# Unusual karyotype in the Malagasy colubrid snake *Mimophis mahfalensis*

Gennaro Aprea[1], Gaetano Odierna[1], Franco Andreone[2], Frank Glaw[3], Miguel Vences[4]

[1] Dipartimento di Biologia Evolutiva e Comparata, Università di Napoli "Federico II", Via Mezzocannone 8, 80134 Napoli, Italy
e-mail: odierna@dgbm.unina.it
[2] Museo Regionale di Scienze Naturali, Via G. Giolitti 36, 10123 Torino, Italy
e-mail: f.andreone@libero.it
[3] Zoologische Staatssammlung, Münchhausenstr. 21, 81247 München, Germany
e-mail: frank.glaw@zsm.mwn.de
[4] Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, Mauritskade 61, 1090 GT Amsterdam, The Netherlands
e-mail: vences@science.uva.nl

The extant reptile fauna of Madagascar has in great parts been shaped by dispersal events from Africa (e.g., Mausfeld et al., 2000; Vences et al., 2001a, b), but no conclusive hypothesis is available at present for the biogeographic origin of the 18 endemic Malagasy colubrid genera. One especially enigmatic genus is *Mimophis* which has been classified in several different subfamilies, e.g. in the Boodontinae and Psammophiinae (Meirte, 1992;

Zaher, 2000). A number of characters from hemipenial and general morphology and natural history indicate close relationships of *Mimophis* to the largely African psammophiines (Brygoo, 1982), and it currently is included in this subfamily (Zaher, 2000). *Mimophis* contains a single species, i.e., *M. mahfalensis*. The status of a second form from central Madagascar, treated as subspecies *M. mahfalensis madagascariensis* (see Glaw and Vences, 1994), is currently uncertain. In this paper we present the first data on the unusual karyotype in *Mimophis mahfalensis*, which in the future may become relevant to assess its phylogenetic relationships.

Our data were obtained from a single female specimen which is currently preserved at the Museo Regionale di Scienze Naturali, Torino (MRSN R1833, from Ambovombe, Tuléar Faritany, leg. F. Andreone and G. Aprea 27.XII.1997). Following the method of Baker et al. (1971), the specimen was injected twice (interval 24 h) with a phytohaemagglutinin solution (dilution 1 : 15 of stock solution; 0.01 ml/g of body weight). 48 h after the first injection the specimen was injected with a 0.5 mg/l colchicine solution (0.01 ml/g of body weight). Two hours later, 0.5-1 ml of blood was extracted from the caudal vein using a heparinized syringe. The collected blood was centrifuged at 500 rpm for 3 min, and the supernatant white cells washed in 5 ml of a salt balanced solution (Hank's solution). Cells were transferred to a 0.7% sodium citrate solution for 30 min and subsequently fixed in acetic methanol (1 part of acetic acid, 3 parts of methanol). Finally they were dropped on clean slides and air dried. Chromosomes were stained for 5 min in a 5% Giemsa solution. Karyotypes were assembled from five metaphase plates and their relative lengths and centromeric indices measured. Additionally we performed C-banding (Sumner, 1972) and sequential C + $CMA_3$ + DAPI staining (Odierna et al., 2000).

The examined specimen had a karyotype of 2n = 44 elements. Of these, 24 were macrochromosomes and 20 were microchromosomes. All macrochromosomes were acrocentric (uniarmed) except one metacentric element (fig. 1). This biarmed element, together with an uniarmed element formed the fourth pair in length. We consider this heteromorphic pair as sex chromosomes, the biarmed chromosome corresponding to the Z element and the uniarmed chromosome to the W element (fig. 1A). Relative chromosome lengths, given as mean ± standard deviation (chromosome pair number in parentheses, was 9.7 ± 0.9 (1), 8.2 ± 0.7 (2), 7.2 ± 0.8 (3), 3.3 ± 0.9 (4Z), 3.6 ± 0.8 (4W), 6.5 ± 0.7 (5), 6.4 ± 0.6 (6), 6.3 ± 0.6 (7), 5.9 ± 0.7 (8), 5.7 ± 0.8 (9), 5.5 ± 0.7 (10), 4.5 ± 0.8 (11), 4.3 ± 0.7 (12), 23.6 ± 2.5 (13-22). The centromeric index was 43.9 ± 3.5 for chromosome 4W, 0 for all the other (acrocentric) chromosomes.

