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Into Africa via docked India: a fossil climbing perch from the Oligocene of Tibet helps solve the anabantid biogeographical puzzle

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ABSTRACT

The northward drift of the Indian Plate and its collision with Eurasia have profoundly impacted the evolutionary history of the terrestrial organisms, especially the ones along the Indian Ocean rim. Climbing perches (Anabantidae) are primary freshwater fishes showing a disjunct south Asian–African distribution, but with an elusive paleobiogeographic history due to the lack of fossil evidence. Here, based on an updated time-calibrated anabantiform phylogeny integrating a number of relevant fossils, the divergence between Asian and African climbing perches is estimated to have occurred in the middle Eocene (ca. 40 Ma, Ma: million years ago), a time when India had already joined with Eurasia. The key fossil lineage is †*Eoanabas*, the oldest anabantid known so far, from the upper Oligocene of the Tibetan Plateau. Ancestral range reconstructions suggest a Southeast Asian origin in the early Eocene (ca. 48 Ma) and subsequent dispersals to Tibet and then India for this group. Thereby we propose their westbound dispersal to Africa via the biotic bridge between India and Africa. If so, climbing perch precursors had probably followed the paleobiogeographical route of snakehead fishes, which have a slightly older divergence between African and Asian taxa. As such, our study echoes some recent molecular analyses in rejecting the previously held “Gondwana continental drift vicariance” or late Mesozoic dispersal scenarios for the climbing perches, but provides a unique biogeographical model to highlight the role of the pre-lift Tibet and the docked India in shaping the disjunct distribution of some air-breathing freshwater fishes around the Indian Ocean.

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1. Introduction

The epic voyage of the Indian Plate after its breakup from the Gondwanan landmass is believed to have transported various Gondwanan species “Out of India” into Asia [1], and is traditionally invoked to explain the evolutionary histories of those organisms with current distribution around the Indian Ocean [2–4]. However, the details of the scenes must be more complex, given the changing biotic links between the Indian Plate and the surrounding landmasses during its northward journey [1,5]. Freshwater fishes are ideal indicators to these biological and geological interplays thanks to their restricted capability for crossing different water drainages and surmounting large marine barriers [6,7].

The labyrinth fishes are primary freshwater fishes now confined mainly to southern Asia and sub-Saharan Africa (Fig. 1) [4]. They

comprise approximately 140 species grouped into three families, Osphronemidae (108 spp.), Helostomidae (1 sp.), and Anabantidae (28 spp.) [6]. The former two families are exclusively Asian, whereas the latter is present on both continents, with *Anabas* (Anabantinae, including two valid species) in Asia and the remaining members (Ctenopominae, including ca. 27 species of three genera, see [11]) in Africa. The African anabantids are all seen in tropical regions except *Sandelia* (only two species) restricted in the temperate Cape region [11]. They usually live in freshwater habitats. Although some climbing perches (e.g., *Anabas*) have been reported in estuaries [12], their ecological preference to the waters with low ion content suggests long-distance transmarine dispersal is unlikely for the group [6].

With this peculiar disjunct distribution, the anabantid historical biogeography has been puzzling and hence controversial [6]. Their distributional pattern was hypothesized by some to arise from the continental drift vicariance associated with the breakup of Gondwana in the Late Jurassic/Early Cretaceous (ca. 165–121 Ma) [13]. Alternatively, this biogeographic pattern was attributed to an

