ISSN NO. 2320-5407

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

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Karyology of the East African caecilian *Schistometopum gregorii* (Amphibia: Gymnophiona) from the Tana River Delta, Kenya

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

Manuscript Info

Manuscript History:

Received: 11 November 2013
Final Accepted: 22 December 2013
Published Online: January 2014

Key words:
Schistometopum gregorii, Giemsa stained chromosomes, meiosis, karyotype.

Abstract

This report presents a male somatic karyotype (2N=22; FN=40) and late meiotic stages of *Schistometopum gregorii* that seems to fall in line with that of other taxa of the family Dermophiidae. In view of a different basic chromosome number prevailing for this species as well for this group, it appears possible to predict that this East African species posits more closely related towards Indian endemic Indotyphlidae.

Introduction

Caecilians are the limbless and elongate amphibians that form the third extant order of Amphibia and are recognised by approximately 190 species (Frost, 2013; Nishikawa et al., 2013). They are sparsely distributed in the wet or moist tropics (except Madagascar) east of the Wallace line (Himstedt, 1996; Kamei et al., 2012). African caecilians (excluding the Seychelles) are represented by about 21 species (Gower et al., 2005), most of which are known from the Eastern Arc Mountains and Coastal Forests biodiversity hotspots (Myers et al., 2000). The coastal forests of eastern Africa contain remarkable levels of biodiversity which have been formally recognised by their reclassification into the Coastal Forests of Eastern Africa, while the Eastern Arc Mountains have been included in the larger Eastern Afromotane hotspot (Myers, 2003; Wilkinson and Nussbaum, 2006; Wilkinson et al., 2003). Although the caecilian fauna of the Eastern Arc Mountains has received recent attention (Loader et al., 2003; Gower et al., 2004a,b; Measey, 2004; Measey and Barot, 2006; Measey et al., 2006), little has been written on species from the coastal Forests of Eastern Africa.

Schistometopum gregorii is one of two species in the African genus *Schistometopum* and the entire known distribution falls within the coastal forests of Eastern Africa hotspot. Nussbaum and Pfrender (1998) reviewed the systematics of the genus, commenting on the strange disjunct nature of the distributions of *S. gregorii* and *S. thomense* from the West African Island of São Tomé (Wilkinson et al., 2003; Loader et al., 2007). While the number of studies on *S. thomense* appear to reflect its abundance on São Tomé (Haft, 1992; Ducey et al., 1993; Delêtre and Measey, 2004; Measey and Van Dongen, 2006), little has been documented on *S. gregorii* since its description. Nussbaum and Pfrender (1998) analysed the morphological differences between collections of *S. gregorii* from the two known sites and showed that animals from Bagamoyo, Tanzania were separated from Kenyan specimens in a multivariate analysis, although too few specimens were available to separate these populations as distinct species. They also commented that despite *S. gregorii* being abundant in anthropogenic habitats in 1934, there have been no subsequent collections (Nussbaum and Pfrender, 1998). However, Loader et al. (2004) commented that they were very common in Bagamoyo, Tanzania in 2002. Measey (2006) found fewer at the same site during an unfavourably dry period in April 2003. Despite searches (Malonza et al., 2006), no *S. gregorii* have been found in the Tana River Delta since Arthur Loveridge collected specimens in May 1933 (Loveridge, 1936). However, during our recent exploration in 2006 we were able to procure ample number of animals from Tana River Delta ($40^o20'$ E, $2^o30'$ S), Kenya.

In this work, based on two male individuals, somatic chromosomal karyotype and late meiotic stages is described.

Materials and Methods

Mitotic metaphase chromosomes and male meiotic stages were obtained by the methods described by Venkatachalaiah and Venu (2002), using intestinal epithelia and testis respectively. Colchicine solution (2mg/ml) was injected intraperitonially (0.1ml/g of body weight) for 24 h at room temperature. Later, the animals were killed by lethal anaesthesia with MS222, the gut and testes were minced and kept in appropriate hypotonic solution for 40 min at room temperature before fixation in 3:1 methanol:glacial acetic acid. Metaphase and meiotic chromosomal spreads were obtained by air drying method and were conventionally stained in 4% Giemsa solution (pH 7.0) for 20 min. Mitotic and meiotic karyotypes were prepared following the protocols of Venu (2008) as earlier conceived from Levan et al. (1964) and of Seto and Nussbaum (1976).

