



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL  
OF ADVANCED RESEARCH

## RESEARCH ARTICLE

## Karyological characteristics of two species of unstriped ichthyophid caecilians (Amphibia:Gymnophiona:Ichthyophiidae) of Western Ghats of India

\* Venu Govindappa

Centre for Applied Genetics, Department of Zoology, Bangalore University, Bangalore, Karnataka, India.

**Manuscript Info****Manuscript History:**

Received: 12 December 2013  
Final Accepted: 19 January 2014  
Published Online: February 2014

**Key words:**

*Ichthyophis malabarensis*,  
*Ichthyophis peninsularis*, Western  
Ghats, Karyotype,  
Chromosomal rearrangement.

**Abstract**

Karyotypic details of two species of unicoloured caecilians namely *Ichthyophis malabarensis* (2n=42-44, FN=60,66) and *Ichthyophis peninsularis* (2n=42, FN=58) collected from Western Ghats of India, is reported for the first time. Role of chromosomal rearrangements involved in karyotypic derivation is discussed. The present chromosomal data suggest that there exists more than one species of unstriped ichthyophid caecilian species in Western Ghats of India.

**\*Corresponding Author****Venu Govindappa**

Copy Right, IJAR, 2013., All rights reserved.

**Introduction**

The moist surface year-round along the South Western coastal region of peninsular India, especially, Western Ghats region have been serving as an ideal home for many caecilian (Gymnophiona) amphibians (Taylor, 1968; Pillai and Ravichandran, 1999; Gower and Wilkinson, 2007; Gower et al., 2008). Thus, taking opportunity of these salubrious biogeographical conditions, two of the ten families of caecilians have been thriving on these hillarious Western Ghats region (Wilkinson et al., 2011; Kamei et al., 2012). As is well-known that uraeotyphlids are solely endemic to this zone (Seshachar, 1939; Wilkinson and Nussbaum, 1996; Gower and Wilkinson, 2007; Gower et al., 2008), while, ichthyophiids could have spread over to South East Asian regions (Gower et al., 2002; San Mauro et al., 2004; Nishikawa et al., 2012a, b, c, 2013), whereas speciose typhlonectid caecilians profusely prevail both western and eastern peninsular India. Among the primitive caecilians belonging to the rankles class Diatriata, the family Ichthyophiidae is highly specious taxa having very wider ramifications possessed by larger expanse of whole of southern Asia (Frost, 2013). However, there are conflicting opinions in regard to ichthyophid biogeographical prominence over these vast region (Gower et al 2002; Zhang and Wake, 2009; Nishikawa et al., 2012c). Two main alternative theories have been put forward to explain their occurrence: 'out of India' and 'out of Laurasia', potentiating their spread upon accretion of Indian subcontinent onto Eurasia. The proponents of 'out of India' hypothesis insist on the expansion of 'Indian origin' ichthyophiids that have moved onto South East Asian regions, whereas the alternative hypothesis impressing upon their dispersal into peninsular India (Gower et al., 2002).

The taxa of Ichthyophiidae seem prevailing in two color morphs: striped (or yellow lateral bands) and non-striped (unicolored) form, even though, no valid phylogenetic basis has been made known. Earlier authors including Taylor (1960, 1961) who have contributed enormously upon caecilian biogeographical discovery were able to document several caecilian taxa including the present one. Wilkinson et al. (2002) while dislodging Taylor's (1960,1968) contention of designating individual species based on external morphological features during the course of their study pertaining to biosystematics and taxonomy of some South Indian uncolored ichthyophiids, instead they recommend upon imminent use of multiple data set along with more number of sampled taxa during such a consideration.

However, Taylor (1960) who had claimed to have given detailed description of four long-tailed and unstriped ichthyophiid species spanning Western Ghats region of South India (viz., *I. peninsularis*, *I. malabarensis*, *I. subterrestris* and *I. bombayensis*), including *I. monochorous* that confined on Eastern Ghats of peninsular India, further, extending far beyond of Borneo. Besides, peninsular India is also known to harbor striped taxa that include *I. beddomei*, *glutinosus*, *tricolor*, *kodaguensis*, *davidi* (Bhatta et al., 2011) and quite recently six more species from eastern India have been added into this list (Mathew and Sen, 2009; Kamei et al., 2009). For considerable numbers of species of striped ichthyophiid taxa, karyological information are available viz., *Ichthyophis bananicus* (Wen and Pang, 1990), *I. beddomei* (Venkatchalaiah 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, 2013) including a lone unicolored species of Sri Lankan origin viz., *I. orthoplicatus* (Seto and Nussbaum, 1976), excepting this, no other species has had any cytological information.

