the Indochinese Peninsula, Philippines, and East Asia; the *javanicus* species group occurs in the eastern part of India and throughout Southeast Asia; and the *celebensis* species group is endemic to Sulawesi Island (figure 2). Among the three species groups, the *latipes* species group is sister to the other two.

#### (b) Divergence time estimates

Adrianichthyidae was estimated to separate from other members of the order Beloniformes around 89 (73–107 in 95% HPD) Mya (figure 2, node 1). The divergence

time of *O. setnai* was estimated around 74 (60–88) Mya (node 2) in the late Mesozoic (see also figure S3). Thereafter, the *latipes* species group split off around 52 (45–60) Mya (node 3), and the subsequent split between the *javanicus* and *celebensis* species groups occurred around 47 (41–52) Mya (node 4).

#### (c) Reconstruction of ancestral areas

The best biogeographical model (Dispersal-Extinction Cladogenesis with founder event speciation model: DEC+J; table S4) estimated that the most probable distribution area for the common ancestor of adrianichthyids was on the Indian subcontinent (i.e. Western Ghats and other parts of India) (figure 2, node 2). The common ancestor of the *latipes*, *javanicus*, and *celebensis* species groups was estimated to be distributed in Southeast Asia (node 3). Thereafter, dispersals from Southeast Asia to East Asia and to Wallacea occurred within the *latipes* species group (node 5), and in the most recent common ancestor of the *celebensis* group (node 6), respectively.

## 4. Discussion

#### (a) Origin and dispersal history of the Adrianichthyidae

We found that *O. setnai*, endemic to the Western Ghats western plains, is sister to all other Adrianichthyidae taxa, which comprises three major groups (the *latipes*, *javanicus*, and *celebensis* species groups) [49,50]. Our results also reveal that the divergence time of this species (60–88 Mya) predates the collision of the Indian subcontinent with Eurasia (35–55 Mya [2–7]), and that the common ancestor of the Adrianichthyidae was estimated to have been distributed on this ancient subcontinent. This lends credibility to the hypothesis that the split of *O. setnai* occurred on the Indian subcontinental ‘raft,’ supporting the ‘out-of-India’ hypothesis.

The divergence of *O. setnai* and its endemism to western India may be related to the formation of the Western Ghats mountain ranges. The western coast of India could have appeared as an abrupt cliff some 1,000 m in elevation ca 65–90 Mya after the Indian subcontinent broke away from Madagascar [51], becoming the present-day Western Ghats. This long (1,600 km) and high mountain range running parallel to the southwestern coast of the Indian subcontinent may have acted as a physical barrier preventing the migration of species between the western and eastern coasts. We think



that the common ancestor of Adrianichthyidae was divided into the west and east by this mountain range, and that the western population would have evolved in isolation as *O. setnai*. The common ancestor of the Eurasian clade probably diverged from the eastern population.

The branch leading to *O. setnai* is notably, disproportionally long, indicating an acceleration in evolutionary rate (figure S2). This acceleration might be related to the geological history of the Western Ghats. It is well known that large-scale, long-term volcanic eruptions occurred along the western coast of the Indian subcontinent during its northward drift, forming the Deccan Traps (e.g., [52]). It is therefore no wonder that *O. setnai* has repeatedly experienced strong bottlenecks caused by recurring eruptions. According to the nearly neutral theory of molecular evolution [53], evolutionary rates of protein-coding genes increase with decreasing population size, which may explain this long branch. A detailed demographic history of *O. setnai* using the nuclear genome is required to test this hypothesis.

The split between the Adrianichthyidae and other members of the Beloniformes; that is, Exocoetoidei, estimated at 73–107 Mya (figure 2, node 1), perhaps occurred in conjunction with the separation of the Indian subcontinent from Africa and Madagascar. Since most extant species in the Exocoetoidei: Belonidae (needlefishes), Exocoetidae (flyingfishes), Hemiramphidae (halfbeaks), and Zenarchopteridae (viviparous halfbeaks) are marine [54], the expansion of coastal areas following the breakup of the Indian subcontinent may have increased opportunities for a common ancestor of Exocoetoidei to pioneer new habitat. In contrast, Adrianichthyidae pioneered the inland areas of the Indian subcontinent, and subsequently those in Eurasia, probably spreading via coastal areas.

