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Chromosome evolution in Indian Caecilians (Amphibia: Gymnophiona) and their phylogeny: A Review

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Abstract

Based on the pooled cytogenetic data obtained from Conventional, C, G, NOR and R-banding technique for twenty three species of Indian gymnophion amphibians procured from Western Ghats regions of peninsular India and one species from Kenya, East Africa, an attempt has been made to construct a tentative scheme for their phylogenetic interrelationships with that of other species of other continents of this group.

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Introduction

Unlike other amphibian fauna, extant gymnophion amphibians are known to thrive from very restrictive but, tropical and moist locales of Southern hemisphere that too with a patchy endemism and hence, of their inconspicuous cytogenetic evaluation. Thus, ecological, morphological and molecular analyses conducted thus far in respect of neobatrachian caecilians reveal that they are found exclusively as South and Central-American clade, and an African clade including Seychelles archipelago and as Southeast-Asian clade including India and Sri Lanka. The broad Gondwanan distribution of caecilians suggests that these limbless forms became isolated in Gondwana in the Jurassic before Southern land mass broke into smaller fragments (Duellman and Trueb, 1986, 1991).

Of the six gymnophion families are known, four families (Rhinatrematidae, Scolecomorphidae, Typhlonectidae and Caeciliidae) are definitely endemic to Gondwanan area, and the fifth and sixth family (i.e., Ichthyophiidae and Uraeotyphlidae) is found on Gondwanan (area) and Laurasian (South East Asia) land masses. Thus, the origin of the South East Asian ichthyophiids has posed a impregnable biogeographic problem (Duellman and Trueb, 1986; Hedges and Maxson, 1993; Hedges *et al.* 1993; Hass *et al.* 1993).

Previous molecular analyses that have been informative and provocative regarding the preavailable interrelationships among caecilian families have led to appraisal of inferences based on nucleotide sequences of mitogenome and nucleogenome (RAG1) (San Mauro *et al.* 2004, 2005; Roelants *et al.* 2007). These studies have revealed that recovering clades comprising of Ichthyophiidae + Uraeotyphlidae and of Nussbaum's (1991) and of Wilkinson and Nussbaum's (2006) higher caecilians (Scolecomorphids and Typhlonectids) and a paraphyletic Caeciliidae are well documented. In agreement with the most recent morphological investigations (Gower and Wilkinson 2005, 2007; Gower *et al.* 2008) their study suggested that Caeciliidae is paraphyletic with respect to perhaps Scolecomorphidae as well as Typhlonectidae. However, many relationships within the higher caecilians were not strongly supported, and further suggest that more molecular and morphological investigations are required to resolve these tangible relationships (San Mauro *et al.* 2004).

The present study was an attempt to procure basic cytogenetic data with regard to some endemic caecilian species of peninsular India, that include the three families: Caeciliidae, Ichthyophiidae and Uraeotyphlidae (Fig. A), and compare them with previously described chromosomal data drawn from other gymnophion caecilians of other continents, exclusively based on 'conventional' chromosome homeology.

In order to obtain 'chromosome homeology', based on the methods utilized during the preparation of traditional karyotypes, this was used as a pointer in the present case. It was also resorted to utilize and rearrange chromosomal set in order to consider as a member of the respective karyotype onto, by means of regrouping them (such as arranging them into A to E order, etc.) and further, the same was adopted for other species karyotypic data and the same was used for purposes of comparison on karyotypes; and thus, we have made use of those data to arrive at 'commonality in approach' methods and the same approach was implicated to acquire 'chromosome homeology search'.

The current karyological data procured so far and their utility in karyotypic evolution in caecilian cytogenetic studies were derived from conventionally stained preparations. This is evident due to difficulties encountered in procuring high quality linear banding differentiation along the metaphase chromosome morphology (for example, G-bands). The use of chromosomal data in understanding phylogenetic relationships (whether of closely or distantly related species) depends on an analysis of information drawn from hierarchical levels of genomic organization: chromosome number, chromosome morphology, linear differentiation and high-resolution banding sequences (in which latter two were not amenable).

