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RESEARCH ARTICLE

Occurrence of chromosomal polymorphism in a striped ichthyophid caecilian (Amphibia: Gymnophiona) from Western Ghats of India

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Abstract

Mitotic and meiotic chromosomes prepared from specimens of Ichthyophis italics *tricolor* collected from either side of Palghat Gap from three different locations of Western Ghats revealed a diploid number (2n=42-44) with Fundamental Number (62-64). Highest diplod number (44) and occurrence of chromosomal polymorphism is documented for the first time for gymnophion amphibians. Role of chromosomal rearrangements involved in karyotypic derivation process is discussed.

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Introduction

The genus italics Ichthyophis (of Ichthyophiidae) is comparatively widespread among primitive caecilians that include about 53 species (Matsui et al., 2006; Wilkinson et al., 2007; Kamei et al., 2009; Mathew and Sen, 2009; Bhatta et al., 2011; Nishikawa et al., 2012a, b, c, 2013; Frost, 2013). Its territorial occurrence ranges throughout Southeast Asian and South Asian regions including peninsular India and Sri Lanka. Earlier studies performed during the past decade following multidisciplinary approaches involving karyological, molecular and morphometric techniques, directed towards revealing that the systematics and taxonomy are more complex than previously thought. These have lent support to a supposition of concurrence of several cryptic species or subspecies (Venu, 2008; Nishikawa et al., 2012c). The biogeographical extent of italics Ichthyophis tricolor Annandale is limited with disjunct distributions essentially confining to southern parts, while italics Ichthyophis beddomei Peters is rather more preponderance to Western Ghats region of South India.

Format In contrast to morphologies of italics Ichthyophis beddomei, italics Ichthyophis tricolor is characterized by presence of continuous stretches of lateral bright yellow band throughout the body length and is wider at collar region. Tentacles are placed more towards eyes and prominent nuchal grooves are confined to ventral and sides of the body whereas transverse folds acquire predominance at dorsum.

Format Some earlier authors have questioned the very validity of italics I. tricolor as an independent species against italics I. beddomei. However, constraints prevailed either as a color variant or a variety (Annandale, 1909), while others implied of a geographical race (Nussbaum and Gans, 1980), eventhough, Taylor (1960) had distinguished this as a valid species. This species for the present purpose was procured from three locations (Table. 1) along the coastal South Western Ghats regions of South India.

Format Cytogenetic analyses of ichthyophiid caecilians based on conventional chromosome staining methods have indicated closer relationships prevailed within the family Ichthyophiidae, even- though only a few species of other two genera viz., italics Uraeotyphlus (Seshachar, 1939; Elayidom et al., 1963; Venu, 2008; Venu et al., 2011; Venu and Venkatachalaiah, 2013) and italics Caudacaecilia (Matsui et al., 2006) are cytologically known. These studies reveal that the genus italics Ichthyophis is karyologically conserved taxa in terms of chromosome number (2n=42) and chromosome arm morphology (FN=60). This report is aimed at presenting karyotype for the taxa derived from three populations while indicating karyological differences.

Materials and Methods

Ten hours prior to chromosomal processing, the animals were inoculated with a suspension of colchicine solution. Mitotic and meiotic chromosomes were procured from liver and testis respectively of specimen, following the procedures described earlier (Venkatachalaiah and Venu, 2002 and Venu and Venkatachalaiah (2005, 2006). Chromosomal morphology was arraigned essentially based on the concept of arm ratio (Levan et al., 1964) and the same was adapted for current purpose (Venu 2008, Venu et al., 2011). The mitotic and meiotic chromosome sets in the karyotype were arranged into broad groups (A-D) based on the relative sizes, shapes and lengths and on the position of their centromeres and were arranged in decreasing order of their total length.