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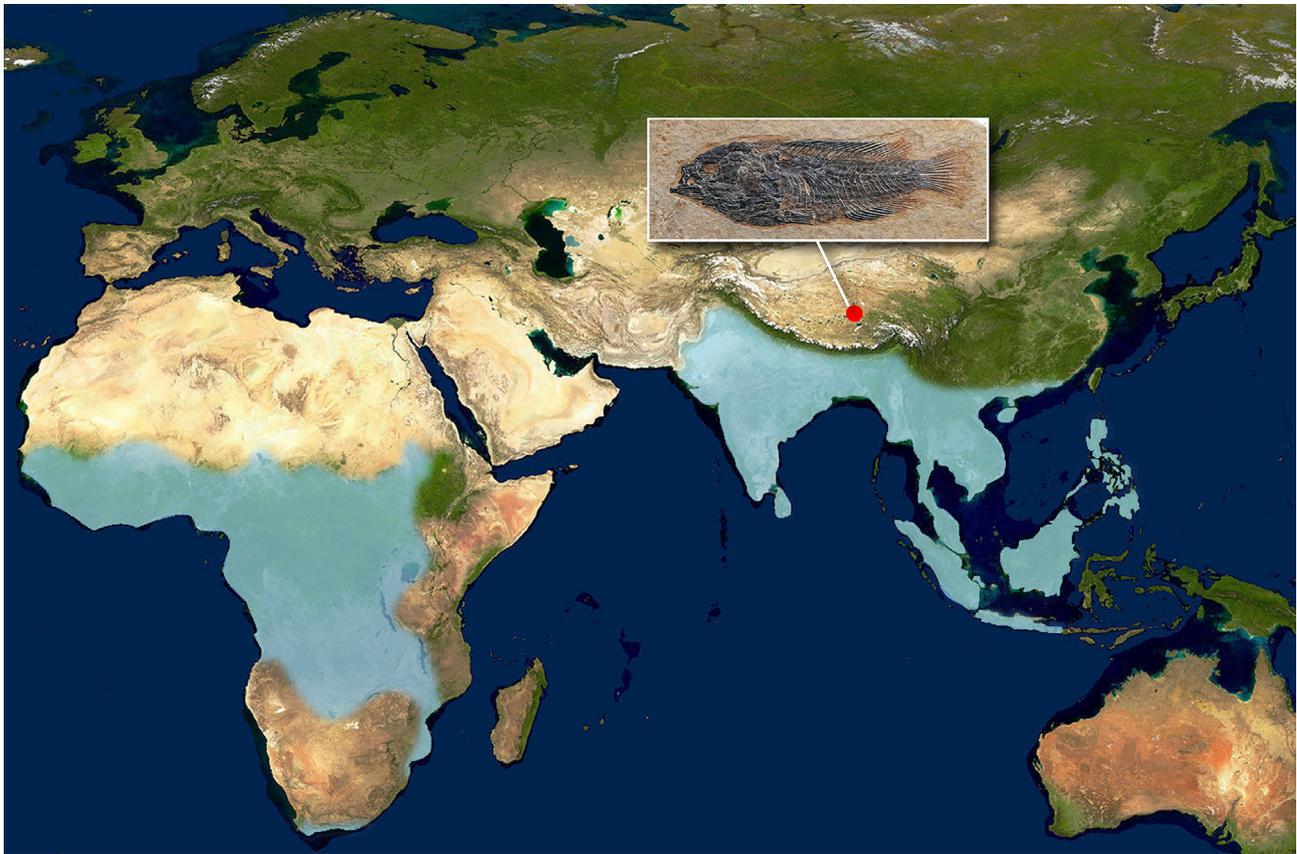


Fig. 1. The current distribution (blue shadowing) of the anabantid fishes (climbing perches), showing an African-south Asian disjunct pattern. Red dot represents the locality where the Tibetan fossil climbing perch was unearthed. The picture of the fossil is the holotype of †*Eoanabas* [8]. Data compiled from [4,8–10], and www.fishbase.org.

Asian origin and subsequent colonization in Africa during the late Eocene, but with little solid evidence and few historical details [12,14,15]. Despite the attempts of molecular analyses, the area of origin area and probable route of dispersal remain unclear [6]. This is due mainly to the extreme scarcity of fossil record of anabantid fishes and their allies [6], which makes it difficult to test those hypotheses. Moreover, those biogeographical scenarios inferred during the pre-cladistic era [9–12] are challenged by the need of the well resolved phylogenetic interrelationships and a temporal framework that simultaneously incorporates both living and fossil species for these fishes.

Past debates on anabantid biogeography have focused on *when* and *how* they diverged [6,7,12]. Although it is possible to reconstruct the general dispersal routes by using paleontological data, time-calibrated molecular phylogeny provides a more reliable temporal framework to constrain the biogeographical scenarios. So far, only Rüber et al. [6] have specified the historical biogeography for the anabantoids by using a molecular phylogenetic time scale, although some other recent molecular analyses have provided indirect evidences for this issue [16–20]. Their results showed that the South Asian and African anabantids might have diverged from each other by either 87.30 Ma (crown calibration, i.e., assigning the fossil gourami to a living lineage of *Osphronemus*) or 30.83 Ma (stem calibration, i.e., assigning the fossil gourami to a basal branch of *Osphronemus*). As these ages are older than that of the closure of the Tethys in the early Miocene (ca. 20–18 Ma) [6], they ruled out the Neogene dispersal hypothesis from Africa to Asia, or *vice versa*, via the Middle East [6]. One of the possibilities proposed by Rüber et al., the dispersal from Asia to Africa probably during the second half of the Paleogene was noted recently in a comprehensive biogeographical review, which hinted a general

Indo-Malayan origin for the anabantid fishes [7]. However, considering the 95% confidence interval of the oldest age estimate (56.43–124.22 Ma) in Rüber et al.'s study [6], it remained an unresolved puzzle: either the hypothesis of Gondwanan continental drift vicariance or that of late Mesozoic dispersal from Africa to Asia, or *vice versa* could be equally possible [6]. Recent molecular clock analyses with the sampling in the scope of the bony fishes [18,19], ray-finned fishes [16,20], or spiny-rayed fishes [17] have provided some upper constraints on the possible timing of anabantid origin and hence do not support the Gondwanan continental vicariance model. Nevertheless, these timescales lack the sampling to address questions about the timing frame of the Asian-African anabantid divergences and untouch the dispersal route within the group [16–20]. Solving the puzzle awaits more fossil anabantoids, especially those of the family Anabantidae with more reliable geological age and higher phylogenetic resolution.

