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First Report of B Chromosomes in Caecilians (Amphibia: Gymnophiona)

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Gymnophion amphibians (caecilians) are less well studied than the other two orders of extant amphibians: Anura and Caudata. We describe the first recorded presence of supernumerary (B) chromosomes in the order Gymnophiona, where a lone intensely stained B chromosome was observed in the somatic metaphase and meiotic complements of the Indian ichthyophiid, *Uraeotyphlus gansi*. Given the rarity of B chromosomes in caecilians and their sporadic presence within *U. gansi*, it is likely that these represent aneuploidy and are a recent acquisition derived from centromeric drive.

B chromosomes are one of the most widespread numerical polymorphisms, or a type of aneuploidy, in the karyotypes of many eukaryotic organisms (Green, 1988; Camacho, 2005; Jones, 2017). B chromosomes occur in approximately 15% of all extant eukaryotes, fungi, animals, and plants that are karyologically known (Melo et al., 2020). B chromosomes do not pair meiotically with A chromosomes but are instead inherited in a non-Mendelian mode and typically composed of constitutive heterochromatin, consisting of highly repetitive DNA sequences characteristic of satellite DNA, ribosomal DNA, and transposable elements (Douglas and Birchler, 2017). B chromosomes are inferred to originate either from intragenomic fragmentations as segmental duplications that acquire the characteristics of a B chromosome or through interspecific hybridization processes with the acquisition of foreign DNA from a closely related species that eventually evolves into a supernumerary chromosome (Camacho et al., 2000; Dhar et al., 2002; Camacho, 2005; Houben et al., 2013).

Over time, B chromosomes diverge quantitatively through sequential evolution and qualitatively through rearrangements. In the absence of selective pressure and reduced recombinational progression, B chromosomes tend to acquire and accommodate mutations. Thus, for the most part, B chromosomes are composed of amplified DNA from either single or several genomic regions. It is now apparent that B chromosomes are transmitted at a higher than expected frequency, increasing in number from one generation to the next, due to acquisition capacity or ‘drive’ mechanisms (Jones, 1995; Houben et al., 2014).

The advent of next generation sequencing (NGS) technology and associated bioinformatics will potentially improve our knowledge of B chromosome architecture (Ahmad and Martins, 2019); for example, the analysis of flow-sorted or micro-dissected B chromosomes has shown they contain substantial amounts of A chromosome-derived DNA sequences including organelle DNA sequences (Leach et al., 2005; Banaei-Moghaddam et al., 2015; Ruban et al., 2017).

These sequential acquisitions of particular DNA sequences by B chromosomes eventually create a multi-chromosomal mosaicism (Palestis et al., 2004a; Borisov, 2014; Houben, 2017; Valente et al., 2017).

Caecilians are elongate, limbless, superficially worm-like organisms mostly inhabiting moist soils in tropical and subtropical regions. The biology of caecilians is less well studied than frogs and salamanders because of their fossorial nature, restricted faunal distribution, difficulty in collection, and difficulty in captive breeding (Taylor, 1968; Gower and Wilkinson, 2005; Wilkinson, 2012). As for other aspects of caecilian biology, there is a paucity of information concerning their cytogenetics (Wen and Pang, 1990; Matsui et al., 2006; Venkatachalaiah et al., 2006; Venu et al., 2011, 2012; Venu and Venkatachalaiah, 2012, 2013; Venu, 2013, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f; Patawang et al., 2016). Cytogenetic investigation of caecilians based on conventional and differential staining protocols revealed their diploid (2n) numbers range between 20–44 (Barrio and Rinaldi De Chieri, 1970; Barrio et al., 1971; Wake and Case, 1975; Wake et al., 1980; Nussbaum and Ducey, 1988; Venu, 2008).

The genus *Uraeotyphlus* with seven nominate species is endemic to southern Western Ghats, India (Wilkinson and Nussbaum, 1996; Gower et al., 2008). It is partitioned into two species groups, viz., the *oxyurus* group (*U. interruptus*, *U. menoni*, *U. narayani*, and *U. oxyurus*) and the *malabaricus* group (*U. gansi*, *U. malabaricus*, and *U. oommeni*; Gower and Wilkinson, 2007). The chromosome numbers for the genus *Uraeotyphlus* range from 36–42 with all the *oxyurus* group species sharing the same diploid number of 36 chromosomes (Seshachar, 1939; Elayidom et al., 1963; Venu and Venkatachalaiah, 2013) and *U. gansi*, the lone cytogenetic representative for the *malabaricus* group with 2n = 42 chromosomes (Venu et al., 2011). The present paper describes the novel finding of B chromosomes in the Gymnophiona based on conventional and differential staining techniques.

