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The Karyology of *Uraeotyphlus gansi*, and Its Implications for the Systematics and Evolution of Uraeotyphlidae (Amphibia: Gymnophiona)

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Caecilians · Chromosomes · India · Karyotype · Systematics · Western Ghats

Abstract
The gross karyotype of the uraeotyphlid caecilian *Uraeotyphlus gansi* is described as comprising 2n = 42 and fundamental number = 58. These are the first karyotype data for any species of *malabaricus*-group *Uraeotyphlus*, and the diploid number is the same as those ichthyophiids thus far studied and differs from the *oxyurus*-group *Uraeotyphlus* (2n = 36). These data support the recognition of two species groups within *Uraeotyphlus*, the monophyly of the *oxyurus* group, and the understanding that the ancestral diatriatan was more ichthyophiid- than uraeotyphlid-like.

The caecilian (Gymnophiona) family Uraeotyphlidae Nussbaum is endemic to the Western Ghats region of peninsular India and contains a single genus, *Uraeotyphlus* Peters. The seven nominate species have been partitioned into two species groups by Gower and Wilkinson [2007; see also Gower et al., 2008]. The *oxyurus* group contains four species *(interruptus, menoni, narayani, oxyurus)* characterized by a derived pattern of annulation in which there is a 1:1 correspondence between primary annuli (and myomeres) and vertebrae. The three species *(gansi, malabaricus, oommeni)* of the *malabaricus* group are characterized by the apparent retention of an ancestral pattern of annulation also seen in the closely related Ichthyophiidae, in which there is no clear differentiation of primary and higher order annuli, and no consistent correspondence between annuli and vertebrae.

Beyond patterns of annulation, the only other notable, documented difference between the two groups of *Uraeotyphlus* is in morphology of the phallodeum [Gower et al., 2008], but comparisons for this and other features are taxonomically incomplete and have been limited by very small sample sizes. Thus, it remains unclear how robust the partition of *Uraeotyphlus* is, whether the two species groups are monophyletic, and whether the *malabaricus* group retains any other ancestral (and ichthyophiid-like) features beyond the annulation pattern that has been lost in the *oxyurus* group. Taxon sampling for molecular phylogenetics has been sparse, but results [Gower et al., 2002] are consistent with the monophyly of *Uraeotyphlus* and of the *oxyurus* group (only a single sample has been included from the *malabaricus* group). Additionally, molecular phylogenies show that the closest relative of *Uraeotyphlus* is the long-tailed, unstriped Indian *Ichthyophis* (*I. bombayensis*) [see Gower et al., 2007], which renders *Ichthyophis* and Ichthyophiidae paraphyletic with respect to *Uraeotyphlus*/Uraeotyphlidae [Gower et al., 2002; Frost et al., 2006]. Here we report the first karyo-