Results

Chromosomal preparations obtained from two male individuals of *S. gregorii* revealed that the diploid number of this species is 22 (2N=22; FN=40). Karyotypic preparation essentially based on Venu (2008) in which the chromosomes were grouped into four categories (A-D) and arranged based on their decreasing lengths. Group A consists of two pairs of submetacentric chromosomes (nos. 1 and 2) and a single pair (3) of metacentric chromosome arranged in the decreasing order of their size. Group B comprises of three pairs (nos. 4-6) of medium sized extreme submetacentrics except the pair no. 5, which is a rather typical submetacentric chromosome. Group C includes four pairs in which pair nos. 7 and 9 are metacentrics; however, pair 8 and 10 are more clearly acrocentrics. Group D includes the last pair (no. 11) of very short metacentric chromosomes (Fig. 1). Morphometric analysis of metaphase chromosomes of *S. gregorii* is provided in Table 1.
Correspondingly, meiotic preparations revealed consisting of 11 bivalents each defined by their distinctive features at late first meiotic stages namely diakinesis (Fig. 2) and metaphase I (Fig. 3).

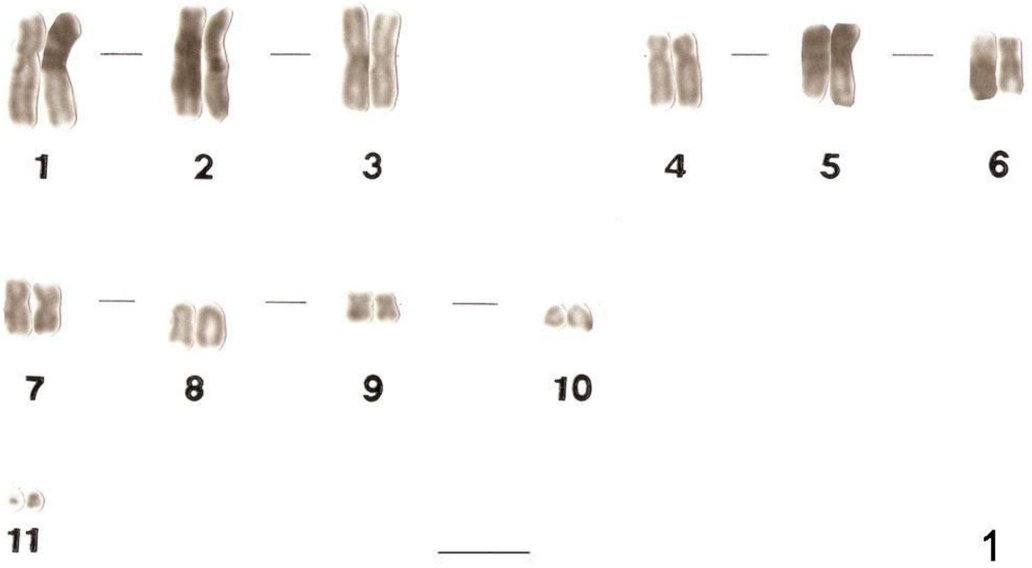

Fig.1 Giemsa stained male somatic metaphase karyotype of *S. gregorii* (Scale bar 10µm)