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Fig. 1. Mitotic male (BUB1202) metaphase complement obtained from intestinal epithelial cells depicting a diploid number ($2n$) of 42 chromosomes along with a B (arrow) chromosome. Scale bar: 10 μm .

MATERIALS AND METHODS

Source of material.—Eight adult individuals (3 females and 5 males) of *Uraeotyphlus gansi* were collected from the type locality at Nalamukku tea estate, Tirunelveli District, Tamil Nadu, India, from July to December, 2007 to 2010. The specimens were intraperitoneally injected with 2 mg/ml colchicine solution (0.1 ml/g body mass), euthanized using MS-222 24 hrs after injection, and then dissected. The specimens were deposited in the museum of Department of Zoology, Bangalore University, Bengaluru, Karnataka, India, bearing the vouchers BUB1202, 1206, 1208, 1209, 1211, 1212, 1226, and 1227. All experiments were carried out according to the guidelines of the ethical committee of the Department of Zoology, Bangalore University, Karnataka, India.

Chromosome preparation.—Somatic metaphase chromosomes from the intestinal epithelia and liver, and male

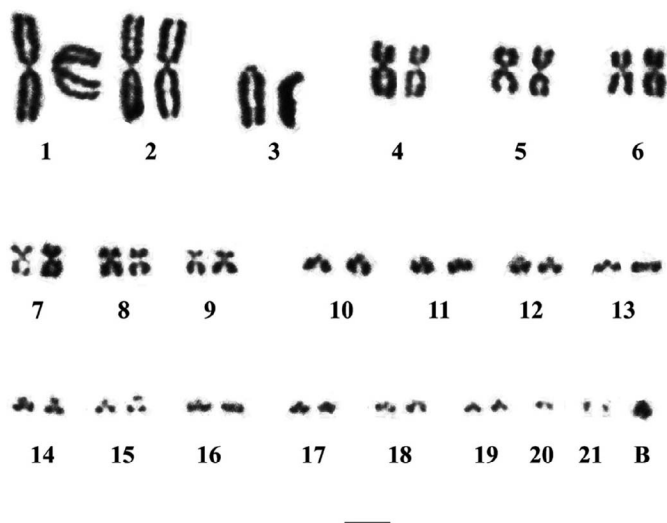


Fig. 2. Somatic male (BUB1202) metaphase karyotype with the karyotypic formula $2n = 42+1B$ and $FN = 62$. The lone B chromosome is the darkest in the karyotype and is placed at the end of the karyotype. Scale bar: 10 μm .

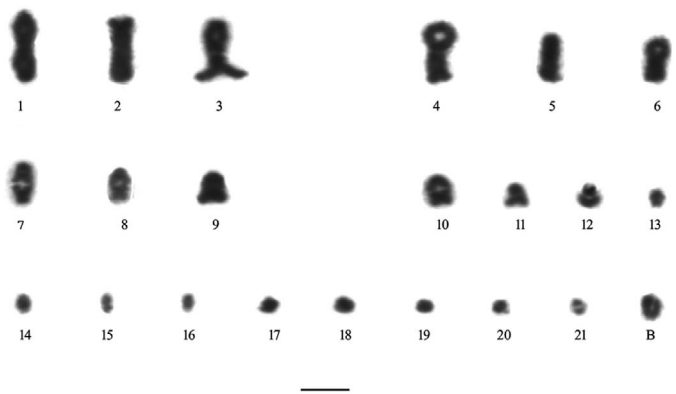


Fig. 3. Male (BUB1202) meiotic diplotene karyotype with 21 bivalents and a B chromosome. Scale bar: 10 μm .

meiotic stage chromosomes from the testes of euthanized specimens prior to fixation in formalin were obtained using modified methods from Venkatachalaiah and Venu (2002), Venu (2008), and Venu et al. (2011). Conventional staining with the help of a diluted Giemsa solution (5%) was used for chromosomal preparations (Venu and Venkatachalaiah, 2005, 2006; Venu et al., 2011). C-banding was accomplished using a slightly modified version of Sumner's (1972) BSG technique, in which air-dried chromosome preparations were hydrolyzed in 0.2 N HCl for a few seconds at room temperature, treated with 10% $\text{Ba}(\text{OH})_2$ for 10 min, re-natured in 2X SSC for 1 h at 60°C, and stained with 10% Giemsa solution for 25 min (Venu, 2013, 2014d).