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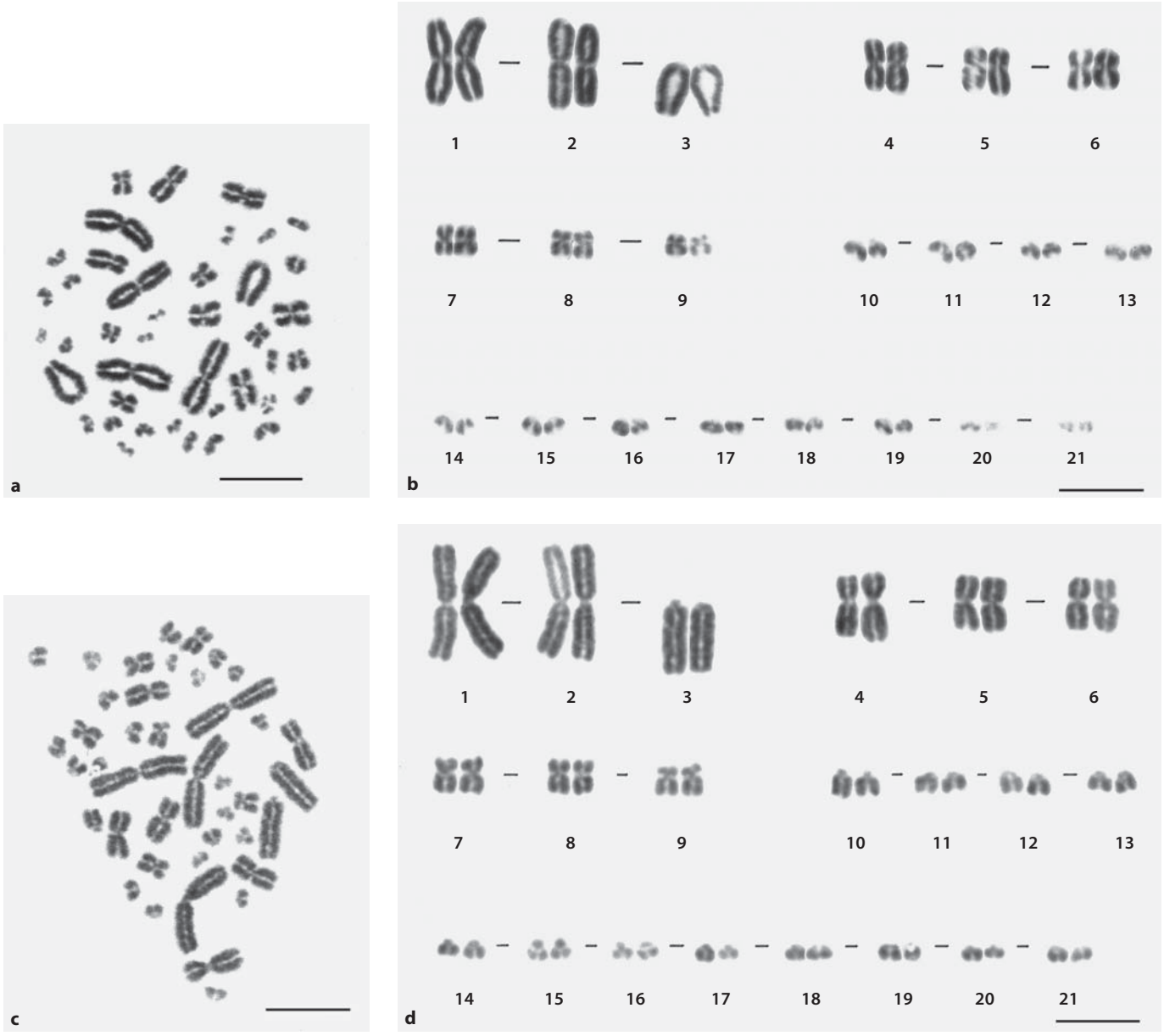

Fig. 1. Giemsa-stained mitotic metaphase karyotype of female (**a**, **b**) and male (**c**, **d**) *Uraeotyphlus gansi* arranged in groups based on size and form. Scale bars 10 μm.

logical data for the *malabaricus* group, and consider their implications for the evolutionary systematics and classification of *Uraeotyphlus*. Some recent studies have not recognized Uraeotyphlidae and instead classified *Uraeotyphlus* within Ichthyophiidae [e.g., Frost et al., 2006], but we follow the classification of Wilkinson and Nussbaum [2006; see also Gower and Wilkinson, 2007; Gower et al., 2008].