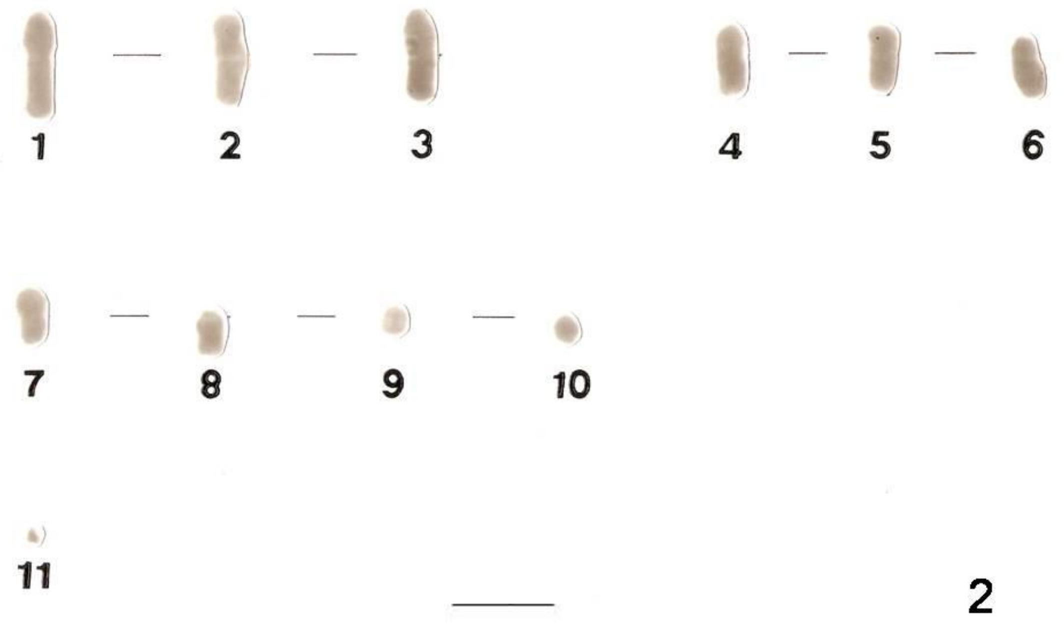

Fig. 2 Giemsa stained male diakinetic karyotype of *S. gregorii* (Scale bar 10µm)

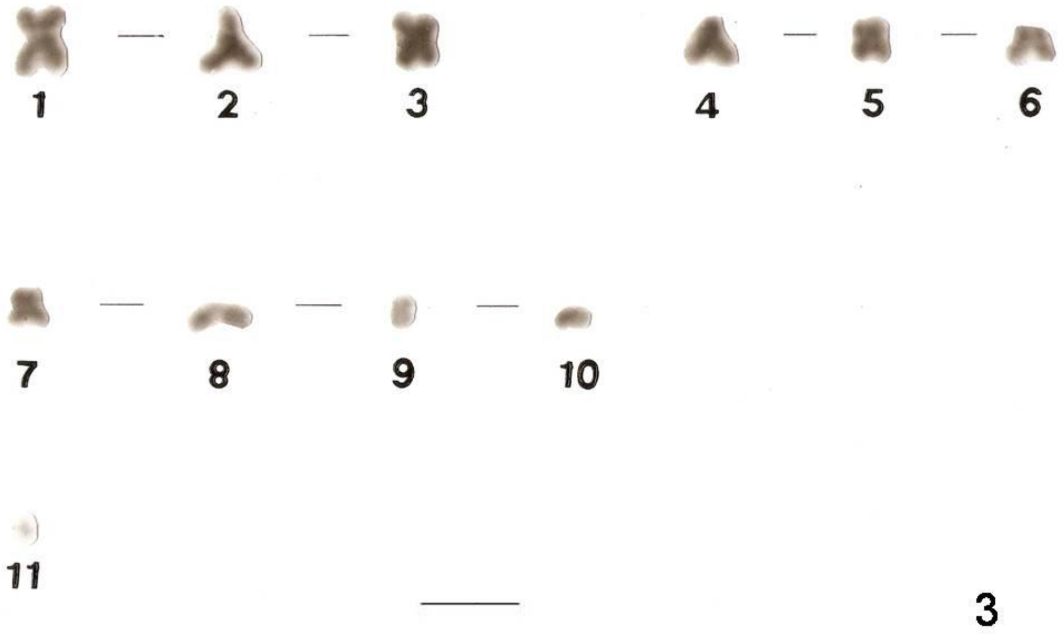

Fig. 3 Giemsa stained male metaphase I karyotype of *S. gregorii* (Scale bar 10µm)

Table 1: Morphometric analysis of metaphase chromosomes of *S. gregorii*

Chromosome pair no.	Total length (I)	Length of short arm (p)	Length of long arm (q)	Arm ratio (q/p)	Centromere Index (px100/I)	Relative length (Ix100/L)
1	22.14	8.61	13.53	1.5714	38.8888	18.2192
2	19.34	8.72	10.62	1.2178	45.0879	15.9150
3	17.54	8.74	8.80	1.0068	49.8289	14.4338
4	12.88	4.85	8.03	1.6556	37.6552	10.5990
5	11.63	3.96	7.67	1.9368	34.0498	9.5704
6	10.90	3.80	7.10	1.8684	34.8623	8.9697
7	8.61	3.60	5.01	1.3916	41.8118	7.0852
8	8.43	0.9	7.53	8.3666	10.6761	6.9371
9	4.19	1.97	2.22	1.1269	47.0167	3.4479
10	3.54	0.6	3.94	4.9000	16.9491	2.9131
11	2.32	1.14	1.18	1.0350	49.1379	1.9091