#### (b) Conclusion: Eurasian biodiversity fuelled by ‘out-of-India’ dispersals

Contrary to Hora’s hypothesis [16,18], Hora’s fish and its relatives originated on the Indian subcontinent and subsequently dispersed east into Southeast and East Asia, where they have greatly diversified. Our review of geographic distributions (table S5) reveals that other ‘out-of-India’ taxa have also diversified more or less in Southeast and East Asia (table 1). This probably reflects that ‘out-of-India’ taxa were newcomers to Eurasia, where they may have found empty niches and/or competitively excluded native

Laurasian taxa. The biodiversity of Eurasia may have been largely fuelled or modified by these taxa which came on the subcontinental raft.

**Data accessibility.** Data available from the Dryad Digital Repository:

<https://doi.org/10.5061/dryad.931zcrjkg> [70]

**Funding.** This study was supported by the University of the Ryukyus Research Project Promotion Grant, the Spatiotemporal Genomics Project promoted by University of the Ryukyus, NIG collaborative grant (B), JSPS KAKENHI Grant Number 17H01675, and JST CREST Grant Number JPMJCR20S2.

**Acknowledgements.** The World Medaka Aquarium and the National BioResource Project ‘Medaka’ kindly provided the laboratory strains. We also thank Steve O’Shea, PhD, from Edanz Group for editing a draft of this manuscript.

## References

1. Torsvik TH, Cocks LRM. 2013 Gondwana from top to base in space and time. *Gondwana Res.* **24**, 999–1030.
2. Storey BC. 1995 The role of mantle plumes in continental breakup: case histories from Gondwanaland. *Nature* **377**, 301–308.
3. Collins WJ. 2003 Slab pull, mantle convection, and Pangaeian assembly and dispersal. *Earth Planet. Sci. Lett.* **205**, 225–237.
4. Aitchison JC, Ali JR, Davis AM. 2007 When and where did India and Asia collide? *J. Geophys. Res.* **112**, B05423.
5. Kumar P, Yuan X, Kumar MR, Kind R, Li X, Chadha RK. 2007. The rapid drift of the Indian tectonic plate. *Nature* **449**, 894–897.
6. van Hinsbergen, DJJ, Steinberger B, Doubrovine PV, Gassmüller R. 2011 Acceleration and deceleration of India-Asia convergence since the Cretaceous: Roles of mantle plumes and continental collision. *J. Geophys. Res.* **116**, B06101.
7. Yoshida M, Hamano Y. 2015 Pangea breakup and northward drift of the Indian subcontinent reproduced by a numerical model of mantle convection *Sci. Rep.* **5**, 8407.
8. Blanford WT. 1901 Distribution of vertebrate animals in India, Ceylon and Burma. *Philos. Trans. R. Soc. B* **194**, 335–436.

9. Hora SL. 1937 Distribution of Himalayan fishes and its bearing on certain palaeogeographical problem. *Rec. Indian Mus.* **39**, 251–259.
10. Hora SL. 1944 On the Malayan affinities of the freshwater fish fauna of Peninsular India, and its bearing on the probable age of the Garo-Rajmahal gap. *Proc. Nat. Inst. Sci. India* **10**, 423–439.
11. Ali S, Ripley SD. 1983 Handbook of the Birds of India and Pakistan. Compact edition. New Delhi, India: Oxford University Press.
12. Daniels RJR. 2001 Endemic fishes of the Western Ghats and the Satpura hypothesis. *Curr. Sci. India* **81**, 240–244.
13. Karanth KP. 2015 An island called India: phylogenetic patterns across multiple taxonomic groups reveal endemic radiations. *Curr. Sci. India* **108**, 1847–1851.
14. Garg S, Biju SD. 2019. New microhylid frog genus from Peninsular India with Southeast Asian affinity suggests multiple Cenozoic biotic exchanges between India and Eurasia. *Sci. Rep.* **9**, 1906.
15. Sidharthan A, Raghavan R, Anoop VK, Keskar A, Dahanukar N. 2021. Phylogenetic position and relationships of mountain loaches (Teleostei: Balitoridae) of the Western Ghats as revealed by CO1 sequences. *Zootaxa* **4926**, 79–92.
16. Hora SL. 1949 Satpura hypothesis of the distribution of the Malayan fauna and flora to peninsular India. *Proc. Nat. Inst. Sci. India* **15**, 309–422.
17. Grismer JL, Schulte JA, Alexander A, Wagner P, Travers SL, Buehler MD, Welton LJ, Brown RM. 2016. The Eurasian invasion: phylogenomic data reveal multiple Southeast Asian origins for Indian Dragon Lizards. *BMC Evol Biol.* **16**, 43.
18. Hora SL. 1953 The Satpura hypothesis. *Sci. Prog.* **162**, 245–255.
19. Bossuyt F, Milinkovitch MC. 2001. Amphibians as indicators of early tertiary “Out-of-India” dispersal of vertebrates *Science* **292**, 93–95.
20. Conti E, Eriksson T, Schonenberger J, Sytsma KJ, Baum DA. 2002 Early tertiary out-of-India dispersal of Crypteroniaceae: evidence from phylogeny and molecular dating. *Evolution* **56**, 1931–1942.
21. Datta-Roy A, Karanth KP. 2009. The Out-of-India hypothesis: What do molecules suggest? *J. Biosci.* **34**, 687–697.
22. Joshi J, Karanth KP. 2011. Cretaceous-Tertiary diversification among select Scolopendrid centipedes of South India. *Mol. Phylogenet. Evol.* **60**, 287–294.