Material and Methods

Five adult *Uraeotyphlus gansi* (four females, one male) were collected from the type locality at Nalamukku tea estate, Tirunelveli District, Tamil Nadu, in 2007. Metaphase chromosome and male meiotic stage preparations were obtained from intestinal epithelia and testis using modified versions of the methods described by Venkatachalaiah and Venu [2002] and Venu and Venkatachalaiah [2005, 2006]. A colchicine solution (2 mg/ml) was injected intraperitonally (0.1 ml/g body mass) for 24 to 48 h before the animals

Table 1. Dimensions (μm) and proportions of metaphase chromosomes of a male *Uraeotyphlus gansi*

Chromosome pair No.	Type	Length of short arm (p)	Length of long arm (q)	Total length (I)	Arm ratio (q/p)	Centromere index (p × 100/I)	Relative length (I × 100/L)
1	m	19.82	19.83	39.65	1.00	49.99	15.90
2	m	18.69	18.75	37.44	1.00	49.92	15.01
3	ac	1.84	22.94	24.78	12.47	7.43	9.94
4	m	8.36	12.37	20.73	1.48	40.33	8.31
5	m	7.89	8.90	16.79	1.13	46.99	6.73
6	m	7.10	9.40	16.50	1.32	43.03	6.62
7	m	5.39	5.60	10.99	1.04	49.04	4.41
8	m	3.97	4.05	8.02	1.02	49.50	3.22
9	st	2.60	5.20	7.80	2.00	33.33	3.13
10	ac	1.32	5.25	6.57	3.98	20.09	2.63
11	ac	1.06	4.85	5.91	4.58	17.94	2.37
12	ac	1.00	4.90	5.90	4.90	16.95	2.37
13	ac	0.96	4.87	5.83	5.07	16.47	2.34
14	ac	0.92	4.82	5.74	5.24	16.03	2.30
15	ac	0.89	4.76	5.65	5.35	15.75	2.27
16	ac	0.83	4.72	5.55	5.69	14.95	2.23
17	ac	0.79	4.68	5.47	5.92	14.44	2.19
18	ac	0.62	4.60	5.22	7.42	11.88	2.09
19	ac	0.58	4.52	5.10	7.79	11.37	2.04
20	ac	0.50	4.47	4.97	8.94	10.06	1.99
21	ac	0.39	4.40	4.79	11.28	8.14	1.92

Total length of chromosomes in the complement = 249.4. m = Metacentric; ac = acrocentric; st = subtelocentric.

were euthanized by anesthesia using MS222 (Sandoz). The gut and testes were macerated and kept in an appropriate hypotonic solution for 40 min before fixation in 3:1 methanol:glacial acetic acid. Metaphase and meiotic chromosomal spreads were prepared by air drying and conventionally stained with a 4% Giemsa solution (pH 7.0) for 20 min. 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 7% $Ba(OH)_2$ for 10 min, renatured in 2× SSC for 1 h at 60°C, and stained with 8% Giemsa solution for 40 min. $AgNO_3$ banding was performed upon conventionally prepared chromosomes according to the 1-step method of Goodpasture and Bloom [1975] with treatment in 50% $AgNO_3$ solution for 1 h at 50°C. Karyotype analyses were performed on 40 well spread metaphase cells and late meiotic stage cells. Voucher specimens are stored in the collections of the Bombay Natural History Society, Mumbai, India. Chromosome morphology follows the classification of Green and Sessions [1991].

Results

The mitotic karyotype of both sexes has a diploid (2n) number of 42. The 21 pairs of homologous chromosomes in the somatic metaphase sets (fig. 1; table 1) can be arranged into four arbitrary groups based on their size, length and position of centromere [Levan et al., 1964; Venkatachalaiah and Venu, 2002; Venu and Venkatachalaiah, 2005, 2006]. Group A comprises three pairs of larger chromosomes, with two subequal metacentric pairs (1–2) and by far the largest acrocentric pair (3) in the karyotype. Group B contains three pairs (4–6) of medium-sized metacentrics, with pair 4 notably larger. Group C consists of two (7–8) smaller pairs of metacentric and one pair (9) of subtelocentric chromosomes. The final 12 pairs (10–21), all small acrocentrics, form Group D which can be subdivided into two groups based on whether they are major acrocentrics with a prominent short arm (pairs 10–13) or lack a distinct short arm (pairs 14–21). There are 12 minichromosomes (i.e. microchromosomes of e.g. Nussbaum [1991]) and the fundamental number (FN) is 58. No morphologically identifiable heteromorphic chromosomes were observed in the two sexes (fig. 1).