The fossil records of anabantids and their relatives with biogeographical bearings were extremely rare. At the time of Rüber et al.'s [6] study, only a fossil gourami from the ?Paleogene of Sumatra could be used, but its morphology was so little known that its taxonomic assignment was unclear [6,21]. Recently, another fossil gourami, †*Ombilinichthys*, was reported from the same locality [22]. Although its anatomy is much clearer, its phylogenetic placement and geological age remain uncertain [22]. As for the Anabantidae, several opercular bones of *Anabas* from the Pleistocene of Java [23] were the only certain fossil record for this family at the time of Rüber et al.'s analyses, but these remains were not considered by those authors [6]. Some teeth from the Eocene of Pakistan were tentatively assigned to anabantids, pending further study [24]. Recently, we described another fossil anabantid, †*Eoanabas*, from the upper Oligocene of the Tibetan Plateau [8]. This is the

only fossil anabantid known from complete skeletons. As the most primitive and oldest anabantid so far known, it extends the geological range of its family back for more than 20 million years (Ma). Morphologically, it shows a mosaic of Asian and African anabantid characters, and hence provides significant clues about the evolution of the group [8].

Here we reconstruct the phylogenetic relationships based on mitochondrial DNA (mtDNA) sequences and tentatively establish a time frame of Labyrinthici. We use node and tip dating approaches to incorporate the latest fossil species of gouramies and anabantids as well as the closely related snakehead fishes to calibrate molecular clock. In addition, we infer ancestral ranges in order to test previous biogeographical hypotheses for the climbing perches and infer the historical scenarios underlying their current disjunct distribution. We focus on two questions: (1) where had the anabantids most likely originated and subsequently dispersed? (2) When and how had Asian anabantids diverged from their confamilial African relatives? We also touch upon, in a broader sense, the biogeographic patterns of the snakehead fishes (Channoidei) with a similar disjunct African-Asian distribution and ecological features, as compared to the results of the case study of the climbing perches.

2. Materials and methods

2.1. Data collection

The core of the taxonomic sampling (Anabantoidei) (Table S1 online) was built on that of Rüber et al. [6], i.e., including the taxa of Anabantoidei *sensu* Lauder and Liem [25], and the presumed sister group to Anabantoidei, the Channoidei (species of *Channa* and *Parachanna*) with the addition of some other representatives of Anabantiformes *sensu* Betancur-R et al. [19] and Collins et al. [26], e.g., *Badis*, *Nandus* and *Dario*. Seven nandoid species were designated as the outgroups for analyses based on phylogenetic relationships in [19].

We downloaded all DNA sequences of Anabantiformes that contain the complete mitochondrial genome or four mitochondrial genes combined (cyt b, 12 rRNA, tRNA-Val and 16s RNA), or two of them except for *Macropodus hongkongensis*, which was only sequenced for cyt b gene. As such, we included as many species as possible while ensuring more gene sequences. The final data set includes 118 sequences of Anabantiformes plus seven nandoid outgroups. The taxon sample includes three suborders (Anabantoidei, Channoidei and Nandoidei), six families (Anabantidae, Helostomatidae, Osphronemidae, Channidae, Badidae and Nandidae), 24 genera and 77 species. Sampled taxa are representatives of six of the seven extant families, 24 of the 27 genera of Anabantiformes (Table S1 online), and include representatives that reflect the geographic distribution of the group as a whole (Africa, India, Southeast and East Asia) (Tables S2 and S3 online). All sequences were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/nucleotide/>).

2.2. Phylogenetic analyses

Sequence data were aligned with ClustalX version 2.0.12 [27]. A total of 2961 base pairs (bp) were included after alignment. Two phylogenetic methods, i.e., maximum-likelihood (ML) [28] and Bayesian inference (BI), were implemented in software RAxML version HPC-PTHREADS_820_32 and MrBayes 3.2.4 [29]. To determine suitable molecular clock and nucleotide substitution model, data were tested in BEAST 2.4.8, using “bModelSelection” package [28–30]. Detailed parameter settings can be found in the Supplementary Information.

2.3. Divergence time estimation

To obtain a comprehensive biogeographical history of Labyrinthici involving extinct taxa (fossils), two dating analyses, node dating and tip dating, were conducted in phylogenetic calibrations.

Node dating (ND) analysis was conducted with birth-death model based on the popular calibration-density method in BEAST v 2.4.8 [30]. A total of nine calibration points was applied to nodes calibrations based on the fossil records and taxonomic placements (Table S4 online). Details on tree prior setting were given in Table S5 (online) in Supplementary Information. We set the upper bound of 163.5 Ma (the beginning of the Late Jurassic) for the parameter value of the calibration nodes, the initial time of the breakup of Gondwana when Madagascan and Indian continent separated from Africa [6].

Tip-dating (TD) analyses were also performed in BEAST v 2.4.8 [30]. We incorporated the phylogenetic information in TD runs by creating monophyletic groups on the basis of the affinity of fossil and extant species lineages (e.g., [8,22,31,32]) (see Table S6 online). More specifically, we set manually the positions of the fossil taxa according to their known affinity. For the taxa with uncertain phylogenetic relationships (e.g., fossil gourami †*Ombilinichthys* and fossil snakehead †*Anchichanna*), we set them in relaxed positions within their own groups, respectively (Table S6 online). Whereas for the taxon with resolved phylogenetic interrelationships (e.g., †*Eoanabas*), we put it in a certain position within its group.