23. Sil M, Aravind NA, Karanth KP. 2019. Role of geography and climatic oscillations in governing into-India dispersal of freshwater snails of the family: Viviparidae. *Mol. Phylogenet. Evol.* **138**, 174–181.
24. Loria SF, Prendini L. 2020. Out of India, thrice: diversification of Asian forest scorpions reveals three colonizations of Southeast Asia. *Sci Rep.* **10**, 22301.
25. Sil M, Aravind NA, Karanth KP. 2020 Into-India or out-of-India? Historical biogeography of the freshwater gastropod genus *Pila* (Caenogastropoda: Ampullariidae). *Biol. J. Linn. Soc.* **129**, 752–764.
26. Praschag P, Schmidt C, Fritsch G, Müller A, Gemel R, Fritz U. 2006. *Geoemyda silvatica*, an enigmatic turtle of the Geoemydidae (Reptilia: Testudines), represents a distinct genus. *Org. Div. & Evol.* **6**, 151–162.
27. Biju SD, Bossuyt F. 2003 New frog family from India reveals an ancient biogeographical link with the Seychelles. *Nature* **425**, 711–714.
28. Anoop VK, Dahanukar N, Philip S, Thomas L, Raghavan R. 2018 Phylogeny of the hillstream loach genus *Mesonoemacheilus* reveals widespread diversification through ancient drainage connections in the Western Ghats Biodiversity Hotspot. *Mol. Phylogenet. Evol.* **129**, 77–84.
29. Chaitanya R, Khandekar A, Caleb DG, Mukherjee N, Ghosh A, Giri V. 2019 Herpetofauna of the Meghamalai Wildlife Sanctuary, southern Western Ghats, India: an updated checklist with annotations on taxonomy and nomenclature. *J. Bombay Nat. Hist. Soc.* **115**, 21–37.
30. Sidharthan A, Raghavan R, Anoop KV, Philip S, Dahanukar N. 2020. Riddle on the riffle: Miocene diversification and biogeography of endemic mountain loaches in the Western Ghats Biodiversity Hotspot. *J. Biogeogr.* **47**, 2741–2754.
31. Parenti LR. 2008 A phylogenetic analyses and taxonomic revision of ricefishes, *Oryzias* and relatives (Belontiiformes, Adrianichthyidae). *Zool. J. Linn. Soc.* **154**, 494–610.
32. Silas EG. 1959 On the natural distribution of the Indian cyprinodont fish *Horaichthys setnai* Kulkarni. *J. Mar. Biol. Ass. India* **1**, 256.
33. Talwar PK, Jhingran AG. 1991 Inland Fishes of India and Adjacent Countries. New Delhi: Oxford and IBH Publishing Co.