Results

Cytotype I

The specimens of **italicize** I. tricolor collected from Chuldmanur, Thiruvananthapuram, Kerala, have served to provide a diploid number of 42 and FN=64 and as well as basal karyotype. Chromosomes were arranged onto groups (A-D) in a karyotype. Group A is comprised of three pairs of longer chromosomal set in which the first and second pairs are metacentrics and the third pair is subtelocentrics. Group B consists of three pairs (4-6) of medium sized metacentrics of uniform size. Group C consists of five pairs of small metacentrics (pairs 7-11). The first set of group D is comprised of two pairs (12-13) of major acrocentrics and the second group is comprised of eight pairs (14-21) of small acrocentrics, all arranged in the decreasing order of their size (Fig. 1).

Cytotype II

Karyotypes prepared from specimens of **italicize** I. tricolor collected from Pathekar, Kollam, Kerala, showed a diploid number of 42 and FN=62. Karyotypic details of this specimen resembled that of cytotype I in all respects with an exception that group C consists of four pairs (pairs 7-10) small metacentrics arranged in decreasing order of their size (Fig. 2).

Cytotype III

Interestingly, the variant karyotype prepared from two specimens of **italicize** I. cf. tricolor collected from Agali, Attapadi Hills, Palakkad district of Kerala, southern India showed consisting of a diploid number of 44 (FN=64). This variant karyotype showed deviations from that of **italicize** I. tricolor standard karyotype by having a) a different diploid number b) subtelocentric chromosome pair 6, c) group C comprised of two pairs (7-8) of small sized submetacentrics, d) the first set of group D comprised of six pairs of major acrocentrics (9-14) and second set comprised of two pairs (15-16) of small sized submetacentrics followed by six pairs (17-22) of small acrocentric chromosomes (Fig.3).

Meiotic chromosomal counts conformed to their mitotic chromosomal counterparts (Fig. 4 and 5).

TABLE 1: Details of collection of italicize Ichthyophis tricolor and italicize Ichthyophis cf. tricolor

Species	Locality	Habitat	Voucher number	No. of animals used	Geographical Coordinates
Ichthyophis tricolor	Chuldmanur, Chathankode, Thembakalu, Thiruvananthapuram (Dt), Kerala, India	Mixed plantations of arecanut, banana, coconut, coffee	BUB 1010, BUB 1011	1 male 1 female	8.4875° N, 76.9525° E
Ichthyophis tricolor	Pathekar, Ottakkal, Kollam (Dt), Kerala, India	By the sides of water canal in a banana and coconut plantation	BUB 1015	1 female	8°58'41"N, 77°3'29"E
Ichthyophis cf. tricolor	Agali, Attapadi Hills, Palakkad (Dt), Kerala, India	Backyard garden with banana plantation	BUB 1079, BUB 1239	1 male 1 female	11°5′0″N, 76°35′0″E

Figure 1 Giemsa stained female metaphase karyotype (cytotype I) of italicize I. tricolor