The spermatogonian meiotic preparations revealed pachytene, diplotene (fig. 2) and second meiotic metaphase complements. The diplotene complement comprised 21 individually identifiable bivalents, with the

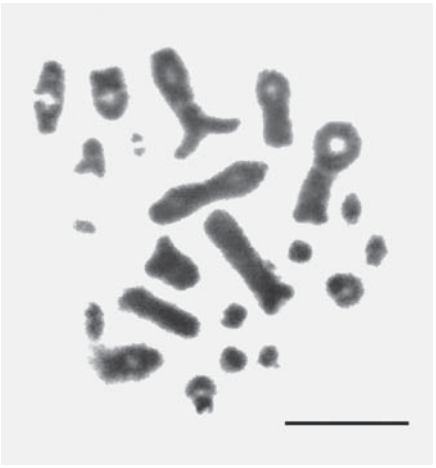

Fig. 2. Diplotene karyotype complement of *Uraeotyphlus gansi*. Scale bar 10 μm.

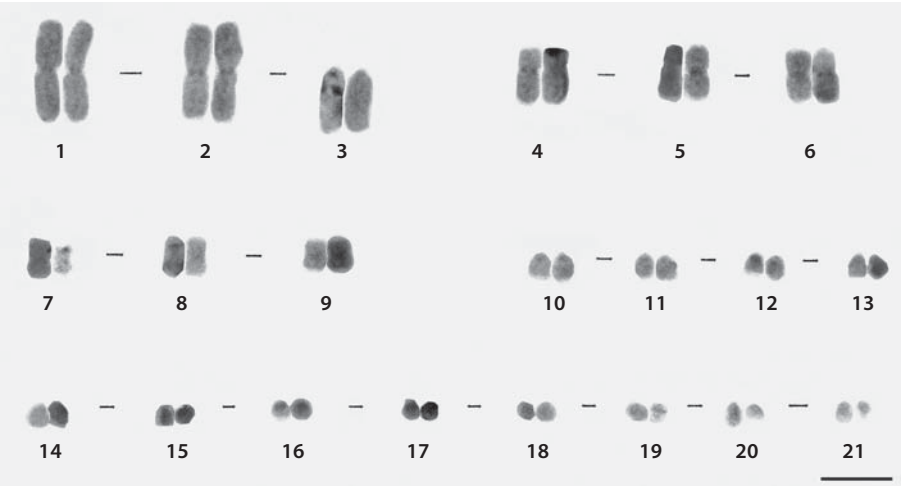

Fig. 3. C-stained somatic metaphase karyotype of *Uraeotyphlus gansi*. Scale bar 10 μm.

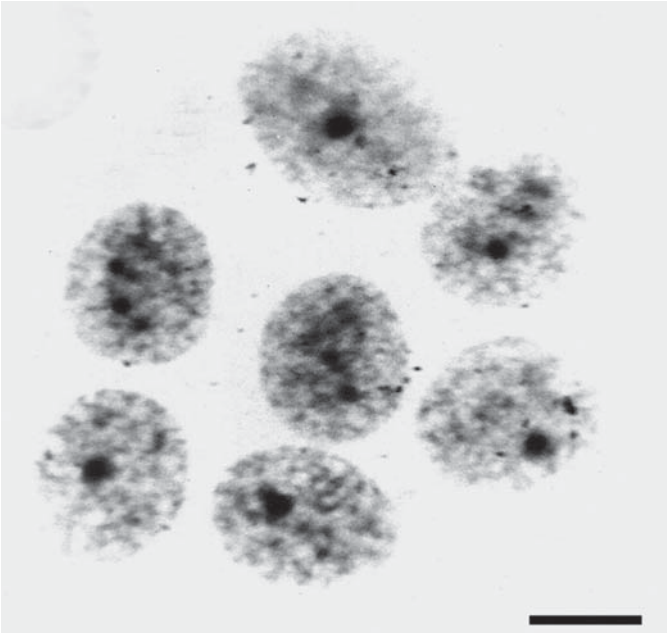

Fig. 4. Silver-stained interphase nuclei of *Uraeotyphlus gansi*. Scale bar 10 μm.