L - Total length of the chromosomes in the complement (121.52)
* All measurements in millimeter

Discussion

Among gymnophions, the family Dermophiidae is projected as one of the well-advanced families and the taxa are characterized by terrestrial habitation and of practicing viviparity in reproduction. Dermophiidae include four genera; *Dermophis*, *Gymnopis*, both of central/south American origin, while *Geotrypetes* and *Schistometopum* are endemic to Africa.

Wake and Case (1975) presented karyotypic information for the representative species of the genus, *Dermophis mexicanus* (Guatemala) comprising of 2N=26 (FN=48) and *Gymnopis multiplicata* (Costa Rica) with 2N=24/26 (FN=48), both of Central America, and closer examination of karyotypes reveals that they are almost similar to each other. The West African genus, *Geotrypetes*, as it was represented by *G. seraphini*, based on well-characterized karyotype is composed of 2N=36-38 (FN=62-64) (Stingo, 1974; Wake and Case, 1975) and has been considered a 'primitive-type karyotype' for an otherwise morphologically derived taxa for this group (incidentally, another East African caecilian *Boulengerula* (*Afrocaecilia*) *taitanus* that has been reported comprising of 2N=**34** (FN=52) (Nussbaum and Ducey, 1988), which appears to be another primitive karyotype. Surprisingly, there is no karyological information available upon the West African dermophid *S. thomense*.

Barrio and Rinaldi de Chieri (1970, 1972) and Wake and Case (1975) have offered in providing karyotypic distinction of South American, *Siphonops paulensis* (of Siphonopidae) comprising of 2N=24 (FN=48) and *Caecilia occidentalis* (of Caeciliidae) with 2N=24 (FN=48). Further, Barrio et al. (1971) and Wake et al. (1980) contributed towards cytogenetic aspects of *Chthonerpeton indistinctum* (2N=20, FN=38) and *Typhlonectes compressicauda* (2N=28 and FN=56) both belonging to Typhlonectidae that are endemic to South America.

Nussbaum and Ducey (1988) have proposed a uniform individualistic karyotypic characteristics for six species of three genera (*Grandisonia*, *Hypogeophis* and *Praslinia*) **that are endemic** to Seychelles Islands belonging to Indotyphlidae bearing a common diploid number of 26 (FN=50-52). Karyotypic dynamicity was highlighted in the case of eight species of Indian genus *Gegeneophis* (Venu and Venkatachalaiah, 2005, 2006; Venu et al., 2012a, 2012b) and in one species of *Indotyphlus* (Venkatachalaiah et al., 2006), all conforming towards in the range of 2N=26 (FN=52) as a basic number, readily recognizable for the family Indotyphlidae.

Karyological characteristics of *S. gregorii* present a distinctive and a new karyotype for this group of taxa (Dermophiidae). This appears obvious in view of possessing a different 2N, FN and in other chromosome structural features. The karyotypic specificity of *S. gregorii* seemed to have achieved this status based upon major chromosomal reorganizations in which number of biarmed and uniarmed chromosomes seemed to have varied to a greater extent.

During the course of karyological elicitation made for each individualistic karyotype described from among the cytologically known taxa of advanced caecilians that have been pointing towards considering chromosome modulation revolving around 2N=26 and thus perhaps serving as a basic diploid but a modal number for this group. Interestingly, having near identical macro chromosomal set (such as meta- and submetacentrics) comprising of pairs (nos. 1-9) in the complement offering a clear distinction of as to their nature of chromosome lengths and each as a set that appear homologous. However, greater variations could be elicited in respect of lower set chromosome pairs (nos. 10-12/13) thereby directing in acquiring chromosome differences and leading towards defining speciation progression.