Microphotography and karyotype construction.—Chromosome preparations were observed using a Zeiss Axioskop 2 plus microscope, and well-spread complements were photographed. Karyotypes were prepared in accordance with Levan et al. (1964), Venkatachalaiah and Venu (2002), and Venu et al. (2011, 2012).

RESULTS

Karyotypic details.—The karyotypes of *U. gansi* constructed from approximately 60 well-spread complements obtained from the females (BUB1206, 1211) and males (BUB1208, 1209, 1212, 1226) had a diploid ($2n$) number of 42 and a fundamental number (FN) of 58. The 21 pairs of homologous chromosomes in the somatic metaphase sets were arranged into groups (A–D) in a karyotype (Venu et al., 2011). No morphologically identifiable sex chromosomes were observed in either sex in the metaphase karyotypes.

Meiotic chromosomes prepared from testes revealed pachytene, diplotene, diakinesis, and second meiotic metaphase stages. The diplotene complements comprised 21 individually identifiable bivalents, with the number of chiasmata per bivalent ranging from 5–6 in the largest, and 2–3 in the medium-small bivalents, and a single chiasma in the smallest acrocentrics. These observations of mitotic and meiotic chromosome karyotypes are in agreement with previously published data for *U. gansi* (Venu et al., 2011).

Mitotic and meiotic chromosomes prepared from specimens bearing voucher number BUB1202 (Figs. 1, 2, 3), and mitotic chromosomes from BUB1227 (Figs. 4, 5), also had karyotypic details matching those described above. In addition, 4–5 complements obtained from mitotic and



Fig. 4. Female (BUB1227) somatic metaphase chromosomal complement prepared from liver cells with $2n = 42$ and a B chromosome (indicated by arrow). Scale bar: 10 μm .

meiotic tissues had the karyotype formula $2n = 42+1B$ and $FN = 62$. Even in the conventionally prepared chromosomes, the additional B chromosomes were darkly stained when compared with A chromosomes. The acrocentric B chromosome was intermediate in size between that of chromosome pairs 11 and 12.

C-staining profile.—The large and medium sized chromosomes in groups A and B failed to exhibit heterochromatin in any region along their lengths. Chromosome pairs 7–9 (group C) and 10–21 (group D) possessed large blocks of heterochromatin localized in their centromeric positions (Venu et al., 2011). The lone B chromosome was the darkest

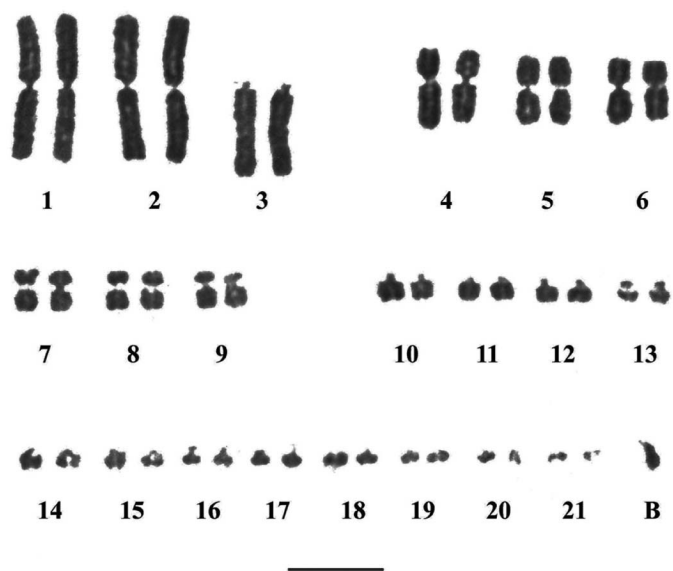


Fig. 5. Female (BUB1227) mitotic karyotype constructed by pairing the homologous chromosomes together and arranged in their decreasing order. The lone supernumerary chromosome (designated as B) is placed at the end of the karyotype. Scale bar: 10 μm .