Gower et al. (2007) while pointing out that during taxonomic revisions pertaining to the description of any species group, they infer that it is essential to take cognizance of multiple approaches prior to initiating some discussions concerning inclusion of more number of valid species. In their recent taxonomic revision based on molecular data that had dramatically changed the aspirations towards long standing demands of systematics of ichthyophiid species and utilizing mitochondrial rRNA sequences (12S and 16S sequences) for fewer unstriped *Ichthyophis* species of South Indian origin, infer that while considering grouping of them into a single species although, earlier they were considered supposedly representing four valid species (Taylor, 1960). However, when comparison was made in contemplation with other representative caecilians including sufficient number of out-group taxa, the results have failed to meet their minimal expectation upon their respective and representative nodes indicating their species insufficiency in effect. The results however, might convey that they comprise a widely distributed but highly interconnected species group.

Any chromosomal studies upon these questionable taxa could help to improve the status of current taxonomic and evolutionary knowledge regarding this group. The objective of the present study, was to provide chromosomal composition of each of the designate species under this group collected from different localities of their occurrence within Western Ghats region of South India and further to understand much better of their cytotoxic relationships.

## Materials and Methods

Details of the specimens used for the present investigation are given under Table 1.

Upon anaesthetizing, the tissues viz., Liver, Gut epithelium and spleen were dissected out. Mitotic chromosomes were prepared from somatic tissues employing the modified techniques of Venkatchalaiah and Venu (2002), Venu (2008, 2013). The slides containing chromosomes were stained in 5% Giemsa solution for ten minutes. Mitotic karyotypes were prepared from well spread metaphase plates following the definitions of Levan et al. (1964).

## Results

### Karyotype of *Ichthyophis malabarensis*

The karyotype of *I. malabarensis* showed a diploid number of 42 and FN = 60. The somatic metaphase chromosome sets in the karyotype were arranged into 4 type-forms based on the major chromosomal identity. The first (A) group (1-3 pairs) includes 2 large metacentric chromosomes and a prominent acrocentric pair. Chromosome pair 1 has a achromatic gap towards distal end followed by a short segment attached to the main arm distinguishing itself as a rearranged chromosome. The second (B) group (4-6) is represented by medium sized metacentrics arranged in decreasing order of their size. The third (C) group includes 4 pairs of submetacentrics (7-10). The first set of group D consists of three (11-13) pairs of acrocentrics followed by the second set with eight (14-21) pairs of acrocentrics arranged in their decreasing order of size (Fig. A).

### Karyotype of *I. cf. malabarensis*

Although the variant karyotype of *I. cf. malabarensis* has more similarities to the karyotype of standard *I. malabarensis*, there are some structural variations as regard to other members in the complement. The karyotype of *I. cf. malabarensis* is consisted of  $2n = 44$  and expectedly of FN = 66. In a typical situation, group A includes two large metacentrics (pair 1 and 2) and a pair of prominent acrocentric chromosomes (no. 3). The second (B) group is represented by three pairs (4-6) of medium sized metacentrics in which fourth pair is comparatively longer than the other two pairs (no. 5 and 6) of chromosomes. The C-group includes five pairs (no. 7 – 11) of which the seventh pair is a very prominent satellited acrocentrics, followed by four pairs of submetacentrics (no. 8, 9, 10 and 11). The first set of group D consists of two pairs of medium sized acrocentrics (no. 12 and 13) followed by the second set represented by nine pairs of chromosomes i.e., eight pairs of small sized acrocentrics (no. 14-20 and 22) in their

decreasing order of their size and a single pair of submetacentrics (no. 21) that are comparatively longer than the latter pair of acrocentrics (Fig. B).