34. Cheng S, Higuchi R, Stoneking M. 1994 Complete mitochondrial genome amplification. *Nat. Genet.* **7**, 350–351.
35. Miya M, Nishida M. 1999 Organization of the mitochondrial genome of a deep-sea fish, *Gonostoma gracile* (Teleostei: Stomiiformes): first example of transfer RNA gene rearrangements in bony fishes. *Mar. Biotechnol.* **1**, 416–426.
36. Iwasaki W, Fukunaga T, Isagozawa R, Yamada K, Maeda Y, Satoh TP, Sado T, Mabuchi K, Takeshima H, Miya M. 2013 MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. *Mol. Biol. Evol.* **30**, 2531–2540.
37. Sato Y, Miya M, Fukunaga T, Sado T, Iwasaki W. 2018 MitoFish and MiFish pipeline: a mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Mol. Biol. Evol.* **35**, 1553–1555.
38. Silvestro D, Michalak I. 2012 RaxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* **12**, 335–337.
39. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, et al. 2019 BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, **15**, e1006650.
40. Murray A.M. 2000 Eocene cichlid fishes from Tanzania, East Africa. *J. Vertebr. Paleontol.* **20**, 651–664.
41. Benton MJ, Donoghue PCJ, Asher RJ, Friedman M, Near TJ, Vinther J. 2015 Constraints on the timescale of animal evolutionary history. *Palaeontol. Electron.* **18.1.1FC**, 1–106.
42. Bannikov A, Parin NV, Pinna G. 1985 *Rhamphexocetus volans*, gen. et sp. nov.: a new beloniform fish (Beloniformes, Exocetoidei) from the Lower Eocene of Italy. *J. Ichthyol.* **25**, 150–155.
43. Frickhinger KA. 1991 Fossilian Atlas. Fische. Melle, Germany: Hans A. Bensch.
44. Horoiwa M, Mandagi IF, Sutra N, Montenegro J, Tantu, FY, Masengi KWA, Nagano AJ, Kusumi J, Yasuda N, Yamahira K. 2021 Mitochondrial introgression by ancient admixture between two distant lacustrine fishes in Sulawesi Island. *PLOS ONE*, in press
45. Moss SJ, Wilson EJ. 1998 Biogeographic implications of the tertiary palaeogeographic evolution of Sulawesi and Borneo. In *Biogeography and*

- geological evolution of SE Asia* (eds R Hall, JD Holloway), pp. 133–163. Leiden, The Netherlands: Backhuys Publishers.
46. Hall R. 2009 Continental growth at the Indonesian margins of southeast Asia. *Arizona Geol. Soc. Digest* **22**, 245–258.
  47. Spakman W, Hall R. 2010 Surface deformation and slab-mantle interaction during Banda Arc subduction rollback. *Nat. Geosci.* **3**, 562–566.
  48. Yu Y, Blair C, He XJ. 2020 RASP 4: ancestral state reconstruction tool for multiple genes and characters. *Mol. Biol. Evol.* **37**, 604–606.
  49. Takehana Y, Naruse K, Sakaizumi M. 2005 Molecular phylogeny of the medaka fishes genus *Oryzias* (Belontiiformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **36**, 417–428.
  50. Naruse K, Yamahira K, Takehana Y. 2019. Medaka and *Oryzias* species as model organisms and the current status of medaka biological resources. In *Medaka. biology, management, and experimental protocols Volume 2* (eds K Murata, M Kinoshita, K Naruse, M Tanaka, Y Kamei), pp. 31–48. Hoboken, NJ: Wiley-Blackwell.
  51. Radhakrishna T, Mohamed AR, Venkateshwarlu M, Soumya GS, Prachiti PK. 2019. Mechanism of rift flank uplift and escarpment formation evidenced by Western Ghats, India. *Sci. Rep.* **9**, 10511.
  52. Schoene B, Eddy MP, Samperton KM, Keller CB, Keller G, Adatte T, Khadri SFR. 2019 U-Pb constraints on pulsed eruption of the Deccan Traps across the end-Cretaceous mass extinction. *Science* **363**, 862–866.
  53. Ohta T. 1973. Slightly deleterious mutant substitutions in evolution. *Nature* **246**, 96–98.
  54. Nelson JS, Grande TC, Wilson MVH. 2016 *Fishes of the World*, 5th Edition. Hoboken, NJ: John Wiley & Sons.
  55. Costa L, Jimenez H, Carvalho R, Carvalho-Sobrinho J, Escobar I, Souza G. 2020 Divide to conquer: evolutionary history of Alliioideae tribes (Amaryllidaceae) is linked to distinct trends of karyotype evolution. *Front. Plant Sci.* **11**, 320.
  56. Chen YS, Meseguer AS, Godefroid M, Zhou Z, Zhang JW, Deng T, Kim JH, Nie ZL, Liu YS, Sun H. 2017. Out-of-India dispersal of *Paliurus* (Rhamnaceae)