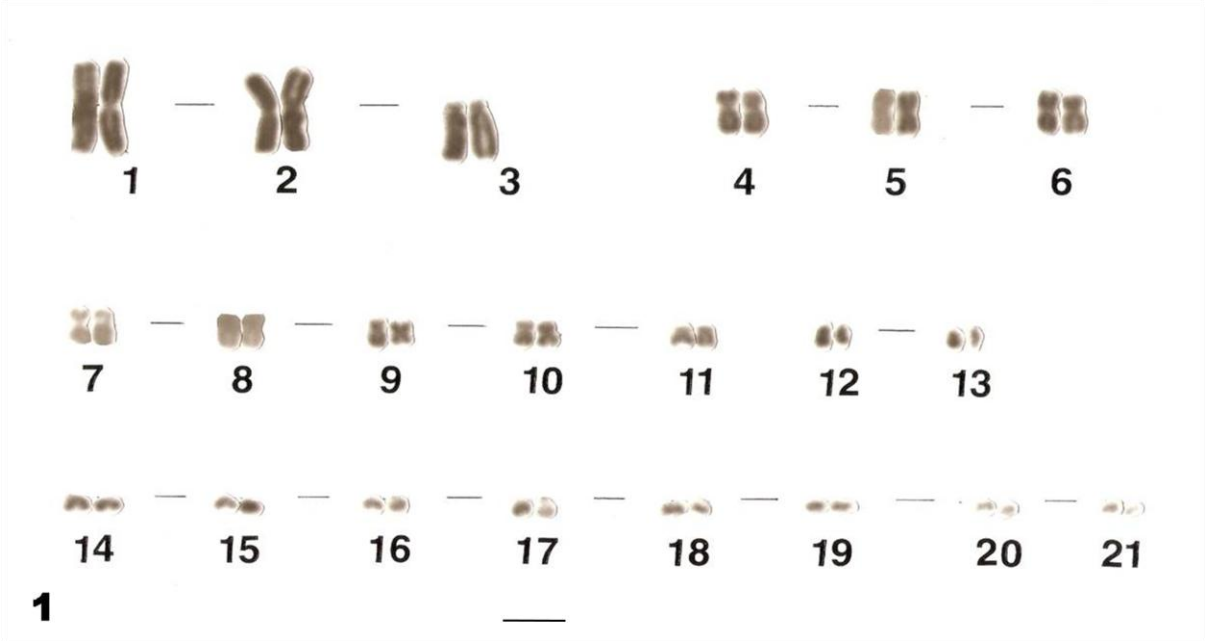


Figure 2 Giemsa stained female metaphase karyotype (cytotype II) of italicize I. tricolor

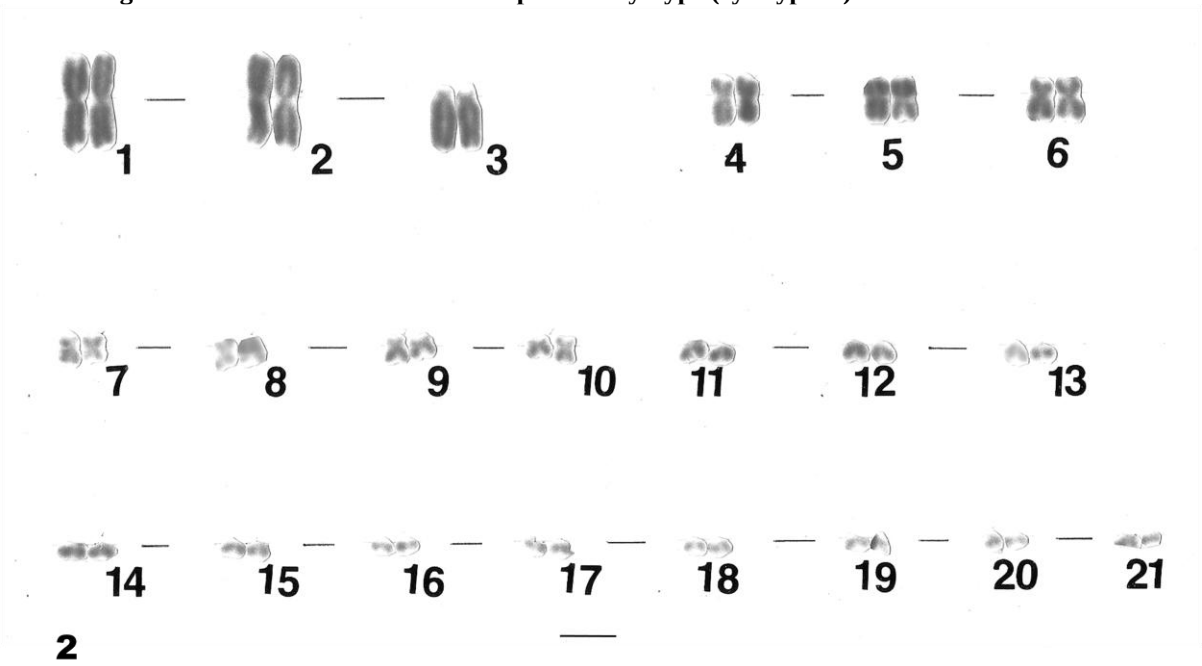


Figure 3 Giemsa stained female metaphase karyotype (cytotype III) of italicize I. cf. tricolor