2.4. Ancestral range reconstruction

Ancestral patterns of distribution for anabantiform lineages using the time-calibrated phylogeny were inferred with the R 3.4.3 module using BioGeoBEARS [33]. This utility builds on the dispersal, extinction and cladogenesis (DEC) model for phylogenetic biogeography [34], incorporating a parameter (J) allowing for founder-event speciation. We conducted the time-stratified analyses under the biogeographical model DEC + J + x, and evaluated the ancestral range possibility, in which J indicates founder-event speciation, and x is the dispersal probability [33,35] (for details see Supplementary Information).

3. Results

3.1. Phylogenetic analyses

The result of the inferred phylogeny of the ML and BI analyses strongly supports the monophyly of the Anabantoidei (*sensu* Lauder and Liem [25] and Nelson et al. [36]), which was placed as the sister group to Channoidei (Channidae) (*sensu* Berra [4]) (Figs. 2 and S1 (online)). The sister-group relationship of these two clades concurs with the definition of Anabantiformes proposed by Wiley and Johnson [37], although this order also includes Nandoidei (Asian leaf-fishes) in Betancur-R et al.’s scheme [19]. This topology is consistent with most of the hypotheses in the aspect of labyrinth fish phylogeny resolved based on morphological or molecular data [5,6,17,19,20,26]. However, it differs from Murray et al.’s morphology-based hypothesis, which did not support the monophyly of Anabantoidei [22].

The topology of the family Anabantidae is similar to that found in previous molecular analyses [6] and the morphology-based phylogeny including fossil lineages [8] but different from those in another morphology-based analysis that only sampled living taxa [11]. In the crown lineage anabantids, the respective monophyly of Asian (Anabantinae) and African (Ctenopominae) species is well supported (Figs. 2 and S1 (online)).