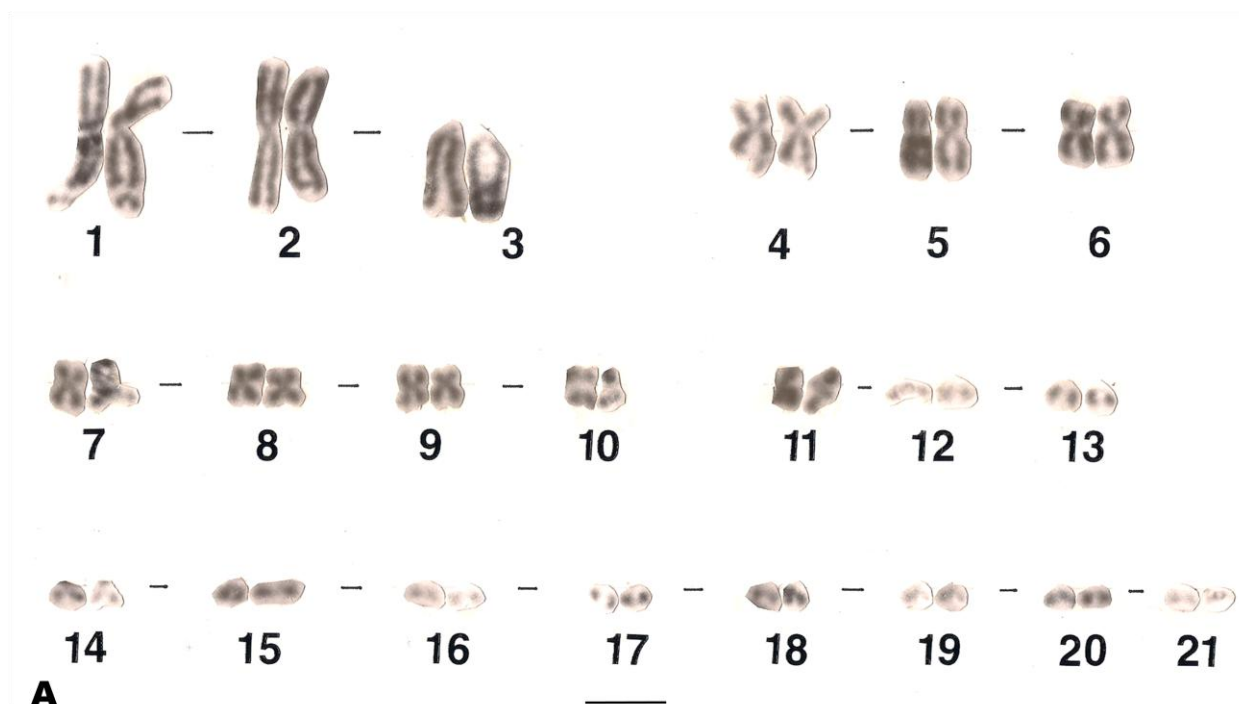
#### Karyotype of *I. peninsularis*

The karyotype of *I. peninsularis* showed a diploid number of 42 and FN = 58. The karyotype of *I. peninsularis* is more similar to the karyotype of *I. malabarensis* in all respects with three exceptions namely, a). The first and fifth chromosomal pairs of *I. peninsularis* are submetacentrics, b). group C of *I. peninsularis* includes three pairs (7-9) of medium sized submetacentrics and c). the first set of group D includes four pairs (10-13) of major acrocentrics (Fig. C).