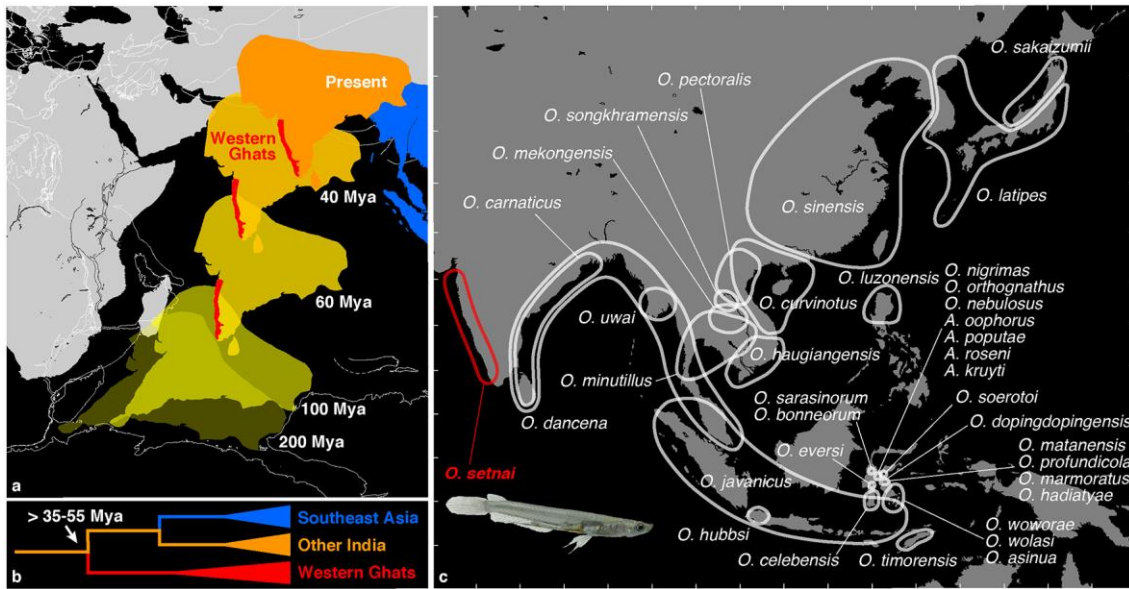
- indicated by combined molecular phylogenetic and fossil evidence. *Taxon* **66**, 78–90.
57. Dayanandan S, Ashton PS, Williams SM, Primack RB. 1999. Phylogeny of the tropical tree family Dipterocarpaceae based on nucleotide sequences of the chloroplast RBCL gene. *Am. J. Bot.* **86**, 1182–1190.
58. Li F, Shao L, Li S. 2020. Tropical niche conservatism explains the Eocene migration from India to Southeast Asia in ochyroceratid spiders. *Syst. Biol.* **69**, 987–998.
59. Prendini L, Loria SF. 2020. Systematic revision of the Asian forest scorpions (Heterometrinae Simon, 1879), revised suprageneric classification of Scorpionidae Latreille, 1802, and revalidation of Rugodentidae Bastawade et al., 2005. *Bull. Am. Mus. Nat. Hist.* **442**, 1–480.
60. Joshi J, Karanth PK, Edgecombe GD. 2020. The out-of-India hypothesis: evidence from an ancient centipede genus, *Rhysida* (Chilopoda: Scolopendromorpha) from the Oriental Region, and systematics of Indian species, *Zool. J. Linn. Soc.* **189**, 828–861.
61. Krosch M, Schutze M, Armstrong K, Graham G, Yeates D, Clarke A. 2012. A molecular phylogeny for the Tribe Dacini (Diptera: Tephritidae): Systematic and biogeographic implications. *Mol. Phylogenet. Evol.* **64**, 513–23.
62. Kumazawa Y, Nishida M. 2000. Molecular phylogeny of osteoglossoids: a new model for Gondwanian origin and plate tectonic transportation of the Asian arowana. *Mol Biol Evol.* **17**, 1869–1878.
63. Barby FF, Ráb P, Lavoué S, Ezaz T, Bertollo LAC, Kilian A, Maruyama SR, de Oliveira EA, Artoni RF, Santos MH, Jegede OI, Hatanaka T, Tanomtong A, Liehr T, Cioffi MB. 2018. From chromosomes to genome: Insights into the evolutionary relationships and biogeography of Old World knifefishes (Notopteridae; Osteoglossiformes). *Genes* **9**, e306.
64. Li X, Musikasinthorn P, Kumazawa Y. 2006. Molecular phylogenetic analyses of snakeheads (Perciformes: Channidae) using mitochondrial DNA sequences. *Ichthyol. Res.* **53**, 148–159.