Pericentromeric heterochromatin was present on the Z chromosome, while the W-element was completely heterochromatic except for an interstitial euchromatic region. The other macrochromosomes showed centromeric heterochromatin, while the microchromosomes were variable: some pairs being euchromatic, some completely or partially heterochromatic (fig. 1B). After combined C-banding and fluorochrome-staining, all heterochromatic regions resulted to be $CMA_3$ and DAPI negative (fig. 1C-D).

The presence of sex chromosomes on the 4th chromosome pair agrees with the state in most snakes, in which usually the Z element is biarmed (metacentric or submetacentric), and the W element displays a large interspecific variation in shape, length and heterochromatin distribution (Singh, 1972; Mengden and Stock, 1980; Olmo, 1986). In contrast, the chromosome number of *Mimophis* differs from the most common situation in colubrids (2n = 36). Also the acrocentric condition of almost all macrochromosomes is unusual.

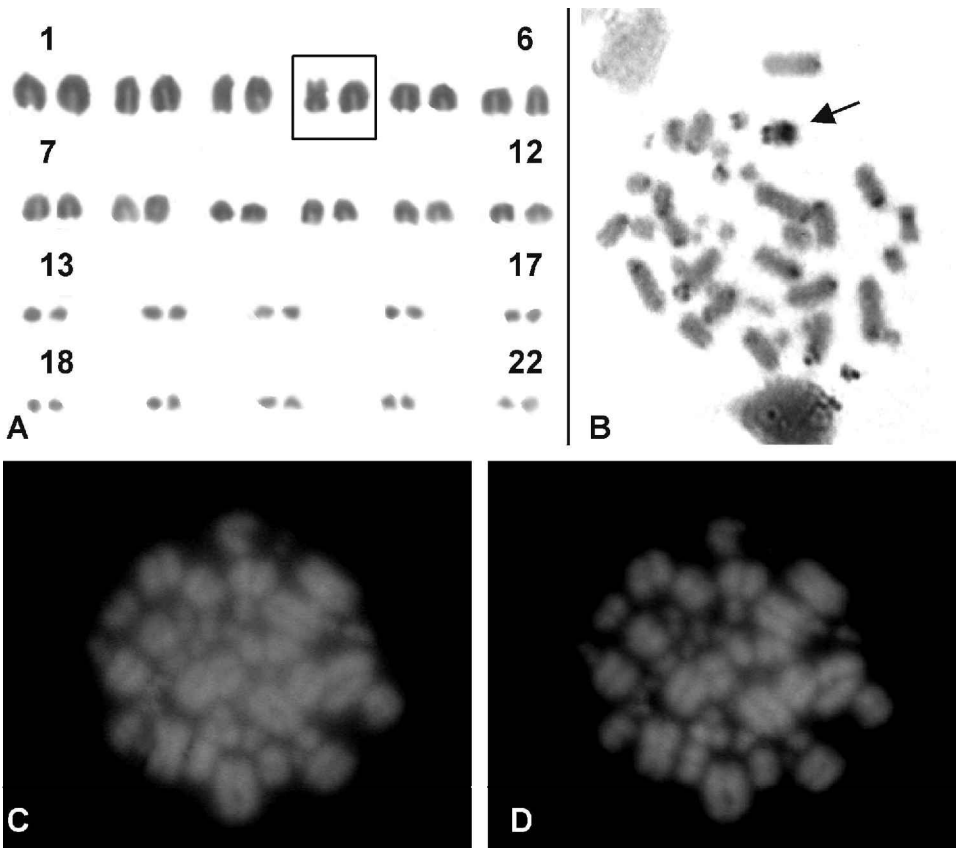**Figure 1.** Metaphase plates of *Mimophis mahfalensis* after conventional Giemsa staining (A), C-banding (B), sequential C + CMA$_3$ staining (C) and sequential C + CMA$_3$ + DAPI staining (D). The square includes the sex chromosome pair, the arrow points to the W sex chromosome.

