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The historical biogeography of the freshwater knifefishes using mitogenomic approaches: A Mesozoic origin of the Asian notopterids (Actinopterygii: Osteoglossomorpha)

Jun G. Inoue [a,*], Yoshinori Kumazawa [b], Masaki Miya [c], Mutsumi Nishida [a]

[a] Ocean Research Institute, University of Tokyo, 1-15-1 Minamidai, Nakano-ku, Tokyo 164-8639, Japan
[b] Department of Information and Biological Sciences, Graduate School of Natural Sciences, Nagoya City University, 1 Yamahohata, Mizuho-cho, Mizuho-ku, Nagoya 467-8501, Japan
[c] Department of Zoology, Natural History Museum & Institute, Chiba, 955-2 Aoba-cho, Chuo-ku, Chiba 260-8682, Japan

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ABSTRACT

The continental distributions of freshwater fishes in the family Notopteridae (Osteoglossomorpha) across Africa, India, and Southeast Asia constitute a long standing and enigmatic problem of freshwater biogeography. The migrational pathway of the Asian notopterids has been discussed in light of two competing schemes: the first posits recent transcontinental dispersal while the second relies on distributions being shaped by ancient vicariance associated with plate-tectonic events. In this study, we determined complete mitochondrial DNA sequences from 10 osteoglossomorph fishes to estimate phylogenetic relationships using partitioned Bayesian and maximum likelihood methods and divergence dates of the family Notopteridae with a partitioned Bayesian approach. We used six species representing the major lineages of the Notopteridae and seven species from the remaining osteoglossomorph families. Fourteen more-derived teleosts, nine basal actinopterygians, two coelacanths, and one shark were used as outgroups. Phylogenetic analyses indicated that the African and Asian notopterids formed a sister group to each other and that these notopterids were a sister to a clade comprising two African families (Mormyridae and Gymnarchidae). Estimated divergence time between the African and Asian notopterids dated back to the early Cretaceous when India–Madagascar separated from the African part of Gondwanaland. Thus, estimated time of divergence based on the molecular evidence is at odds with the recent dispersal model. It can be reconciled with the geological and paleontological evidence to support the vicariance model in which the Asian notopterids diverged from the African notopterids in Gondwanaland and migrated into Eurasia on the Indian subcontinent from the Cretaceous to the Tertiary. However, we could not exclude an alternative explanation that the African and Asian notopterids diverged in Pangea before its complete separation into Laurasia and Gondwanaland, to which these two lineages were later confined, respectively.

1. Introduction

Recent technical developments in DNA sequencing and molecular dating have enabled examination of biogeographical hypotheses based on more realistic 'relaxed clock' assumptions of molecular evolution (Avise, 2000; Yang, 2007). The "out of India" hypothesis holds that some Asian biotic elements have an ancient Gondwanan origin and arrived in Asia by rafting on the Indian plate (McKenna, 1973). Recent molecular phylogenetic and dating analyses also suggested an "out-of-India" migration in ranid frogs (Bossuyt and Milinkovitch, 2001), ratite birds (Cooper et al., 2001), arowanas (Kumazawa and Nishida, 2000), channids (Li et al., 2006), and cichlids (Zardoya et al., 1996; Farias et al., 2000; Sparks and Smith, 2004; Azuma et al., 2008). However, it has been suggested that extensive volcanism that produced on the India–Seychelles landmass the largest continental lava deposit (Deccan Traps) of the past 200 Myr had a heavy impact on the fauna and erased many taxa from the isolated Indian subcontinent itself (Thewissen and McKenna, 1992). Freshwater fishes form an important aspect of biogeographical studies, because they do not disperse easily through saltwater areas, and thus their evolution may be tightly linked to the geological histories of landmasses on which the evolution took place (Lundberg, 1993).

The family Notopteridae, commonly referred to as Old World knifefishes, comprises eight species contained in four genera. They are found in freshwater from western and central Africa and from India and Southeast Asia (Fig. 1). Only *Notopterus notopterus* is

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* Corresponding author. Present address: Department of Biology, University College London, Darwin Building, Gower Street, London WC1E 6BT, UK. Fax: +44 20 7679 7096.
E-mail address: j.inoue@ucl.ac.uk (J.G. Inoue).

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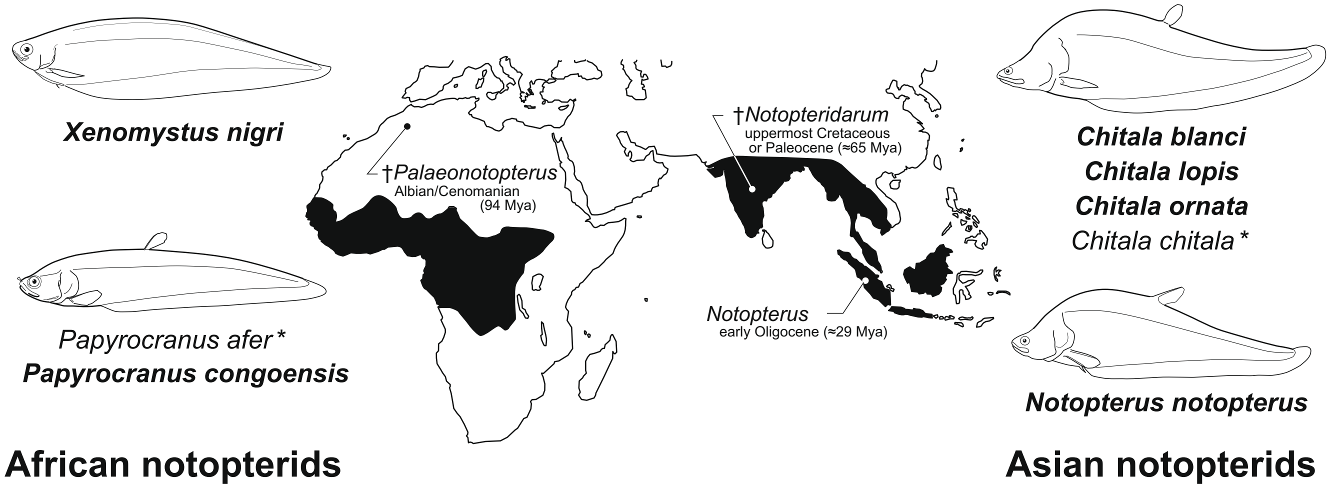