Recently, comparative chromosome painting, the method of choice for genome-wide comparison at the molecular cytogenetic level has been successfully applied for comparative cytogenetic studies of various animal and plant genome analysis. Such an approach would involve modern chromosome technology studies which could offer a highly robust data set for the study of evaluation of karyotypic evolution and its use in phylogenetic assessments.

The primitive caecilian family, among Indian caecilians, viz. Ichthyophiidae include only two genera (*Ichthyophis* and *Caudacaecilia*) consisting of $2n=42$ with chromosomal constitution characterized by asymmetrical (combination of biarmed, acro/telocentrics and microchromosomes) karyotypes. Although, $2n=42$ karyotype of *Ichthyophis* exhibits no interspecific variations in number but in respect of relative size and shape they differ obviously from one species to another, even though they seem to be endowed with two colour morphs (striped and non-striped forms). Even if the taxonomy and systematics of the genus *Ichthyophis* is much clearer now-a-days than a few years ago many questions still remain unanswered.

Infact, cytogenetic studies have revealed a highly interesting pattern of chromosomal evolution and the occurrence of several chromosomal races or species; however, the range of this genus is very wide and a definitive and complete scenario of variation across the genus is still not possible.

Ichthyophiids seem to provide a novel example of a gymnophion group whose diversification is associated with an episode of extensive chromosomal polymorphism. *Ichthyophis* karyotypes are remarkably constant within each taxon but seem to vary between taxa. This suggests that chromosome rearrangements are not neutral and that homozygosity may offer selective advantages. It also suggests occurrence of novel rearrangements are not being generated at a high frequency. Thus, *Ichthyophis* population probably experience only brief periods of chromosomal polymorphisms before fixation or elimination is achieved (Fig. B).

The large-chromosome-number karyotype with diploid number ranging from 36 to 40 chromosomes is represented by Uraeotyphlidae, considered to be a sister group in ichthyophiid – uraeotyphlid clade. Karyotypic analyses with regard to the species belonging to the genus *Uraeotyphlus*, surprisingly exhibit 'karyotypic conservatism' to such an extent to consider them to be a unique karyotype in the family in spite of their local endemism to peninsular India and nowhere else (Fig. C).

In stark contrast, the higher caecilian family, Caeciliidae includes the large-chromosome number group, with diploid numbers ranging from 20 to 36/38 chromosomes of diverse size and shape. Presently, this taxa is consisted of peninsular Indian genus, *Gegeneophis* ($2n=26-30$), *Indotyphlus* ($2n=26$), six species group taxa of Seychelles islands ($2n=26$); *Afrocaecilia* ($2n=34$), *Geotrypetes* ($2n=36/38$) of African counterpart; and of South American counterpart represented by *Dermophis* ($2n=26$), *Gymnopsis* ($2n=22-24$) and *Siphonops*. This assemblage of Caeciliidae are not only the most speciose of the group but are chromosomally by far the most diverse family that spread across three

Southern continents for the order gymnophion amphibians including very wider range of chromosomal numbers for the higher order caecilians. Majority of them are endowed with karyologically variable groups of caeciliids with the karyotype mostly of biarmed chromosomes that are relatively uniform structural size and shape but in the decreasing order with no microchromosomes, thereby qualifying themselves to be derivative ones (Fig. D).

The next most variable gymnophion family, in terms of chromosome number and morphology is the members belonging to the family Typhlonectidae that having resorted to aquatic and semi aquatic habituation, while only two species of two genera are known for their karyology, viz, *Chthonerpeton indistinctum* ($2n=20$) and *Typhlonectes compressicauda* ($2n=28$) and all are biarmed chromosomes and no microchromosomes and are of South American origin.