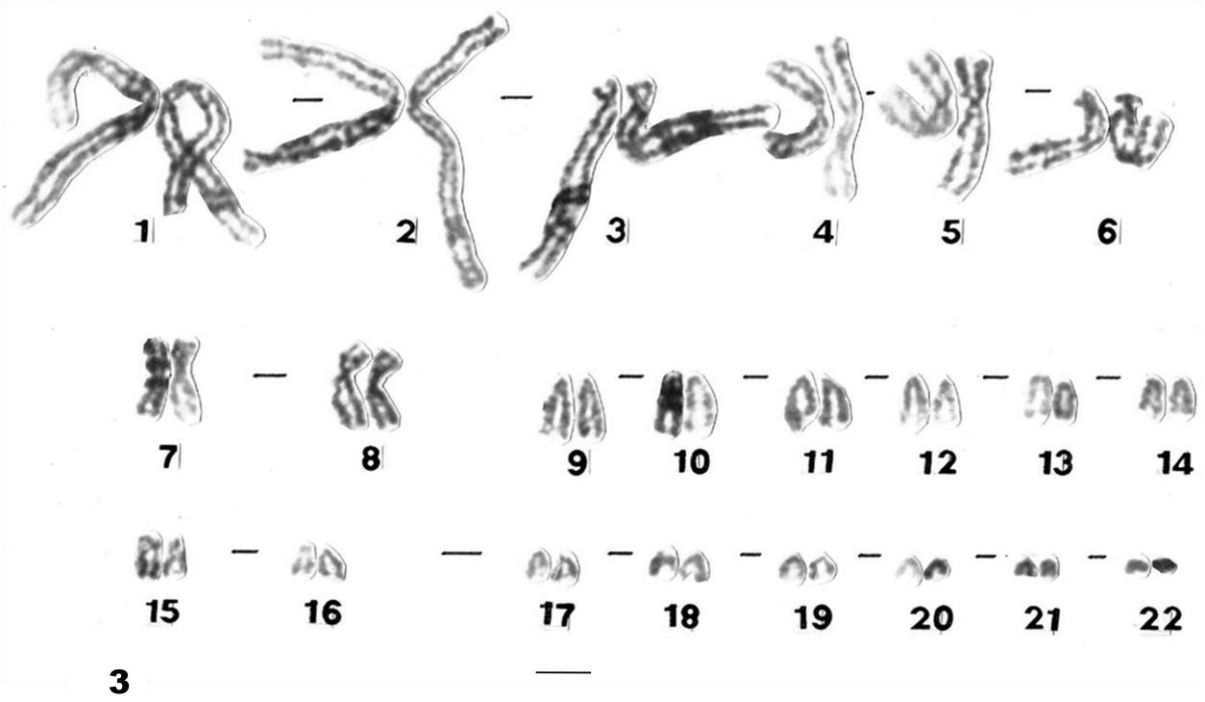


Figure 4 Giemsa stained male diplotene karyotype (cytotype I) of italicize I. tricolor