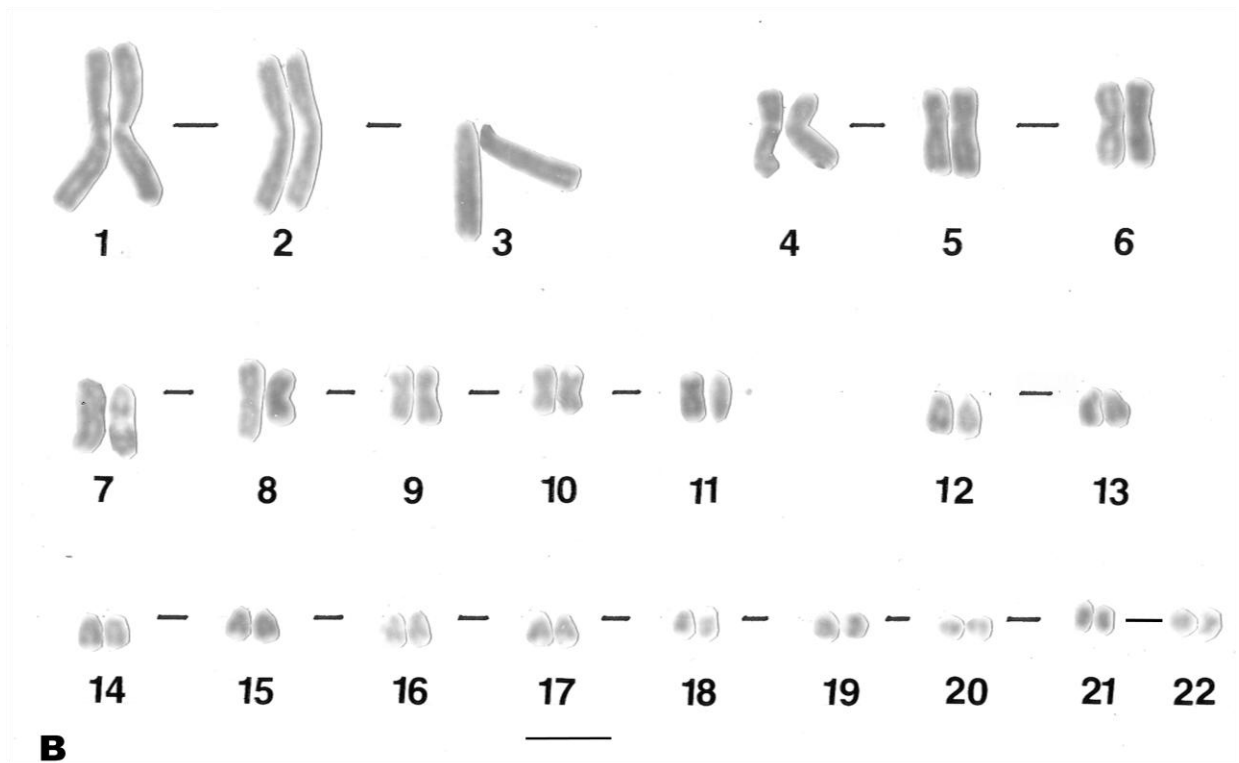
**TABLE 1: Details of collection of *Ichthyophis malabarensis*, *I. cf. malabarensis* and *I. peninsularis***

Species	Locality	Habitat	Voucher number	No. of animals used	Geographical coordinates
<i>Ichthyophis malabarensis</i>	Seeke village, Balehonnur, Chikkamagalur (Dt.), Karnataka, India	Mixed plantations of arecanut, coffee, pepper, banana, cardamom	BUB 1051	1 female	13.3500° N, 75.4600° E
<i>Ichthyophis cf. malabarensis</i>	Koodam village, Kannur (Dt.), Kerala, India	Backyard garden with banana, coffee plantations	BUB 1040	1 female	12.1076° N, 75.1944° E
<i>Ichthyophis peninsularis</i>	Vettilapara, Ernakulam (Dt.), Kerala, India	Inside a well in an agricultural farm	BUB 1054	1 female	10°17'55"N, 76°29'19"E