Morescalchi's (1973, 1975) proposed chromosome model number for amphibian cytogenetic scrutiny implies that karyotype with relative large chromosome numbers, asymmetrical chromosome morphology and presence of microchromosomes; characterize primitive (plesiomorphic) members of each of the three amphibian orders. Although the observed karyological variation in amphibians as a whole had been termed complicated than other wise, but the pattern of karyological variation in caecilians does seem to fit in well with Morescalchi's dictum (1975) with some exceptions. Evolutionary changes in and as asymmetrical and bimodal karyotype are evolved as ancestral in some caecilians and obvious, both ichthyophiids and uraeotyphlids seem to retain the primitive condition, whereas that of higher caecilian examples studies reflect upon their carved derivative state.

Moreover, based on the chromosome 'homeology' that were observed upon examples of higher order caecilians of Indian taxa including that of South American, African clade of Gondwanan origin, are in concordance with both reduced chromosome number and morphology.

Caecilian amphibians appear to provide further evidence based on much needed cytological parameter in this pursuit of evolutionary decrease in chromosome number. The highest chromosome numbers are found in the diverse array of plesiomorphic genera including *Caudacaecilia*, *Ichthyophis* and *Uraeotyphlus* and have reduced basic numbers in the more derived and successful genera, such as, Indian *Gegeneophis*, Seychellean caeciliids, African *Geotrypetes*, South American *Gymnopsis*, *Caecilia*, *Siphonops* and others.

Another interesting parameter that seemed offering in the current understanding of caecilian genomic architecture has been the prolific variations in C-banding profiles. Variation in C-band heterochromatin has also been a topic of interest in caecilians, in the light of variations encountered that has potentiated in their respective genomic expression. Most caeciliid amphibians seem to possess very limited extent of C-banding expression with most of the highly dark staining portion confining to the centromeric and pericentromeric regions in the complement.

From the point view of application of C-banding sequences, presented a pictorial legacy of evolution of chromosome structure in which centromeric DNA first accumulates *de novo* around centromeres and later disperses outward onto the chromosome arms. Thus classical C-bands were found exuberantly expressed in case of plesiomorphic complement (such as ichthyophiids to a greater extent and to lesser extent in uraeotyphlid genomes), whereas in other higher caeciliids they virtually subsides distributionally to a speck (perhaps at kinetochore-specific regions alone) that disburses towards other portions of the chromosome arms rather than at centromeres.

Macgregor and Sessions (1986) suggest a model that is more akin to salamanders but extension of it could as well serve purposes of the present case. The model subscribe to the view that pericentromeric satellite sequences would be older and show more interspecific homologies than centromeric sequences, wherein, that of centromeric sequences are well preserved between species whereas centromeric-specific satellite DNA are not. Pertaining to the pertinent observations made among the Indian caecilian examples investigated it is possible to offer possibilities that those of taxa having Gondwanan origin perhaps could have had their genome oppressed due to tectonic and oceanic turbulences resulting in repression of profusely stainable C-banding expression; whereas those of the taxa originating from Laurasian groups have had lesser effect upon their genome and thus led into profusely stained cytological expression patten (i.e. C-positive bands).

As the nature of differential $AgNO_3$ expression among the species surveyed it could be interpreted as due to occurrence of chromosome repatterning via translocations of the conserved loci in the genome. Variation in NOR positions in different species, although do not offer much needed insights during the studies pertaining to species

differentiation, it becomes obvious to note that homosequentiality of NORs in which apparent repatterning is achieved without translocations through growth are declined in the number of repeats in clusters of gene sequences that were already more or less widely scattered throughout the ancestral chromosome. Macgregor and Sherwood (1979) implied to the 'homosequentiality hypothesis', insisting upon variation in cytologically visible ribosomal gene loci reflect differential expression pattern in sizes of gene clusters generated by unequal crossing over within the clusters.