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. Chromosome pairs 7–9 and 10–21 (groups C, D) possessed large blocks of heterochromatin localized in their centromeric positions (fig. 3), a situation similar to that seen in the C-stained chromosomes of several *oxyurus*-group species of *Uraeotyphlus* [G. Venu and G. Venkatachalaiah, unpublished data]. The large and medium sized chromosomes of groups A and B failed to exhibit heterochromatin in any region along their lengths. With silver staining, interphase nuclei with one to two secondary constriction spots were observed (fig. 4).

Discussion

Only two previous studies have reported karyological features of *Uraeotyphlus*, both of species from the *oxyurus* group [Seshachar, 1939; Elayidom, 1963]. These studies found 2n = 36, with the three largest pairs being metacentric and about 7–10 pairs of minichromosomes. The precise specific identification of *Uraeotyphlus* can be problematic, but the karyotype of several species of what are clearly *oxyurus*-group *Uraeotyphlus* have recently been examined and found to have 2n = 36 and FN = 60 [Venu, 2008].

The karyotypes of rhinatrematids, the sister group of all other caecilians [e.g. Wilkinson and Nussbaum, 2006], are not known. All ichthyophiids *(Ichthyophis* and *Caudacaecilia)* examined to date have a diploid number of 42 and FN of 58–64 [Nussbaum, 1991; Venkatachalaiah and Venu, 2002; Matsui et al., 2006]. The only exception to this diploid number reported in the literature (2n = 36; FN = 60) [Venkatachalaiah and Venu, 2002] was in fact observed in material of an *oxyurus*-group species of