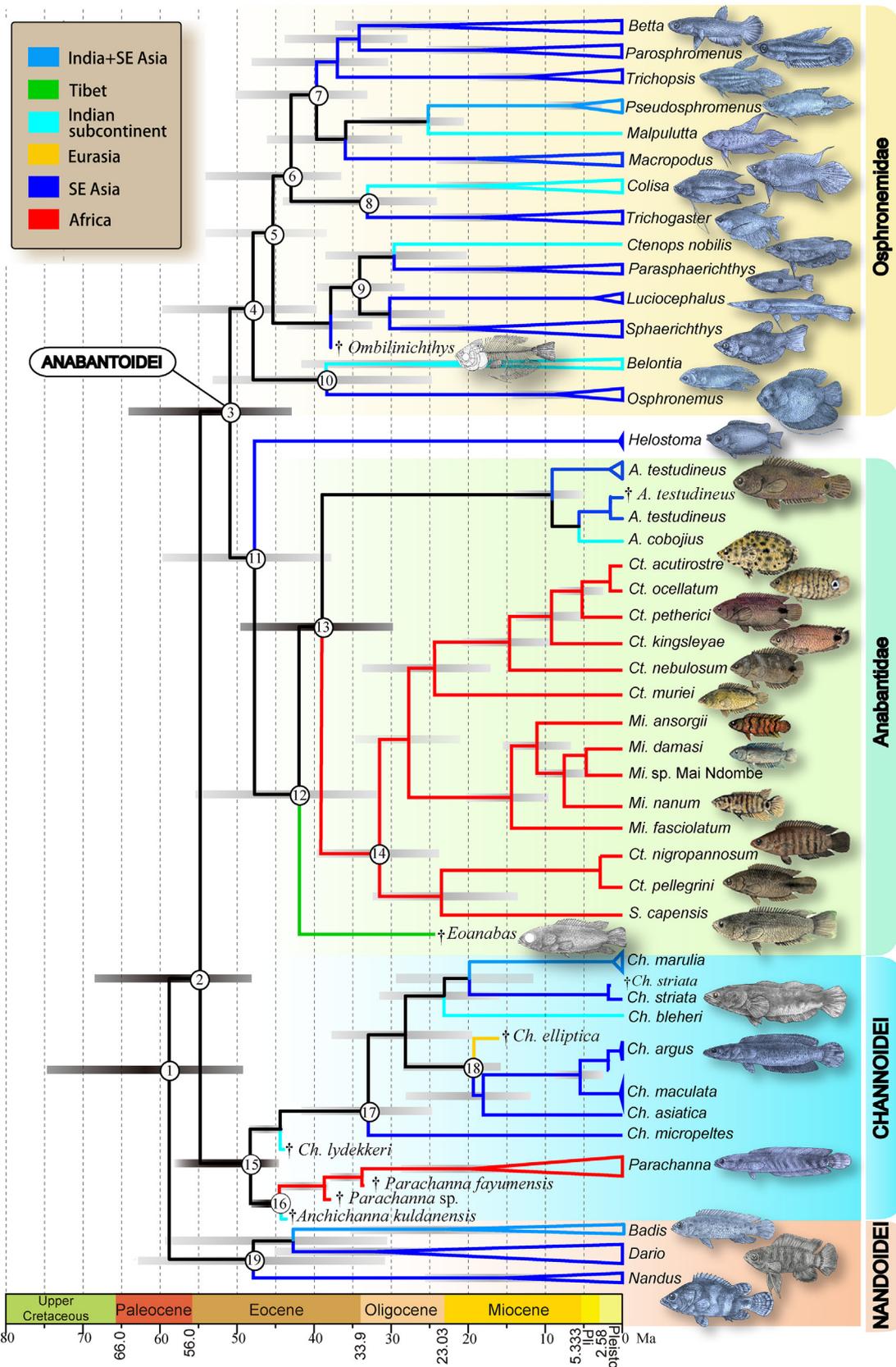


Fig. 2. Chronogram of anabantiform phylogeny estimated with tip dating (TD) using BEAST with fossilized birth-death process. Names of fossil taxa are preceded by a dagger symbol (†) and placed at their chronological horizon and systematic position. Gray bars represent 95% HPD intervals. Black bars are the 95% HPD intervals of the key nodes in this study. Lineage colors represents the distribution ranges, for explanations see the inserted box in the top left corner. Abbreviations: A. = Anabas; Ch. = Channa; Ct = Ctenopoma; Mi = Microctenopoma; S. = Sandelia; Pli. = Pliocene; Pleisto. = Pleistocene. Fish drawings are not to scale and those of the target group, Anabantidae are colorful.

3.2. Divergence age estimations

Generally, the ages estimated by tip dating were very close to those made by node dating for all nodes (Table S7 online), contrasting previous opinions that tip dating may overestimate the divergence ages in calibrated phylogenies, e.g., ca. 185–119 Ma (tip dating results) vs ca. 86–96 Ma (node dating results) for the origin of the tetraodontiform fishes (trigger fish and trunkfish) [38]. At the root of the Anabantiformes *sensu* Betancur-R et al. [19], the leaf fishes (Nandoidei) diverged from other anabantiforms at approximately 59 Ma (95% HPD: 48.90–74.67 Ma (Fig. 2, Table S7 (online))). The age of the most recent common ancestor (MRCA) of Channoidei and Anabantoidei is estimated as approximately 55 Ma (95% HPD: 47.60–68.45 Ma). Channoidei diverged into Asia (including India) and African lineages in the early Eocene (mean 44.5 Ma, 95% HPD: 44.5–47.08 Ma), roughly 11 and 25 Ma before the origins of crown-group *Channa* (mean 33.21 Ma (95% HPD: 24.41–42.39 Ma) and *Parachanna* (mean 19.42 Ma (95% HPD: 10.73–28.13 Ma), respectively (Fig. 2, Table S7 (online))).

Within the Anabantoidei, the crown age was estimated to be approximately 51 Ma (95% HPD: 43.26–64.07 Ma) (Fig. 2, Table S7 (online)). The MRCA of crown osphronemids was estimated to occur in the early Eocene (mean 47.0 Ma, 95% HPD: 39.47–59.28 Ma). Within the Anabantidae, the stem lineage, †*Eoanabas* was estimated to have diverged from other anabantids in the middle Eocene (mean 42.19 Ma, 95% HPD: 32.13–55.38 Ma). The estimated timing of the separation of African and Asian anabantids is approximately 40 Ma, mean 38.51, 95% HPD: 29.74–49.42 Ma), i.e., only ca. 3 Ma after the initial divergence between †*Eoanabas* and its confamilial relatives (Fig. 2, Table S7 (online)). This estimate is roughly 49 Ma younger or 8 Ma older than that estimated by previous molecular analyses, depending on the stem or crown calibration of the single fossil †*Osphronemus* [6].

3.3. Ancestral area reconstructions

The results of our analyses suggest Southeast Asian + Indian subcontinent as the ancestral area for the MRCA of the Anabantiformes and the Anabantomorpha (Channoidei + Anabantoidei) (Figs. 3 and S3 (online)). The snakehead fishes (Channoidei) likely originated in the Indian subcontinent, by the late Paleocene (Figs. 3 and S2 (online)). For the Anabantoidei, and within it the families Osphronemidae and Helostomidae, a Southeast Asian origin was supported (Figs. 3, S2, and Table S8 (online)). Within the family Anabantidae, Tibet was reconstructed as the most likely range for the MRCA of †*Eoanabas* and the crown anabantids and stem anabantid represented by the ancestor of †*Eoanabas* (Figs. 3, S3 and Table S8 (online)). For the MRCA of the crown lineage anabantids, an Indian origin is most likely (Figs. 3, S2, and Table S8 (online)). The Ctenopominae and Anabantinae appear to be primarily African and Indian + Southeast Asian in origin, and by the early Oligocene and the late Miocene, respectively (Figs. 3, S2, and Table S8 (online)). These results suggest that the inclusion of the fossil lineages reveals the role of the pre-uplift Tibet in the early diversification of anabantids, particularly during the early Eocene to the late Oligocene, though far from conclusive.