Based on this type of incriminating chromosomal tendency, it becomes possible to acquire onto a requisite type of karyotype, by invoking a probable role of pericentric inversion and a Robertsonian fusion processes. Thus, there is a possibility of procuring *Gymnopis multiplicata* karyotype from that of *Dermophis mexicanus*. Implying to a similar kind of chromosome kinetics, it is also possible to obtain karyotypic specificity of *Siphonops paulensis* from *Dermophis mexicanus*, except for change in the morphology of chromosome pair no. 1. A closer appraisal of *S. gregorii* karyotype reveals that it is also possible to derive a karyotype from that of *Gymnopis multiplicata* karyotype by inferring an involvement of minimal chromosome structural rearrangements that include at least a pericentric inversion and a Robertsonian fusion process. On a similar account, one could also derive karyotypic structure of *Chthonerpeton indistinctum* from *S. gregorii* by adopting the similar but a different type of chromosome reduction trend that could be involving appropriate designate chromosomes.

Morescalchi (1983) and Nussbaum (1991) observed that those of morphologically primitive caecilians were characteristic for representing 'primitive- type karyotype' as against advanced –type possessing more derivative morphologies. On this regard, Wake et al. (1980) exasperates that this generalization as yet a putative one.

Kamei et al. (2012) have opined that they were propelled to imply upon establishing closer lineological relationships with African herpelid caecilians to establish sisterly-group relation to that of recently explored northeast Indian Chikilidae taxa rather than to that of endemic Indotyphlidae. In this extent, it would appear very interesting to await further chromosomal and other features made available within this extent, so as to make a comprehensive generalization upon their biosystematics.

The preceding discussion are in line with the opinions of King (1990, 1993) who emphasized that the criteria for determining ancestral or basal karyotypes suffer from a number of basic tenets when attempts were made to compare at higher level taxonomics and further to incite that the above generic level comparison of taxa may become unrecognizable in terms of chromosome homologies. King (1991) and Sessions (2008) further argue that differences and similarities may be used to infer phylogeny only if chromosome number, morphology, C-bands and sites of secondary constrictions or other markers can be traced to a commonality or an ancestral karyotype in a monophyletic lineage.

The karyotypic specificity of *S. gregorii* seems pointing towards pretentious relations to other Dermophiidae than to herphelid taxa.

Acknowledgement

The author is grateful to John Measey, Patrick K. Malonza, Berryl for helping with logistics and Wilson (Wundayani, Kenya) for field assistance. **Also thankful to Prof. G. Venkatachalaiah for his critical comments on the draft version of the manuscript.**

References

BARRIO, A. AND RINALDI DE CHIERI, P. (1970): Estudio chromosomico de *Caecilia occidentalis* (Gymnophiona: Caeciliidae). Physis., 30: 305-307.

BARRIO, A., SAEZ, F. A., AND RINALDI DE CHIERI, P. (1971): The cytogenetics of *Chthonerpeton indistinctum* (Amphibia: Gymnophiona). Caryologia., 24: 435-445.

BARRIO, A. AND RINALDI DE CHIERI, P. (1972): El complemento chromosomico de *Siphonops paulensis* (Gymnophiona: Caeciliidae). Physis., 31: 273-276.

DELETRE, M. AND MEASEY, G. J. (2004): Sexual selection vs. ecological causation in a sexually dimorphic caecilian, *Schistometopum thomense* (Amphibia: Gymnophiona: Caeciliidae). Etho. Ecol. Evol., 16: 243-253.

DUCEY, P. K., FORNANOWICZ, D. R., BOYET, L., MAILLOUX, J., AND NUSSBAUM, R. A. (1993): Experimental examination of burrowing behavior in caecilians (Amphibia: Gymnophiona): Effects of soil compaction on burrowing ability of four species. Herpetologica., 49: 450-457.

FROST, D. 2013. Amphibian species of the World: an Online Reference. Version 5.6 (9 January 2013). American Museum of Natural History, New York, U.S.A.

GOWER, D. J., LOADER, S. P., AND WILKINSON, M. (2004a): Assessing the conservation status of soil-dwelling vertebrates: insights from the rediscovery of *Typhlops uluguruensis* (Reptilia: Serpentes: Typhlopidae). Syst. Biodi., 2: 79-82.