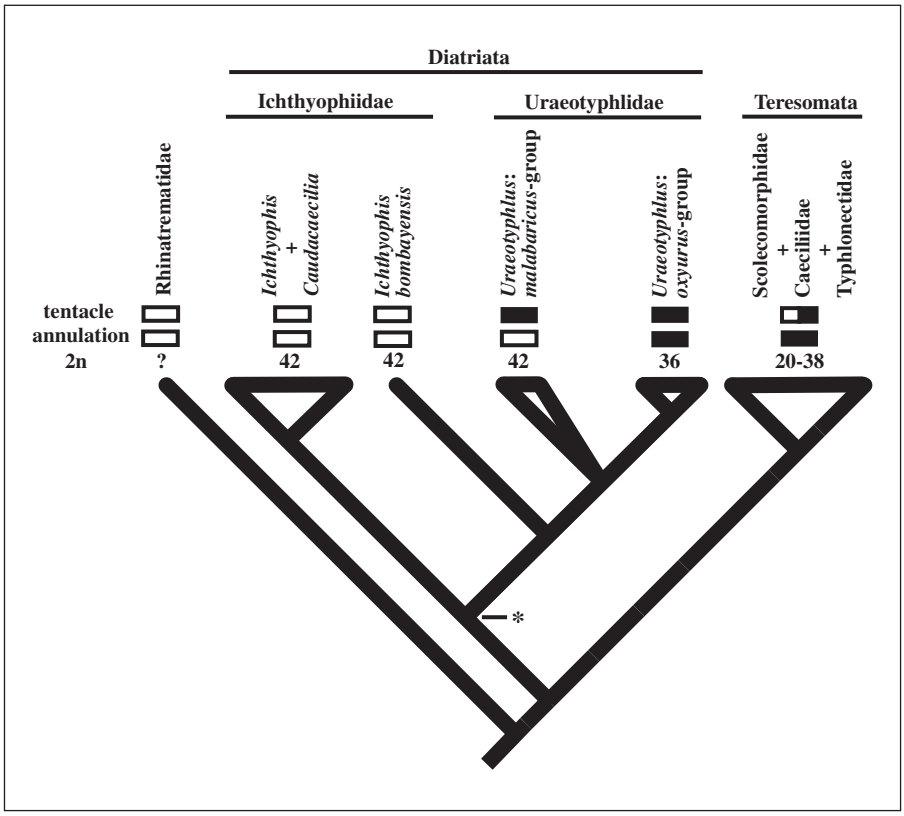

Fig. 5. Taxonomic distribution of states of three characters mapped onto a caecilian phylogeny. The three characters are position of tentacle (white = between eye and (behind) nostril; black = below nostril); pattern of annulation (white = no consistent correlation between annuli and underlying myomeres/vertebrae; black = primary annuli corresponding with myomeres/vertebrae); diploid chromosome complement. * = ancestral diatriatan. Phylogeny based on morphological [Wilkinson and Nussbaum, 1996] and molecular [Gower et al., 2002; Wilkinson et al., 2002; Frost et al., 2006; Roelants et al., 2007] analyses. It is unknown whether *malabaricus*-group *Uraeotyphlus* are mono- or paraphyletic.

Uraeotyphlus (based on D.J.G.'s examination of voucher specimens) that had been misidentified as *I. malabarensis* (= *I. bombayensis*) [see Gower et al., 2007]. Lower 2n values (20–38) have been reported for all other, teresomatan (scolecomorphid, caeciliid and typhlonectid) caecilians examined thus far [Wake and Case, 1975; Wake et al., 1980; Nussbaum, 1991; Venkatachalaiah and Venu, 2002; Venu and Venkatachalaiah, 2005, 2006; Venkatachalaiah et al., 2006]. One further difference between *oxyurus*-group *Uraeotyphlus* on the one hand and ichthyophiids plus *malabaricus*-group *Uraeotyphlus* (or at least *U. gansi*) on the other is that in the former, C-positive bands are visible only sparingly and at the centromeric regions of acrocentrics [Venu, 2008] but in the latter they are visible at the centromeric regions of all chromosomes in the complement. Although the karyotype of *U. gansi* is thus most similar to those of ichthyophiids, one notable difference is that the third largest pair (A3 here) is acrocentric in *U. gansi* but meta- or subtelocentric in ichthyophiids examined to date.

Mapping basic karyotype features onto a phylogeny (fig. 5) indicates that *U. gansi* has likely retained the ancestral diatriatan 2n complement and that in this feature, as with annulation, the *malabaricus*-group species of *Uraeotyphlus* resemble *Ichthyophis* more than the derived *oxyurus*-group *Uraeotyphlus*. This adds further support to Gower and Wilkinson's [2007] partition of *Uraeotyphlus*, and their conclusion that the ancestral diatriatan would likely have resembled extant ichthyophiids more than extant *Uraeotyphlus*. Monophyly of the *malabaricus*-group species of *Uraeotyphlus* is suspected, but has yet to be adequately tested because most of the characters in which they are known to differ from the *oxyurus* group (axial musculature, phallodeum, DNA sequences, karyotype) are plesiomorphic or have yet to be examined in more than one of the three known species. Further understanding of phylogeny and of the karyotypes of other *malabaricus*-group species is required to gain a better understanding of chromosomal evolution in Diatriata. Some attempts have been made to infer phylogeny and/or more detailed aspects of karyotypes than chromosomal numbers and gross features [e.g. Venu and Venkatachalaiah, 2006], but in the absence of more precise banding data, denser taxon sampling, and a more complete phylogenetic framework we refrain from that here.

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