65. Murphy WJ, Collier GE. 1997. A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): the role of vicariance and the origins of annualism. *Mol. Biol. Evol.* **14**, 790–799.
66. Gower DJ, Kupfer A, Oommen OV, Himstedt W, Nussbaum RA, Loader SP, Presswell B, Müller H, Krishna SB, Boistel R, Wilkinson M. 2002. A molecular phylogeny of ichthyophiid caecilians (Amphibia: Gymnophiona: Ichthyophiidae): out of India or out of South East Asia? *Proc. Biol. Sci.* **269**, 1563–1569.
67. Garg S, Biju SD. 2019. New microhylid frog genus from Peninsular India with Southeast Asian affinity suggests multiple Cenozoic biotic exchanges between India and Eurasia. *Sci. Rep.* **9**, 1906.
68. 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 ranidae. *Syst. Biol.* **55**, 579–594.
69. Macey JR, Schulte II JA, Larson A, Ananjeva NB, Wang Y, Pethiyagoda R, Rastegar-Pouyani N, Papenfuss TJ. 2000. Evaluating trans-Tethys migration: an example using acrodont lizard phylogenetics. *Syst. Biol.* **49**, 233–256.
70. Yamahira K, Ansai S, Kakioka R, Yaguchi H, et al. 2021 Data from: Mesozoic origin and ‘out-of-India’ radiation of ricefishes (Adrianichthyidae). Dryad Digital Repository. <https://doi.org/10.5061/dryad.931zcrjkg>