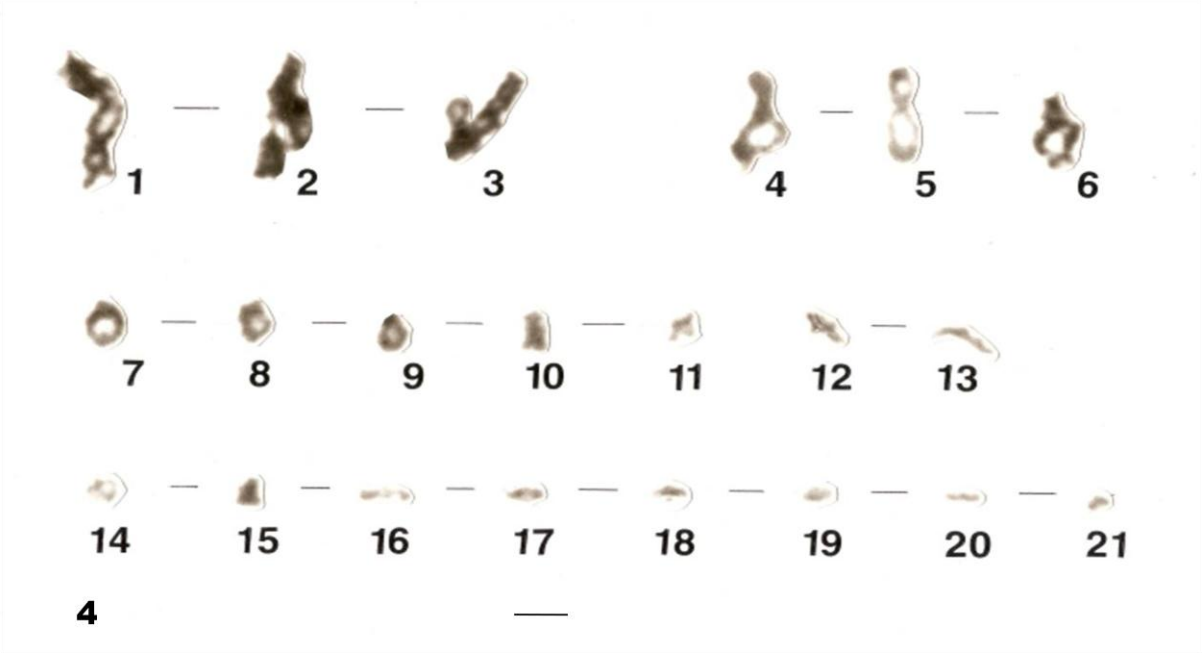
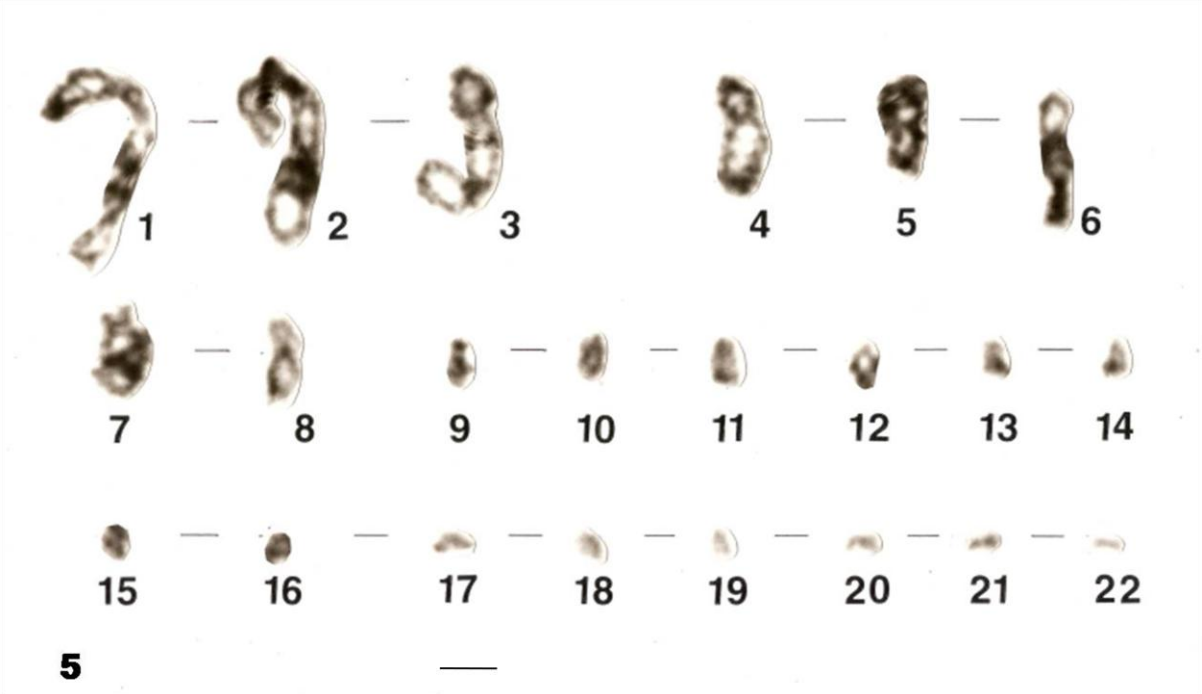