Fig. 1. The geographic distribution of the family Notopteridae (Roberts, 1992; Berra, 2001). *Papyrocranus afer* and *Chitala chitala* were not available in this study (asterisks). Possible notopterid fossils include the following: †*Palaeonotopterus* from Albian/Cenomanian Kem Kem Beds (94 Mya) of Taouz area, southern Morocco (Forey, 1997; Cavin and Forey, 2001), †*Notopteridarum* from the uppermost Cretaceous or Paleocene freshwater deposits (≈65 Mya) near Hyderabad, central India (Rana, 1988), and *Notopterus* from the early Oligocene freshwater deposits (≈29 Mya) near Padang, Sumatra (Sanders, 1934; Barber et al., 2005). Fossil taxa are designated with daggers (†).

known to enter brackish water occasionally (Roberts, 1992). One of the long standing enigmas in freshwater zoogeography has been the disjunct distributions of notopterids. Bănărescu (1991) suggested that the Asian notopterids either (1) dispersed after the Miocene contact between Africa and Europe or (2) were carried by the Indian Plate from Africa to southeastern Asia during the Cretaceous. Phylogeny reconstruction and molecular dating of osteoglossomorphs may provide new information to evaluate these competing biogeographical hypotheses.

There remains controversy over the interrelationships and the associated divergence times of osteoglossomorphs (reviewed in Hilton, 2003; Lavoué and Sullivan, 2004). The Osteoglossomorpha is widely recognized as a monophyletic group composing six families (Greenwood et al., 1966) and has a long evolutionary history dating back at least to the middle Jurassic (Arratia, 2004). Considering the poor fossil record of extant major osteoglossomorph groups and the fact that they may have acquired specialized morphology early in their evolutionary histories (Li and Wilson, 1996), it is no wonder that comparative anatomical approaches have faced difficulties in addressing their origins and relationships. Previous molecular studies based on limited numbers of sites and taxonomic representation have shed new light on the phylogenetic relationships among osteoglossomorph members (Lavoué and Sullivan, 2004) but did not have enough power to estimate the date of divergence and the biogeographical questions still remain unclear.

The purpose of the present study is to estimate the phylogenetic relationships and divergence times of Notopteridae using longer DNA sequences than previously used. We used whole mitochondrial genome (mitogenome) sequences because they have successfully been utilized not only for the phylogenetic analysis (see, e.g., Inoue et al., 2003; Miya et al., 2003; Zardoya et al., 2003) but also for the divergence time estimation (see, e.g., Kumazawa et al., 2004; Inoue et al., 2005; Zhang et al., 2005; Pereira and Baker, 2006; Kumazawa, 2007; Azuma et al., 2008) with deep branch divergences that occurred more than 100 million years ago (Mya). In addition to previously determined mitogenome sequences from 23 actinopterygians and two species of coelacanths, we determined mitogenome sequences for 10 new osteoglossomorphs including seven notopterids representing all major lineages of the Notopteridae. Based on the molecular, geological, and paleontological evidence, we evaluated alternative hypotheses concerning the origin and migrational pathway of the Asian notopterids.

2. Materials and methods

2.1. Taxon sampling

We chose seven individuals representing six out of eight described species of the Notopteridae and one or two species from all the other osteoglossomorph families (Table 1). We included two individuals from *Notopterus notopterus* to cover a broad geographical range of the species from India to Malaysia. We chose at least two species from all major lineages of the basal teleosts (Salmoniformes and more-derived teleosts) to minimize any long branch attraction that might exist. Two coelacanths, were chosen as the representatives of sarcopterygians. We chose three polypteriforms, three actinopterygians, two lepisosteids, and an *Amia* as the representatives of more basal actinopterygians, 11 species (four elopomorphs, two clupeiforms, two cypriniforms, and three salmoniforms) as the representatives of the other basal teleosts, and three species as the representatives of neoteleosts. Final rooting was done using a shark *Scyliorhinus canicula*. All species used in this study are shown in Table S1, with their DDBJ/EMBL/GenBank Accession Nos. (Supplementary material available online at http://www.systematicbiology.org). Voucher specimens were deposited in the Fish Collection, National Science Museum, Tokyo (NSMT-P) and Natural History Museum & Institute, Chiba (CBM-ZF) with registered numbers shown in Table 1.

2.2. DNA extraction

A portion of the epaxial musculature (ca. 0.25 g) was excised from fresh specimens of each species and immediately preserved in 99.5% ethanol. Total genomic DNA was extracted using the Qiagen DNeasy tissue kit following the manufacturer's protocol.

2.3. PCR and sequencing

Whole mitogenome sequences from the 10 osteoglossomorphs were determined following the method described by Miya and Nishida (1999). The mitogenomes of the 10 specimens were amplified in their entirety using a long-PCR technique (Cheng et al., 1994). We divided the mitogenome into two overlapping segments that can be amplified with four primers. Combinations for the LA-PCR primers (Inoue et al., 2003; Miya et al., 2003) were: L2508-