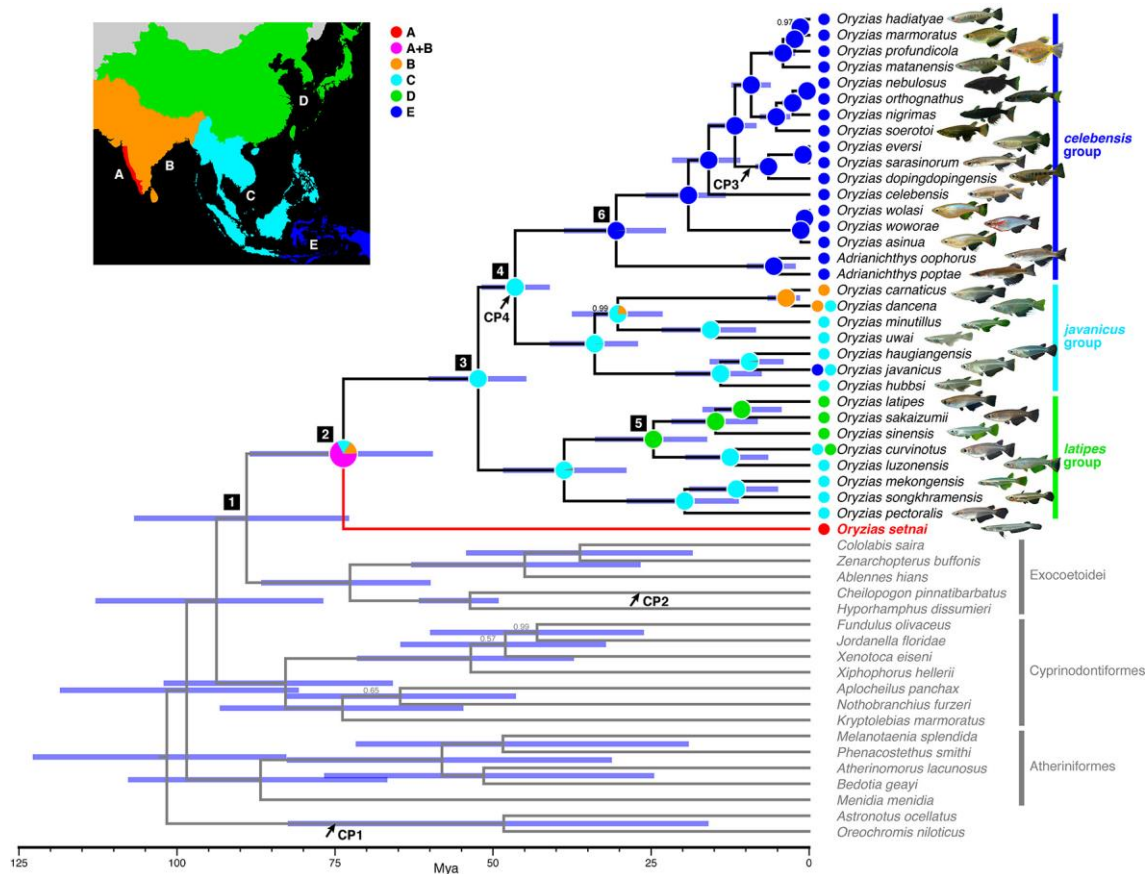


**Table 1.** Geographic distributions of taxa demonstrated to be “out-of-India” by molecular studies (i.e., “out-of-India” taxa demonstrated solely by taxonomic and/or paleontological studies are not included). Numbers represent the numbers of species in each geographic area. Asterisks indicate that the number of genera was used for the taxa. Bold numbers represent modes. See table S5 for the list of species and genera used, and their geographic ranges.

Taxa [reference]	Geographic areas				
	Western Ghats (A)	Indian subcontinent (B)	Southeastern Asia (C)	Eastern Asia (D)	Wallacea and New Guinea (E)
Plants					
Allioideae* [55]	0	0	0	<b>3</b>	0
<i>Paliurus</i> [56]	0	0	0	<b>4</b>	0
Crypteroniaceae [20]	0	2	<b>11</b>	1	2
Dipterocarpaceae* [57]	3	7	<b>10</b>	3	4
Invertebrates					
Theotiminae (spiders) [58]	0	1	<b>49</b>	7	7
Heterometrinae (scorpions) [59]	5	<b>31</b>	9	0	0
Oriental <i>Rhysida</i> (centipedes) [60]	<b>8</b>	6	2	0	0
Tribe Dacini (fruit flies) [61]	44	78	238	75	<b>384</b>
Freshwater fishes					
<i>Scleropages</i> [62]	0	0	<b>5</b>	0	0
Notopteridae [63]	0	2	<b>6</b>	0	0
Channoidei [64]	5	<b>23</b>	22	6	0
Aplocheilidae [65]	4	<b>6</b>	1	0	1
Adrianichthyidae [This study]	1	2	11	4	<b>22</b>
Amphibians					
Ichthyopiidae [66]	13	11	<b>31</b>	1	0
Microhylidae [67]	16	22	<b>58</b>	13	2
Ranoidea* [68]	19	23	<b>43</b>	25	15
Reptiles					
Agamidae* [69]	8	15	<b>26</b>	10	10



**Figure 1.** (a) Time sequence of drifting continental blocks at each age from 200 Mya to present (map provided by and modified from [7] with permission). (b) Schematic phylogeny under the scenario ‘out-of-India.’ (c) Map depicting the geographic distributions of all species in the family Adrianichthyidae.



**Figure 2.** Bayesian chronogram of adrianichthyid taxa based on mitochondrial and nuclear sequences. Bars represent 95% high posterior density. Ancestral areas reconstructed using a DEC+J model are shown as pies at each node. Numbers on branches are Bayesian posterior probabilities (BPP); branches with no number represent BPP = 1. Arrows indicate calibration points; CP1: †*Mahengechromis* for the node between the two cichlids, CP2: †*Rhamphexocoetus volans* for the branch leading to the flyingfish, CP3: †*Lithopoecilus brouweri* for the node between *Oryzias sarasinorum* and *O. eversi*, and CP4: the opening of the Makassar Strait (see text for details).