2. Tentative scheme for phylogenetic tree

White (1968, 1973, 1978), a proponent of stasipatric (according to some, parapatric) mode of speciation emphasized that in many groups of animals of restricted vagility, very closely related species not only differ in karyotypes, but also exhibit very extensive differences due to the establishment of 'chains' of several or many structural chromosomal change. Several classifiable model systems, (for example, mice, grasshoppers *etc.*) came in support of such a testament, which primarily relies on sequential establishment of chromosomal rearrangements each within the range of the previous one. By these means, the genetic isolation of the area population is progressively reinforced. It is suggested that an important reason for the establishment of these chromosomal rearrangements is their role in protecting coadapted gene complexes (area effects) from disruption by introgression from neighboring population. In the context of present work, it seems probable to note that the role played by chromosomal rearrangements perceived points towards a particular mode of speciating mechanism operating in caecilian examples. The chromosomal mechanics of speciation seem to be especially prevalent in the organisms of restricted vagility whose population consists of numerous local demes that persists in the same area for many generations. The cytogenetic methodology employed in the present work permitted to gain valuable karyological data for the species characterized. Although a meager cytogenetic data in context of major model systems is operating based on the foregoing account of Indian caecilian chromosomal analyses, it is possible to construct a probable phylogenetic tree with the implications that manifest in Western Ghats region for gymnophion fauna constituting a sizable community of populational (biological) stratagem (Fig. E).

In essence, in the taxa of Indian caecilians, chromosomal evolution involves rearrangements of both repetitive sequences (constitutive heterochromatin) and other chromosome structural variations. It could be inferred that structural rearrangements are facilitated by reorganization of heterochromatin. Heterochromatin would then appear as a driving force of chromosomal evolution. In caecilians, with the reorganization of heterochromatin which may indeed also represent the visualization of a general process involving all chromosomal structures. As to the nature of karyology of caecilians, no general picture could be drawn, since some species in some genera possesses lowest heterochromatin segments and thus they seem to fall into derivative categories with more interspecific variations (for example, *Gegeneophis* species groups).

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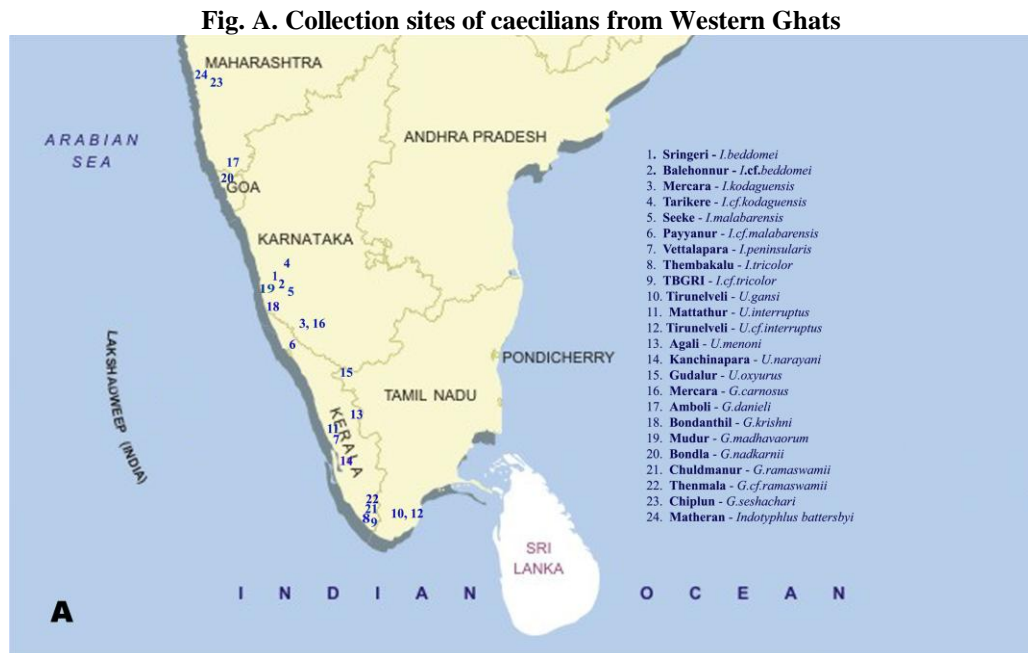