4. Discussion and conclusion

4.1. Divergence time estimates

Our dating results for the key nodes are partially inconsistent with the previous estimates. For example, in Rüber et al.'s labyrinth fish phylogeny [6], divergence time estimates of the Asian-African anabantid separation ranged from 87.30 (95%: 56.43–

124.22) to 30.83 (95%: 25.31–36.96) Ma and those in the root of the Anabantoidei ranged from 103.44 (95%: 67.75–145.37) to 37.70 (95%: 32.15–43.70) Ma depending on the analytical strategies [6], whereas the estimates of the Asian-African anabantid divergence time are 38.51 (95%: 29.74–49.42) Ma and those of age of the Anabantoidei are 51.05 (95%: 43.26–64.07) Ma (Table S7 online). For channoid fishes, the Asian-African divergence time estimates were 40–50 Ma [31] or ca. 120 Ma [38], whereas our estimations are ca. 45 Ma (Table S7 online). For a broader comparison of the age estimates, we referred to some influential molecular analyses which incorporated the anabantiform phylogeny [16–20]. As those studies aimed to resolve the phylogeny in the scope of the bony fishes, ray-finned fishes, or the spiny-rayed fishes, they sampled few species of Anabantomorpha *sensu* Near et al. [17] (Anabantoidei + Channoidei) [16–20]. For example, our estimations (mean) of the age of Anabantomorpha are ca. 55 Ma (95%: 47.60–68.45 Ma), and those of Anabantoidei are 51 Ma (95%: 43.26–64.07) Ma (for details see Table S7 online); whereas these two estimated ages are ca. 68.8 Ma and ca. 59 Ma in [17], ca. 69.1 Ma and ca. 62.3 Ma in [19], and ca. 60 Ma and ca. 45 Ma in [20], respectively.

The accuracy of molecular age estimates is positively correlated with the number of the calibrating fossil taxa and the proximity of the fossil lineages to the nodes under assessment [38]. The time-calibrated phylogeny herein differs from the previous estimates within Anabantoidei or Channoidei [6,39] in the following aspects. First, we included more fossil lineages (nine within Anabantiformes versus only one in [6] and none in [16–20,39]) distributed both in Anabantoidei and Channoidei as the tip taxa to calibrate the age estimation of relevant nodes. Second, we used †*Eoanabas*, a stem anabantid (versus a possible fossil gourami with uncertain systematic assignment in [6]) and therefore incorporated the uncertainties in the systematic placement and geological age of the key fossil calibration. And finally, the relevance of †*Eoanabas* to crown anabantids makes it an ideal candidate for constraining the disjunct biogeographical pattern for the family. The fossil calibration regime and the extensive sampling in the target groups (Anabantoidei and Channoidei) increase the precision of the results of the analyses. Unexpectedly, the general conflict between the age estimates of the node dating and tip dating seen in molecular clock analyses [38] is reconciled in the results of our study (Table S7 online). Therefore, the divergence time estimates here represent a reasonable approximation of the historic paleobiogeographical scenarios of the target groups in testing those hypothesized in the previous studies (e.g., [6,34]).

4.2. Historical biogeography

By integrating a number of relevant fossil lineages, our analyses provide a higher resolution for anabantoid paleobiogeographic history than the previous relevant molecular analyses [6] and traditional hypotheses [12,14]. Based on our analyses, Southeast Asia and Indian subcontinent, especially the former might be the cradle of the anabantiform fishes. This hypothesis is compatible with the distribution pattern of Nandoidei (Asian leaffishes), Channoidei (snakeheads) and Anabantoidei (labyrinth fishes), of which the diversity center is all in Southeast Asia [4,31] (Tables S2 and S3 online). The relevance of Indian subcontinent as the origin area of the root of the Anabantiformes might be contributed by the sampled extant species in eastern India (Fig. 2, Tables S2 and S3 (online)).

After the divergence of the Asian leaffishes (Nandoidei), the Anabantoidei and its sister clade Channoidei probably had arisen in Southeast Asia and India, respectively, by the early Cenozoic when the Indian subcontinent had already collided with Eurasia [40]. From the Indian subcontinent, the ancestral snakehead fishes