Figure 5 Giemsa stained male diplotene karyotype (cytotype II) of italicize I. cf. tricolor



Discussion

Five species of the genus *Ichthyophis* viz., *Ichthyophis bananicus* (Wen and Pang, 1990), *I. beddomei* (Venkatachalaiah and Venu, 2002), *I. glutinosus* (Seshachar, 1936, 1937a, b; Wake and Case, 1975, Nussbaum and Treisman, 1981), *I. kohtaoensis* (Nussbaum and Treisman, 1981), *I. kodaguensis* (Venu, 2008, 2013), *I. orthoplicatus* (Seto and Nussbaum, 1976) have been karyotyped using conventional chromosome staining methodology.

The karyotype of *I. tricolor* (standard) in the present study was found very similar to that of the karyotype described earlier for *I. beddomei* (Venkatachalaiah and Venu, 2002), except for the change in the morphology of chromosome #3 and #11. Thus, the subtelocentric chromosome pair 3 and submetacentric chromosome pair #11 of *tricolor* could have been derived from *beddomei* karyotype with the involvement of chromosome rearrangements in respect of chromosome #3 and #4 respectively. Apart from this change, rest of the chromosomes in the karyotype remains homologous between the two species. An extent of intrusion of a pericentric inversion is necessary in realizing of subtelocentric pair #3 that could be obtained from acrocentric pair #3. An intrusion of a pericentric inversion and transposition of such a segment realizing in reduction in chromosome arm length of chromosome #4 and in the acquisition of chromosome pair #11 to submetacentric would satisfy derivation processes for variant morphology.

This study also presents that *I. tricolor* is evolving with variable karyotypes (or cytotypes) provided that the pronounced differences observed both in chromosome number (2n=42 to 44) and chromosomal arms (FN=62 to 64). Based on variations observed in relation to changes in chromosome arms and centromere positions, two different interpopulational karyotypic formulae could be obtained. The present cytological data point towards an assertion that this species might have shown to represent interspecific chromosomal polymorphism.

Karyotypic differences observed among these cytotypes implicitly pertain to chromosomes bearing m-sm and st-types, could be envisaged as indicating the occurrence of polymorphism within *I. tricolor*.

The cytogenetic data obtained for *I. tricolor*, of Agali population is representing a new basal number of 2n=44 chromosomes for the genus *Ichthyophis*. Since, this karyotype is bestowed with a modified chromosomal set involving more number of chromosomal rearrangements, thus defining derivative features from that of either Pathekar or the basal karyotype. Interestingly, biogeographical position of these ichthyophid taxa surveyed that took their origin makes an interesting scenario, since these taxa were derived from either side of the 'Palghat Gap' region that separates Western Ghats region into two zones, *viz.*, northern and southern zones. Since these three populations of the present study falls within this range and hence, make an interesting phenomenon. Agali, Attapadi Hills region is significantly distant from those two other populations (*viz.*, Thiruvananthapuram and Pathekar) taking origin South of 'Palghat Gap' region.

In the current study karyological differences in diploid number and chromosome arms (2n=42-44; FN=62-64) reveal three distinct sympatric syntopic cytotypes (I and II) for the specimen collected from south western India for the cytological assessment. Results point out that centric fusion and other structural chromosomal rearrangements could have driven towards the diversification attained of the different cytotypes.

A direct comparison of karyotypes of other ichthyophiid species karyotypes makes it clear that the occurrence of a succession of chromosomal rearrangements, involving mainly of pericentric inversions, Robertsonian translocations and fission/fusions was found necessary in order to acquire altered karyotype.

Both the populations (Pathekar and Agali) could have been derived from the same stock or in succession from the basal stock, *i.e.*, standard space karyotype. This situation warrants upon similar adaptations acquired by different segments of a single stock, evolving in response to similar but different environmental conditions.

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