GOWER, D. J., LOADER, S. P., WILKINSON, M., AND MONTCRIEFF, C. B. (2004b): Niche separation and comparative abundance of *Boulengerula boulengeri* and *Scolecomorphus vittatus* (Amphibia: Gymnophiona) in an East Usamabara forest, Tanzania. Afr. J. Herpt., 53: 183-190.

GOWER, D. J., STUART, S. N., BHATTA, G., COX, N., GERLACH, J., LOADER, S. P., LONG, J., LYNCH, J. D., MEASEY, G. J., NUSSBAUM, R. A., OOMMEN, O. V., MANAMENDRA-ARACHCHI, K., RAVICHANDRAN, M. S., WILKINSON, M., AND YOUNG, B. E. (2005): Caecilians and the 2004 Global Amphibian Assessment. Cons. Biol., 19: 54-55.

HAFT, J. (1992): Bemerkungen zu den Blindwühlen der Gattung *Schistometopum* von Sao Tomé (Gymnophiona, Caecilidae). Bon. Zool. Beit., 43: 477- 479.

HIMSTEDT, W. (1996): Die Blindwühlen. Magdeburg, Westarp-Wissenschaften, p. 160.

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.

KING, M. (1990): Amphibia. p. 1–241. *In*: B. John (ed.), *Animal Cytogenetics*. Gebrüder Borntraeger.

KING, M. (1993): Species Evolution: The role of chromosome change. p. 1-336. Cambridge University Press, New York.

LEVAN, A., FREDGA, K., AND SANDBERG, A. A. (1964): Nomenclature for centromeric position on chromosomes. Hereditas., 52: 201-220.

LOADER, S., WILKINSON, M., GOWER, D. J., AND MSUYA, C. (2003): A remarkable young *Scolecomorphus vittatus* (Amphibia: Gymnophiona: Scolecomorphidae) from the North Pare Mountains, Tanzania. J. Zoo., 259: 93-101.

LOADER, S., HOWELL, K., GOWER, D. J., MEASEY, G. J., AND WILKINSON, M. 2004. *Schistometopum gregorii*. in: IUCN (2013): IUCN Red List of Threatened Species. Version 2013.1. www.iucnredlist.org. Downloaded on 24 September 2013.

LOADER, S. P., PISANI, D., COTTON, J. A., GOWER, D. J., DAY, J. J., AND LOVERIDGE, A. (1936): Scientific results of an expedition to rain forest regions in eastern Africa. VII. Amphibians. Bull. Mus. Comp. Zool., 79: 369-430.

MALONZA, P. K., WASONGA, V. D., MUCHAI, V., ROTICH, D., BWONG, B. A., AND BAUER, A. M. (2006): Diversity and biogeography of herpetofauna of the Tana River Primate National Reserve, Kenya. J. East. Afr. Nat. Hist., 95: 95-109.

MEASEY, G. J. (2004): Are caecilians rare? An East African perspective. J. East. Afr. Nat. Hist., 93: 1-21.

MEASEY, G. J. (2006): Surveying biodiversity of soil herpetofauna: towards a standard quantitative methodology. Eur. J. Soi. Biol., 42: S103-S110.

MEASEY, G. J. AND BAROT, S. (2006): Evidence of seasonal migration in a tropical subterranean vertebrate. J. Zool., 269: 29-37.

MEASEY, G. J. AND VAN DONGEN, S. (2006): Bergmann's rule and the terrestrial caecilian *Schistometopum thomense* (Amphibia: Gymnophiona: Caeciliidae). Evol. Eco. Res., 8: 1049-1059.

MEASEY, G. J., MEJISSA, J., AND MULLER, H. (2006): Notes on the distribution and abundance of the caecilian *Boulengerula uluguruensis* (Amphibia: Gymnophiona: Caeciliidae) in the Uluguru Mountains, Tanzania. Afr. J. Eco., 44: 6-13.

MORESCALCHI, A. (1983): Citogenetica e storia naturale degli Anfibi. Rivi. Di. Biolo., 76: 379-408.

MYERS, N. (2003): Biodiversity hotspots revisited. Bio. Sci., 53: 916-917.

MYERS, N., MITTERMEIER, R., MITTERMEIER, C., FONSECA, G. D., AND KENT, J. (2000): Biodiversity hotspots for conservation priorities. Nature., 403: 853-858.