Fig. B. Karyotypic differentiation in Ichthyophiidae

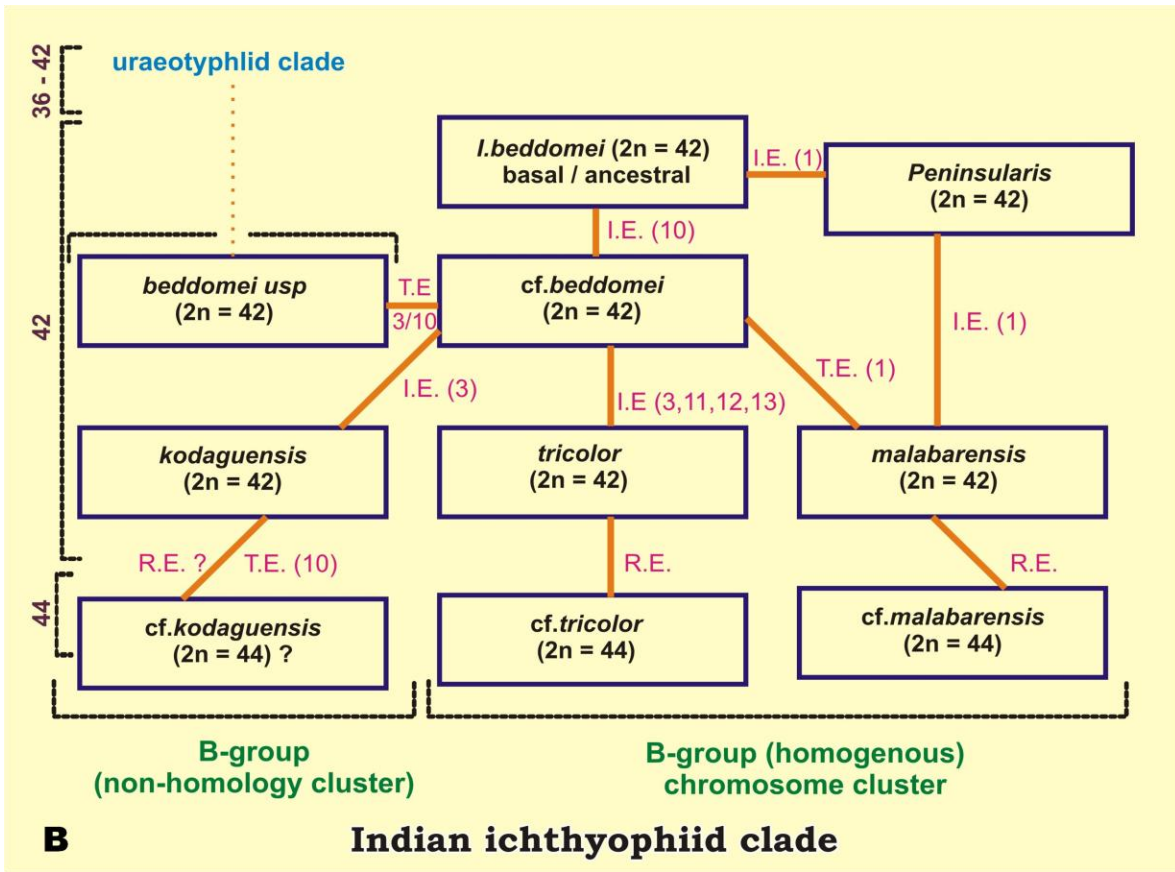


Fig. C. Karyotypic differentiation in Uraeotyphlidae

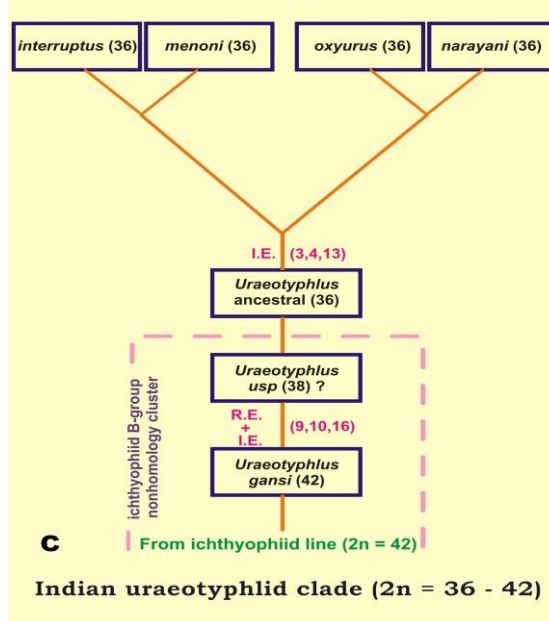