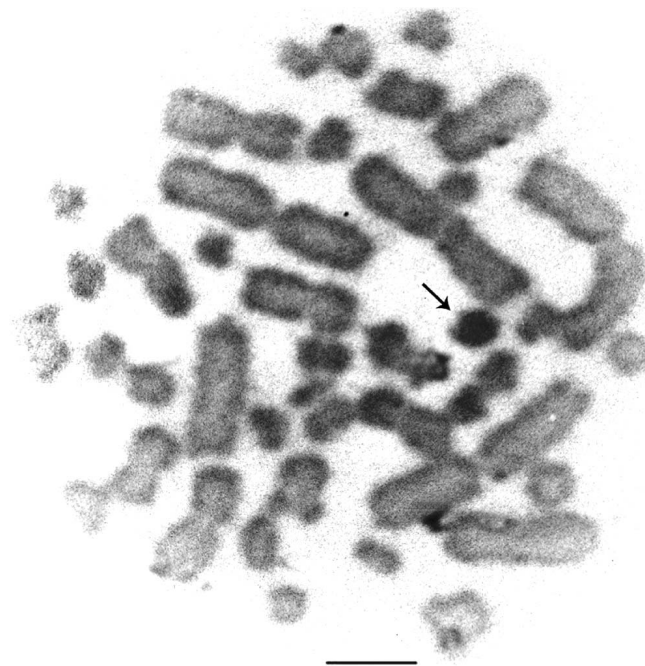


Fig. 6. C-banded somatic metaphase complement. The darkest chromosome (arrowhead) in the complement corresponds to the B chromosome. Scale bar: 10 μm .

body in the complement, and the whole chromosome was heteropycnotic in nature (Fig. 6).

DISCUSSION

Amphibian chromosomes are unique among animals because they possess inflated amounts of repetitive DNA, and they are composed of an accumulation of noncoding DNA along with tandem duplications of their short mobile genetic elements (Schmid et al., 2012). At this level of structure, the karyotypes of the anurans *Leiopelma hochstetteri* (Sharbel et al., 1998) and *Gastrotheca espeletia* (Schmid et al., 2002) and the salamander *Dicamptodon tenebrosus* (Brinkman et al., 2000) strongly suggest the acquisition of variable repetitive DNA sequences in respect to their size, shape, and number of B chromosomes. Legless amphibians commonly known as caecilians have moderately large genome sizes that fall between frogs and salamanders (Gregory, 2015).

Most amphibian B chromosomes are represented as minor elements in the karyotype, but there are instances of anuran (Rosa et al., 2003; Green, 2004; Medeiros et al., 2006; Milani et al., 2010; Ferro et al., 2012, 2016; Mezzasalma et al., 2015) and salamander (Sessions and Kezer, 1991; Brinkman et al., 2000; Green, 2004; Jones, 2017) B chromosomes differing in their number, morphology, and structural characteristics. Previous studies (Palestis et al., 2004b; Houben et al., 2013; Tosta et al., 2014; Douglas and Birchler, 2017) have inferred from B chromosome origins and evolutionary trajectories that B chromosomes are found primarily in taxa with elevated levels of chromosome reshuffling and in phylogenetic groups with unstable chromosome numbers. Several authors have suggested that species with mainly acrocentrics in karyotypes would be more prone to possess B chromosomes than those comprised mainly of biarmed chromosomes because female meiosis seems to favor centromeric tendencies over fewer acrocentrics (Palestis et al., 2004a,

2004b; Houben et al., 2014). Our finding based on limited cytogenetic data does not enable inferences of the dynamics or relationships of caecilian B chromosomes within the nucleus and/or among populations.

Our current report of the occurrence and prevalence of B chromosomes in *U. gansi* is the first of its kind for Gymnophiona. Most caecilian karyotypes have been documented on the basis of relatively small sample sizes, and fewer caecilian karyotypes have been documented than for anurans and salamanders. Nonetheless, B chromosomes seem to be less common in caecilians, as we have not previously observed them in chromosomal preparations from hundreds of specimens of Indian caecilians (Venkatachalaiah and Venu, 2002; Venu and Venkatachalaiah, 2005, 2006, 2013; Venkatachalaiah et al., 2006; Venu et al., 2011, 2012; Venu, 2013, 2014b, 2014c, 2014d, 2014e, 2014f), and in *U. gansi* they were only sporadically present. Thus, B chromosomes in *U. gansi* might be taxonomically highly restricted and intraspecifically variable, and thus possibly represent a recent acquisition based on centromeric drive and hence, in an aneuploidy condition. This can be tested with additional karyological data for additional individuals, including from other species, and including observations of heterochromatinization accrued in the B chromosomes.

DATA ACCESSIBILITY

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