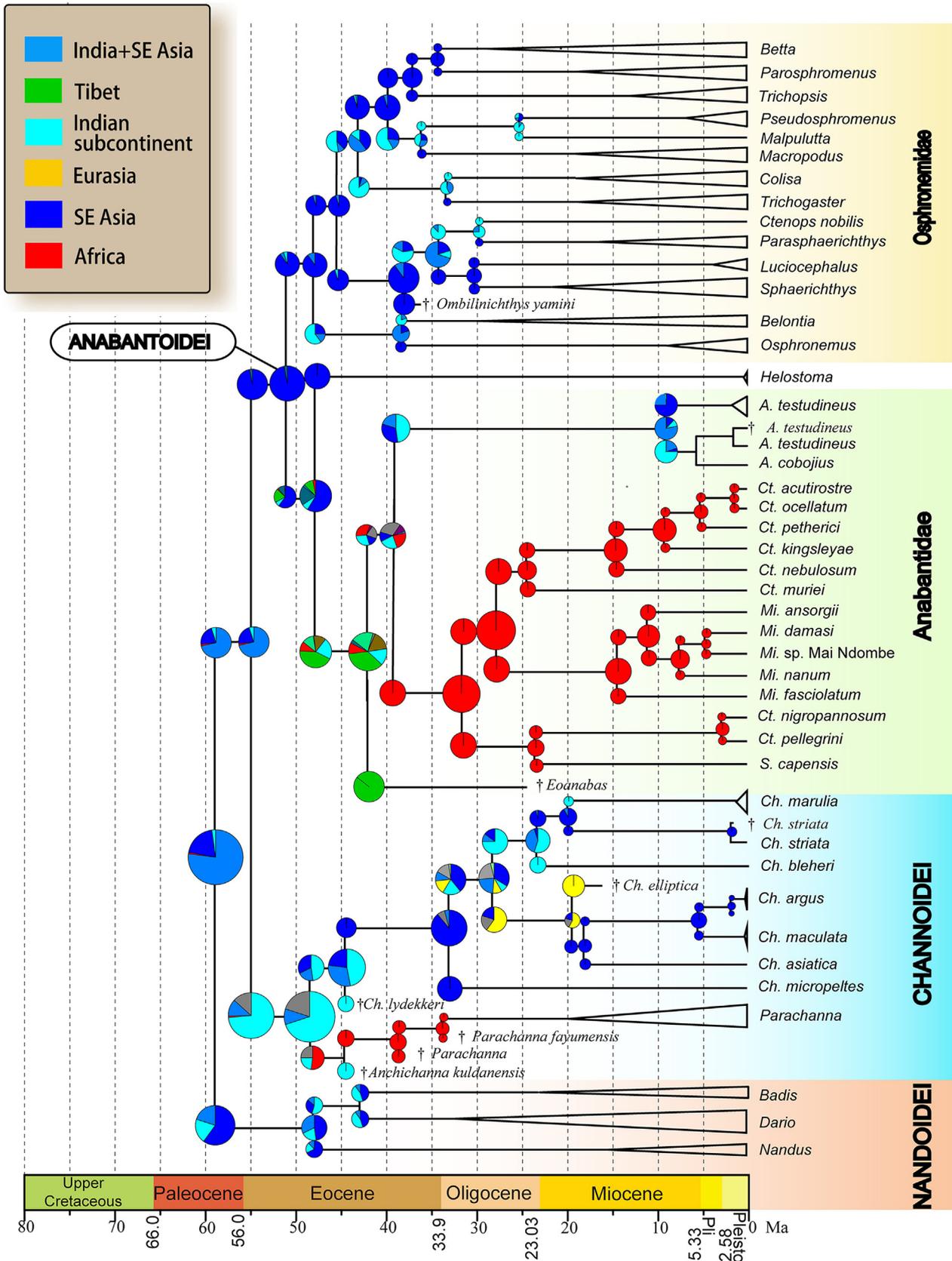


Fig. 3. Ancestral range estimations inferred using the DEC + J + x model based on a time-calibrated Bayesian phylogeny of Anabantiformes species. States at branch tips indicate the current geographical distributions and fossil localities of taxa, whereas states at nodes indicate the inferred ancestral distributions before speciation (middle) and after (corner). The region divisions in this analysis include the following: Southeast Asia (including southern East Asia), Eurasia (Europe and Central Asia), Indian subcontinent, Tibet, and sub-Saharan Africa, see the inserted box for the explanation.

migrated to Africa by the late early Eocene (ca. 45 Ma) (Figs. 2, 3 and S2 (online)), several million years earlier than the arrival of the climbing perches. A Southeast Asian origin for the labyrinth fishes in general and Anabantidae in particular is compatible with the viewpoints of Darlington [14], Liem [12], and Mayekiso [41] and hence contradicts the ancient Gondwanan origin held by other scholars, e.g., Skelton [13]. Furthermore, our results have refined the history of anabantid geographical range evolution. The emerging anabantid ancestor might have occurred in Southeast Asia by the early Eocene (ca. 47 Ma), followed by dispersal to Tibet and then the Indian subcontinent (Figs. 3 and S2 (online)), given the presence of some favorable wet and lowland habitat along the southern and central part of Tibet [42–46] which were positioned at the paleolatitudes of 21°–24°N then [47], and a river system connecting Southeast Asia and Tibet during the Paleocene to Eocene [48], and the completion of the India-Asia collision [40]. The lineage represented by †*Eoanabas* had inhabited Tibet at least until the late Oligocene and eventually it was eliminated by the cooling linked to the rise of the plateau [49]. It is also quite likely that the climbing perches had a more northward extension in East Asia during the Paleogene than today. In that warmer world, the excursion of the thermal equator may have been several degrees greater than now and even brought seasonal rainfalls to latitudes of ~30° [50]. However, their range shrank probably as a response to the Neogene intensification of the winter East Asian monsoon [43,51–53]. In contrast, the lineage in India lived much longer and marched onto a new destination.