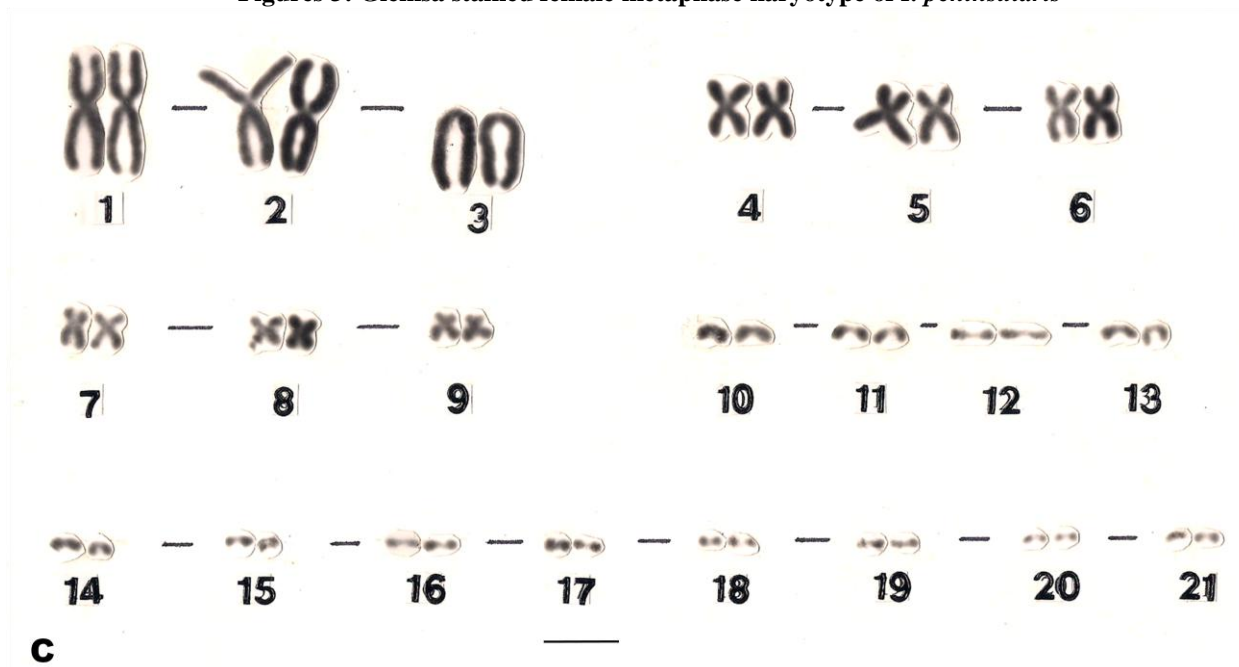
**Figures 1: Giemsa stained female metaphase karyotype of *I. malabarensis***



Figures 2: Giemsa stained female metaphase karyotype of *I. cf. malabarensis*



Figures 3: Giemsa stained female metaphase karyotype of *I. peninsularis*



## Discussion

The karyotype specificities of unstriped *I. peninsularis* ( $2n=42$ , FN=58) is similar to the karyotype described by Seto and Nussbaum (1976) for Sri Lankan unstriped taxon, *I. orthoplicatus* ( $2n=42$ , FN=60), but differ in respect of chromosome pair #3 and #10, in which a probable involvement of a pericentric inversion to acquire the derivative state of chromosome morphology in each species. But it seems more simple to procure the karyotype of *I. beddomei* ( $2n=42$ ; FN=58) from *I. peninsularis* by involving a role of pericentric inversion to procure acrocentric chromosome #3 from that of a sub-metacentric chromosome #3; apart from this change in the complement, all the other chromosomes are homologous to a greater extent.

In order to obtain *I. malabarensis* (standard) karyotype from *I. peninsularis*, it is essential to invoke individual pericentric inversion to effect the specificities of *malabarensis* type that were characterized by metacentric #1, submetacentric #4, and subtelocentric pair #10.

A derivative karyotype of *I. cf. malabarensis* could be obtained from the standard karyotype by inducing a pericentric inversion to procure metacentric pair #4, in which the distal segment to add on, from that of its predecessors submetacentric chromosome and subsequent progression of an inversion process and the distal end could have translocated on to the distal end of the long arm of metacentric chromosome #1, probably by means of a tandem fusion process. Besides, submetacentric chromosome #11 could have attained from the predecessors acrocentric chromosome.

Results of the present study suggest the probable role of centric fusions / or fissions, or tandem fusions and other structural chromosomal rearrangements could have incurred during the diversification of different chromosomal morphologies. On the other hand, for *I. malabarensis*, it was suggested of a completely distinct karyoevolutionary pathway. Despite the maintenance of constant diploid number ( $2n=42$ ), several banded chromosomes were detected in *I. cf. malabarensis* (FN=62-68) thereby diverging from the primitive fundamental number (FN=58/60) proposed for ichthyophiid species (Venu, 2008). A closer perusal of the karyotypic compositional alignment reveals, at least, role of one / or two pericentric inversions seems imminent, a common chromosomal reorganization mechanism responsible for most karyotypical differentiation observed in this group.