Among colubrids the same condition was described only in the colubrine *Elaphe subocularis* (Baker et al., 1971; Mengden and Stock, 1980) and the xenodontine *Clelia clelia* (as *C. occipitolutea*) (Beçak, 1965). *Elaphe subocularis* has 40 chromosomes (3 biarmed and 7 uniarmed pairs of macrochromosomes and 10 pairs of microchromosomes), and *C. clelia* has 50 chromosomes (1 biarmed and 14 uniarmed pairs of macrochromosomes and 10 pairs of microchromosomes).

Two alternative hypotheses have been drawn to explain their distinctive karyotypes. The 2n = 36 karyotype with all biarmed chromosomes has been assumed to be plesiomorphic (Beçak and Beçak, 1969). If this was the case, then chromosome sets such as those of *C. clelia*, *E. subocularis* and *M. mahfalensis* would have originated by means of fissions and inversions of the macrochromosomes. In contrast, Mengden and Stock (1980) proposed to consider karyotypes with all biarmed macrochromosomes as derived at least in some cases.
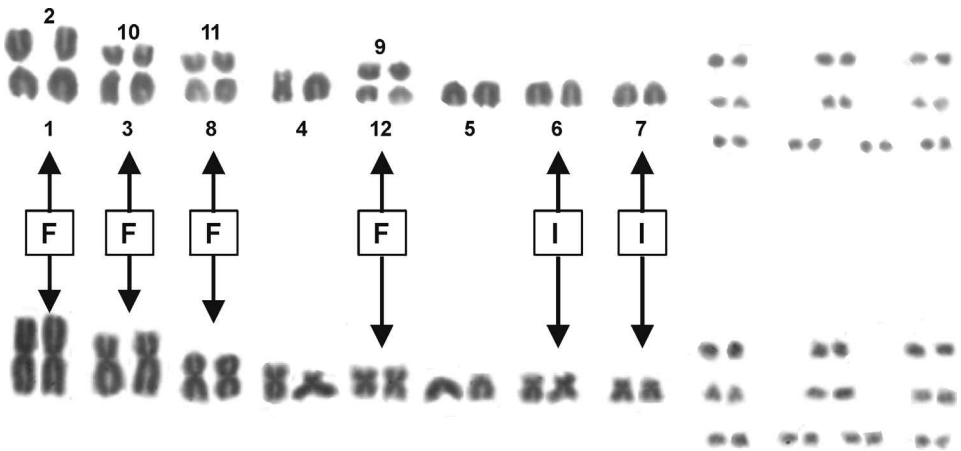
**Figure 2.** Schematic hypothesis of evolution of the *Mimophis* karyotype from the standard colubrid karyotype (as represented by *Coronella austriaca*; Odierna, unpubl.), or vice-versa.

For the karyotype of *Mimophis mahfalensis*, four fissions (or fusions) and two pericentric inversions could be involved (fig. 2).

Regarding the relationships of *Mimophis,* it remains to be assessed whether its unusual karyotype bears phylogenetic information. South African psammophines have karyotypes ranging from 44-56 chromosomes (Branch, 1980), but no further reliable information is available to date (Branch, in litteris 2002). *Malpolon moilensis* and *M. monspessulanus*, another two psammophiine species, as *Mimophis mahfalensis* also have a chromosome set of 2n = 42 elements (22 macro- plus 20 microchromosomes), but differ because the first 11 macrochromosome pairs are biarmed (Matthey, 1931, 1970; Branch, 1980; De Haan, 1999).

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