The split of Asian and African anabantids was estimated to have occurred in around 40 Ma, and ancestral range reconstructions suggest that these fishes might have dispersed to Africa also from the Indian subcontinent as did the snakehead fishes (Figs. 3 and S2 (online)). This is congruent with the paleobiogeographical relationships between these two landmasses during that time. Instead of an isolated island, the Indian plate was more like a “passenger ship with a mobile gangplank” during its northward journey since its breakup from the Gondwana (see p. 3, 7, 73 in [1]). Some filter bridge existed, as the biotic link to Afro-Arabia was maintained by the Indian Plate as it drifted northward, even after its collision with Eurasia [1]. Four-legged animals including some placental animals had dispersed from India to North Africa via this “gangplank”, which was still in place by the middle Eocene [1]. The presence of the amphibious protocetid whales both in Northeast Africa and the Indian subcontinent during the middle Eocene (49–37 Ma) [54,55] also hints at the limited marine barriers between these two landmasses. Moreover, the Eocene freshwater snakehead fishes indicate the paleoichthyofaunal links between Northeast Africa and Indo-Pakistan [24], which is consistent with the split between Asian and African snakehead fishes during the late early Eocene (Fig. 2). Although several million years later, the anabantid fishes possibly dispersed via the same route. The capability of breathing air and short-distance overland excursion of extant species [4,11] (air-breathing structure also present in fossil anabantid †*Eoanabas* [8]) corroborates this possibility. On the one hand, the colonization of Africa, a relatively isolated continent since the Late Cretaceous [56], might have provided these anabantid precursors new ecological opportunities and thereby promoted rapid diversifications leading to the current diversity of African climbing perches [4,11]. This may partially account for the uneven distribution of anabantid species richness between Africa (28 spp.) and Asia (2 spp.) [11]. On the other hand, despite the biotic exchange between India and Madagascar during the early Paleocene and early Eocene as indicated by the history of some Malagasy frogs [57,58], it appears that no anabantiform fishes had followed this route to invade Madagascar, where there are no snakehead fishes or climbing perches today [4,6].

Admittedly, despite the absence so far of fossil anabantids from Western Asia, another route to Africa cannot be ruled out. Had the

ancestral anabantids expanded their distribution from India and Tibet to Western Asia, e.g., Iran, the trans-Tethyan dispersal might not have been impossible. The intermittent biotic links between Africa and adjacent landmasses, particularly Laurasia, during the Late Cretaceous to early Miocene, facilitated a series of animal dispersals into Africa [56]. More specifically, the agamid lizard and a number of placental mammals had migrated from Asia to colonize Africa via parts of the emergent Mediterranean Tethyan Sill, which was exposed during the intervals of low sea level [56,59]. Coincidentally, there was indeed a relatively lowstand period [60] around our estimated age of the African-Asian anabantid split (Fig. 2). However, whether or not the early anabantid fishes took this route to reach Africa is inconclusive.

It is also notable that biotic exchange between Africa and India-Asia is likely unidirectional, as indicated by the respective monophyly of the African and Asian anabantids. This implies that there should be no inbound migration or new immigrations after their arrival in Africa, even though the Afro-Arabian Plate was reconnected with Eurasia during the early Miocene (ca. 20–18 Ma) [61,62] and some other land animals and plants had successfully taken this route (i.e., the *Gomphotherium* land bridge) to disperse from Africa to Asia, or *vice versa* [62–64]. It might be due to the continuing northward convergence of India with Asia, the collision of Afro-Arabian Plate with Eurasia, and their mountain-making consequences in adjacent areas [1,60,65], which would have hampered or stopped such back-and-forth dispersals for freshwater fishes. Moreover, a strong paleoenvironmental change seems to have also started affecting the paleoichthyofauna in North Africa and Arabia since the Miocene which eventually wiped out the typical equatorial and tropical fish species there [66], hence practically obstructed dispersal of humid-adapted fishes, such as the anabantid fishes.

4.3. Conclusions

Reconstructions of the ancestral areas and an updated time-calibrated phylogeny of the labyrinth fishes resolve the ambiguous paleobiogeographical history of the climbing perches. Our results suggest a Southeast Asian origin for climbing perches during the middle Eocene followed by their dispersal to Tibet and India, and estimate the divergence of African and Asian climbing perches occurring in the middle Eocene (ca. 40 Ma). This relatively young split thus contradicts the attribution of their African-Asian disjunct distribution to the ancient Gondwana continental drift vicariance or the Late Cretaceous dispersal event from Africa to Asia, or *vice versa* [6] as did some other recent molecular clock analyses [16–20]. To explain this, we propose a westbound dispersal to Africa via the Indian subcontinent for the climbing perches, probably along some biotic exchange pathway between these two landmasses and following the biogeographical route of the snakehead fishes, on the basis of a similarly disjunct distribution and peculiar air-breathing capability. The utility of the fossil climbing perch †*Eoanabas* in the analyses is vital and for the first time highlights the role of pre-uplift Tibet in the early history of the labyrinth fishes. Therefore, looking ahead, we expect more discoveries of relevant fossils on that underexplored plateau.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

F. Wu designed the research and wrote the paper; F. Wu and D. He performed the research and analyzed the data. D. He, G. Fang and T. Deng participated in the research and writing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scib.2019.03.029>.

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