NISHIKAWA, K., MATSUI, M., SUDIN, A., AND WONG, A. (2013): A new striped *Ichthyophis* (Amphibia: Gymnophiona) from Mt. Kinabalu, Sabah, Malaysia. Curr. Herpt., 32: 159-169.

NUSSBAUM, R. A. (1991): Cytotaxonomy of caecilians. p. 22-76. *In*: S. K. Sessions and D. M. Green (eds.), *Amphibian Cytogenetics and Evolution*. Academic Press, San Diego, USA.

NUSSBAUM, R. A. AND DUCEY, P. K., (1988): Cytological evidence for monophyly of the caecilians (Amphibia: Gymnophiona) for the Seychelles Archipelago. Herpetolo., 44: 290-296.

NUSSBAUM, R. A. AND PFRENDER, M. E. (1998): Revision of the African caecilian genus *Schistometopum* Parker (Amphibia: Gymnophiona: Caeciliidae). Misc. Publ. Mus. Zool., Uni. Michi., I-IV: 1-32.

SESSIONS, S. K. (2008): Evolutionary cytogenetics in Salamanders. Chromo. Res., 16: 183-201.

SETO, T. AND NUSSBAUM, R. A. (1976): Mitotic and meiotic chromosomes of *Ichthyophis orthoplicatus* Taylor (Amphibia: Gymnophiona). Caryolog., 29: 317-331.

STINGO, V. (1974): Karyology of *Geotrypetes seraphini* (Amphibia: Apoda). Caryolog., 27: 485-492.

VENKATACHALAIAH, G. AND VENU, G. (2002): Karyology of three species of Indian caecilians (Amphibia: Gymnophiona). Cytolog., 67: 191-198.

VENKATACHALAIAH, G., VENU, G., GIRI, V., AND WILKINSON, M. (2006): Studies on the karyotype of *Indotyphlus battersbyi* Taylor (Amphibia: Gymnophiona: Caeciliidae). J. Cyt. Gen., 7: 47-56.

VENU, G. (2008): Cytogenetic studies of Indian caecilians with regard to their evolutionary implications. Unpublished doctoral dissertation. Bangalore University, Bangalore, Karnataka, India.

VENU, G. AND VENKATACHALAIAH, G. (2005): Karyology of two species of caecilians (Caeciliidae: Gymnophiona); evolution through tandem fusion and sex chromosome dimorphism. Caryolog., 58: 140-151.

VENU, G. AND VENKATACHALAIAH, G. (2006): Karyological characteristics of two species of Indian *Gegeneophis* (Amphibia: Gymnophiona: Caeciliidae). Amp. Rept., 27: 130-134.

VENU, G., BHATTA, G., AND VENKATACHALAIAH, G. (2012a): Chromosomal studies of two species of the genus *Gegeneophis* (Amphibia: Gymnophiona: Caeciliidae). Curr. Herpeto., 31: 14-20.

VENU, G., GIRI, V., AND VENKATACHALAIAH, G. (2012b): Karyology of two species of the genus *Gegeneophis* (Amphibia: Gymnophiona: Indotyphlidae). J. Cyt. Gen., 12: 41-48.

WAKE, M. H. AND CASE, S. M. (1975): The chromosomes of caecilians (Amphibia: Gymnophiona). **Copeia.,** 1975: 510-516.

WAKE, M. H, HAFNER, J. C, HAFNER, M. S., KLOSTERMAN, L. L., AND PATTON, J. L. (1980): The karyotype of *Typhlonectus compressicauda* (Amphibia: Gymnophiona) with comments on chromosome evolution in caecilians. Experent., 36: 171-172.

WILKINSON, M. (2007): Relative timescales reveal multiple origins of parallel disjunct distributions of African caecilian amphibians. Biolo. Lett., 3: 505-508.

WILKINSON, M., LOADER, S. P., GOWER, D. J., SHEPS, J. A., AND COHEN, B. L. (2003): Phylogenetic relationships of African Caecilians (Amphibia: Gymnophiona): insights from mitochondrial rRNA gene sequences. Afr. J. Herpetolo., 52: 83-92.

WILKINSON, M. AND NUSSBAUM, R. A. (2006): Caecilian phylogeny and classification. p. 39-78. *In*: J. M. Exbrayat, (ed.), *Reproductive Biology and Phylogeny of Gymnophiona*. Science Publishers, New Hampshire, USA.