A closer scrutiny of the karyological variations observed with particular reference to each taxon surveyed and its comparison among the selected taxa, seem to throw some light on karyotypic bifurcation of the chromosome types, as it was possible to distinguish each as a southern and northern chromosome- types, based on "Palghat Gap" that bisect the Western Ghats region into South and northern portions as such. It is interesting to note that the chromosome rearrangements detected for the karyotypic characteristics and of the encountered chromosomal variability are attributable to the consequences meted out of role of pericentric inversions, and of in tandem fusion/fission events. In total, in those of the taxa scrutinized, they did show at least four, distinct karyotypic forms (Venu, 2008).

Currently, the cytological studies have revealed a highly interesting pattern of chromosomal evolution and the occurrence of such karyological events that could lead to considering each representing either as a chromosomal race or a species.

Inclusion of such a vast biogeographical regional occurrence for this taxa (genus *Ichthyophis*) which is rather for stipulate comprehension very wide one, thus a definitive and a complete scenario of variations across this grouped range is still not possible as to the explicit nature of chromosomal reorganization based on the available chromosomal data. But to the least, it is possible to subscribe that in relations to their external morphological features, there is much greater chromosomal divergence prevailing among the taxa analyzed.

Currently, it seems possible to conceive that there exist some disparate opinions with regard to their biosystematics issues relating to the unicolored ichthyophiid taxa comprising South Indian Western Ghats region. Based on external morphology, it was identified they were consisting of four distinct species (Taylor, 1960). However, molecular analyses especially of mitochondrial ribosomal (12S and 16S) sequences, it was projected that they were considered consisting of a single species viz., *I. bombayensis* but having spread across wider regions for having included and contained through junior synonyms (Gower et al., 2007).

However there are opinions that predict that morphological variations are always predictable and tenable from that of molecular evidence. But it is also tenable that various regions of nuclear genes are known to show unequal rates of substitutions in different model organisms. Differences and similarities in rates of mitochondrial rDNA sequence evolution that were taken into account during the survey of phylogenetic assessments, even though they have been heralded in non-congruent pathways. Pertaining to the present case, it becomes evident during the realignment processes that morphological and molecular data are uncoupled (Gower et al., 2001).

There are various explanations why different characters may evolve at different rates in ichthyophiids. As is well-known that morphological evolution may be governed by environmental processes and localized adaptation to specific ecological niches. Heterogeneity in molecular clock has been attributable to generation effects, DNA repair



efficiency, GC contents, metabolic rates and effective population sizes and body sizes (Spradling et al., 2001). Many of the variability might be similar between different species, however, rapid efficiencies could well vary and metabolic rates could differ dramatically through microenvironments that they inhabit realizing in variation in the tempo and mode of chromosomal evolution has been well documented among higher vertebrates, less so in others, even though precise mechanistic explanations are wanting.

On the contrary, morphometric studies suggested that adaptation to local environments could have played an important role in phenotypic expression and therefore they agree in part with other approaches to phylogeny.

### Acknowledgement:

The author is grateful to Naveen Misquith (Seeke), Vijayakumar, Gangadhar (Koodam village, Pyyanur), Sharon, Jom (Vettilapara) and their family members for permitting to do field work in their agricultural fields and also for assisting me in the field. My sincere thanks to Prof. Venkatachalaiah for his critical comments on the draft version of the manuscript.