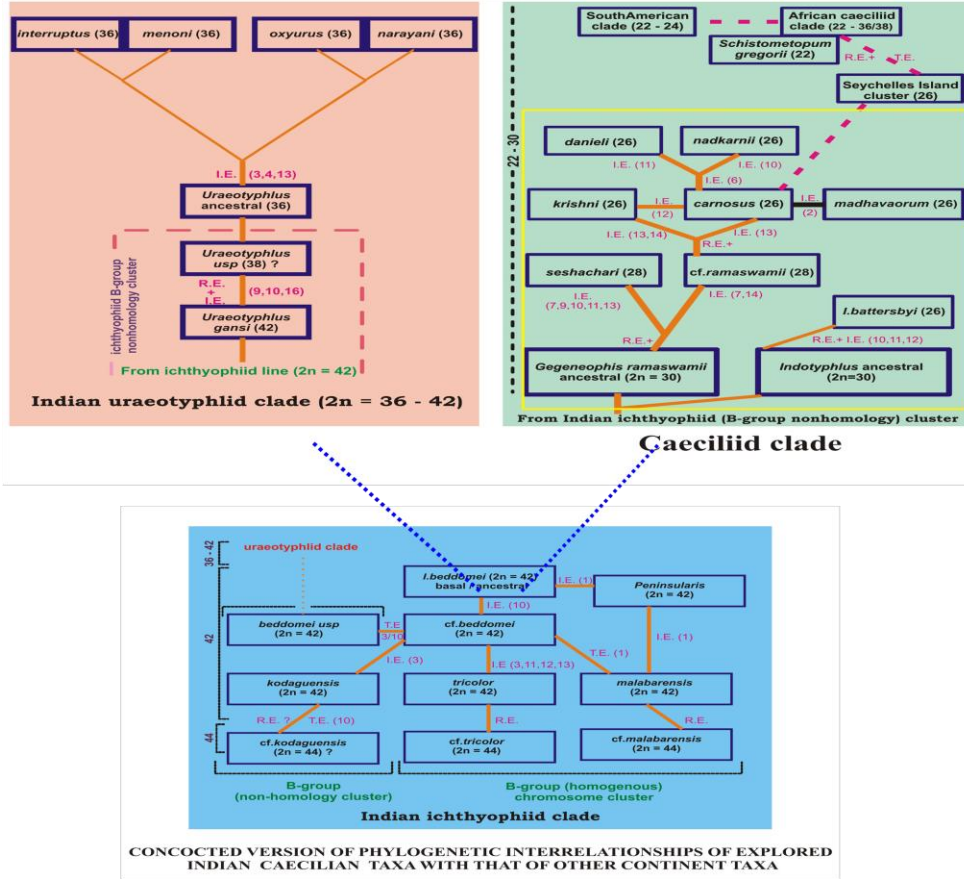


Fig. D. Phylogenetic interrelationships of Indian caecilian taxa with that of other continent taxa



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Fig E is missing