### References

- Bhatta, G., Dinesh, K.P., Prashanth, P., Kulkarni, N. and Radhakrishnan, C. (2011): A new caecilian *Ichthyophis davidi* (Gymnophiona: Ichthyophiidae): the largest striped caecilian from the Western Ghats. *Curr. Sci.*, 101: 1015-1019.
- Frost, D. (2013): Amphibian species of the World: an online reference. Version 5.6. Electronic database accessible at <http://research.amnh.org/vz/herpetology/amphibia/>. American Museum of Natural History, New York, U.S.A.
- Gower, D.J. and Wilkinson, M. (2007): Species groups in the Indian caecilian genus *Uraeotyphlus* Peters (Amphibia: Gymnophiona: Uraeotyphlidae), with the description of a new species. *Herpetologica.*, 63: 401-410.
- Gower, D.J., Wilkinson, M. and Oommen, O.V. (2001): Molecular and morphological systematic of Uraeotyphlidae (Amphibia: Gymnophiona). Abstracts, Fourth World Congress of Herpetology, Supplement. Gampola, Sri Lanka: AMP Print Shop.
- Gower, D.J., Kupfer, A., Oommen, O.V., Himstedt, W., Nussbaum, R.A., Loader, S.P., Presswell, B., Muller, H., Krishna, S.B., Boistel, R. and Wilkinson, M. (2002): A molecular phylogeny of ichthyophiid caecilians (Amphibia: Gymnophiona: Ichthyophiidae): Out of India or out of southeast Asia? *Proc. Roy. Soc. Lon.*, 269: 1563-1569.
- Gower, D. J., Dharne, M., Bhatta, G., Giri, V., Vyas, R., Govindappa, V., Oommen, O.V., George, J., Shouche, Y. and Wilkinson, M. (2007): Remarkable genetic homogeneity in unstriped, long-tailed *Ichthyophis* along 1500km of the Western Ghats, India. *J. Zoo.*, 272: 266-275.
- Gower, D. J., Rajendran, A., Nussbaum, R. A. and Wilkinson, M. (2008): A new species of *Uraeotyphlus* (Amphibia: Gymnophiona: Uraeotyphlidae) of the malabaricus group. *Herpetologica.*, 64: 235-245.
- Kamei, R.G., Wilkinson, M., Gower, D.J. and Biju, S.D. (2009): Three new species of striped *Ichthyophis* (Amphibia: Gymnophiona: Ichthyophiidae) from the northeast Indian states of Manipur and Nagaland. *Zootaxa.*, 2267: 26-42.
- Kamei, R.G., San Mauro, D., Gower, D.J., Van Bocxlaer, I., Sherratt, E., Thomas, A., Babu, S., Bossuyt, F., Wilkinson, M. and Biju, S.D. (2012): Discovery of a new family of amphibians from Northeast India with ancient links to Africa. *Proc. Roy. Soc.*, 279: 2396-2401.
- Levan, A., Fredga, K. and Sandberg A.A. (1964): Nomenclature for centromeric position on chromosomes. *Hereditas.*, 52: 201-220.
- Mathew, R. and Sen, N. (2009): Studies on Caecilians (Amphibia: Gymnophiona: Ichthyophiidae) of North East India with description of three new species of *Ichthyophis* from Garo hills, Meghalaya and additional information on *Ichthyophis garoensis* Pillai and Ravichandran, 1999. In: Records of the Zoological Survey of India. Occasional Paper no. 172, Director, Ed., Zoological Survey of India, Kolkata, p. 1-56.
- Nishikawa, K., Matsui, M. and Orlov, N. L. (2012a): A new striped *Ichthyophis* (Amphibia: Gymnophiona: Ichthyophiidae) from Kon Tum Plateau, Vietnam. *Curr. Herpet.*, 31: 28-37.
- Nishikawa, K., Matsui, M. and Yambun, P. (2012b): A new unstriped *Ichthyophis* (Amphibia: Gymnophiona: Ichthyophiidae) from Mt. Kinabalu, Sabah, Malaysia. *Curr. Herpet.*, 31: 67-77.
- Nishikawa, K., Matsui, M., Yong, H.S., Ahmad, N., Yambun, P., Belabut, D.M., Sudin, A., Hamidy, A., Orlov, N.L., Ota, H., Yoshikawa, N., Tominaga, A. and Shimada, T. (2012c): Molecular phylogeny and biogeography of caecilians from Southeast Asia (Amphibia, Gymnophiona, Ichthyophiidae), with special reference to high cryptic species diversity in Sundaland. *Mol. Phy. Evol.*, 63: 714-723.
- Nishikawa, K., Matsui, M., Sudin, A. and Wong, A. (2013): A New Striped *Ichthyophis* (Amphibia: Gymnophiona) from Mt. Kinabalu, Sabah, Malaysia. *Curr. Herpet.*, 32(2): 159-169.

- Nussbaum, R.A. and Treisman, G. (1981): Cytotaxonomy of *Ichthyophis glutinosus* and *I. kohtaoensis*, two primitive caecilians from Southeast Asia. *J. Herpet.*, 15: 109-113.
- Pillai, R.S. and Ravichandran, M.S. (1999): Gymnophiona (Amphibia) of India. A taxonomic study. *Rec. Zool. Sur. Ind. occa. pap.*, 172: 1-117.
- San Mauro, D.S., Gower, D.J., Oommen, O.V., Wilkinson, M. and Zardoya, R. (2004): Phylogeny of caecilian amphibians (Gymnophiona) based on complete mitochondrial genomes and nuclear RAG I. *Mol. Phyl. Evol.* 33: 413-427.
- Seshachar, B.R. (1936): The spermatogenesis of *Ichthyophis glutinosus* (Linn.) Part1. The spermatogenesis and their distribution. *Z. Zellf.*, 24: 662-706.
- Seshachar, B.R. (1937a): The chromosomes of *Ichthyophis glutinosus* (Linn.). *Cytologia.*, 8: 327-330.
- Seshachar, B.R. (1937b): The spermatogenesis of *Ichthyophis glutinosus* (Linn.) Part2. The meiotic divisions. *Z. Zellf.*, 26: 133-158.
- Seshachar, B.R. (1939): The spermatogenesis of *Uraeotyphlus narayani* Seshachar. *La. Cellu.*, 48: 63-76.
- Seto, T. and Nussbaum, R.A. (1976): Mitotic and meiotic chromosomes of *Ichthyophis orthoplicatus* Taylor (Amphibia: Gymnophiona). *Caryologia.*, 29: 317-331.
- Spradling, A., Drummond-Barbosa, D. and Kai, T. (2001): Stem cells find their niche. *Nature.*, 414: 98-104.
- Taylor, E.H. (1960): A new caecilian genus in India. *Uni. Kan. Sci. Bull.*, 40: 1-36.
- Taylor, E.H. (1961): Notes on Indian Caecilians. *J. Bom. Nat. Hist. Soc.*, 58: 355-365.
- Taylor, E.H. (1968): The caecilians of the world: A taxonomic review. Kansas Press, Lawrence, Kansas.
- Venkatachalaiah, G and Venu, G. (2002): Karyology of three species of Indian caecilians (Amphibia: Gymnophiona). *Cytologia.*, 67: 191-198.
- Venu, G. (2008): Cytogenetic studies of Indian caecilians with regard to their evolutionary Implications. Ph.D. Dissertation, Centre for Applied Genetics, Bangalore University, Bangalore, Karnataka, India, p. 1-248.
- Venu, G. (2013): The karyotype of *Ichthyophis kodaguensis* – A Striped Ichthyophiid Caecilian from Western Ghats of Peninsular India (Amphibia: Gymnophiona: Ichthyophiidae). *Curr. Herpet.*, 32(2): 197-202.
- Wake, M.H. and Case, S.M. (1975): The chromosomes of caecilians (Amphibia: Gymnophiona). *Copeia.*, 1975: 510-516.
- Wen, Y. and Pang, Q. (1990): The karyotype of *Ichthyophis bannanica* and comparison with *Ichthyophis glutinosus*. *Zool. Res.*, 11: 121-125.
- Wilkinson, M. and Nussbaum, R.A. (1996): On the phylogenetic position of the Uraeotyphlidae (Amphibia: Gymnophiona). *Copeia.*, (1996): 550-562.
- Wilkinson, M., Oommen, O.V., Sheps, J.A. and Cohen, B.L. (2002): Phylogenetic relationships of Indian caecilians (Amphibia: Gymnophiona) inferred from mitochondrial rRNA sequences. *Mol. Phyl. Evol.*, 23: 401-407.
- Wilkinson, M., San Mauro, D.S., Sherratt, E. Gower, D.J. (2011): A nine- family classification of caecilians (Amphibia: Gymnophiona). *Zootaxa.*, 2874: 41-64.
- Zhang, P. and Wake, M.H. (2009): A mitogenomic perspective on the phylogeny and biogeography of living caecilians (Amphibia: Gymnophiona). *Mol. Phyl. Evol.* 53: 479-491.