Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tcar20

The Karyology of Hemisus Marmoratus (Amphibia, Salientia)

Ettore Olmo[a]

[a] Istituto di Istologia ed Embriologia, Università di Napoli, Napoli, Italy

Published online: 30 Jan 2014.

To cite this article: Ettore Olmo (1972) The Karyology of Hemisus Marmoratus (Amphibia, Salientia), Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics, 25:1, 33-42, DOI: 10.1080/00087114.1972.10796463

To link to this article: http://dx.doi.org/10.1080/00087114.1972.10796463

THE KARYOLOGY OF *HEMISUS MARMORATUS* (AMPHIBIA, SALIENTIA) *

ETTORE OLMO

Istituto di Istologia ed Embriologia, Università di Napoli, Napoli, Italy

Received: 17th May 1971

INTRODUCTION

The taxonomic position occupied by the genus *Hemisus* Günther a burrowing Anuran Amphibian that is widespread in the savannahs of tropical and southern Africa, is uncertain and debated by various authors.

Initially, it was included by NOBLE (1931) in the family of the *Brevicipitidae (Microhylidae)*, but it was later considered by DE VILLIERS (1931, 1933) and PARKER (1934) as belonging to the *Ranidae*, chiefly on the basis of its larval form. LAURENT (1951) maintains, though with certain reservations, that it belongs to a highly specialized branch of the family of the *Hyperoliidae*; various present-day authors, however, consider it as one of the *Ranidae* (cf. COCHRAN 1961 and SCHIØTZ 1967).

The chromosome set of *Hemisus* has not yet been described in detail; therefore I have thought it useful to do so, with the object of making a karyological contribution to the problems involved in the systematics of this genus. A short note on the chromosomes of *Hemisus marmoratus* has already been published (cf. MORESCALCHI *et al.* 1970); here I shall investigate the morphology of the individual pairs of homologues of this species and make a morphometric comparison between the karyotype of *Hemisus* and that of species belonging to the families that, according to the systematists, are the nearest to this genus: *Breviceps gibbosus (Microhylidae)*; *Kassina senegalensis (Hyperoliidae)* and *Rana arvalis (Ranidae)*. The choice of these species depends on the fact that they have same chromosome number as *Hemisus*.

* Research carried out through a contribution from the C.N.R. (Genetics Enterprise).

MATERIAL AND METHODS

I have studied the chromosome set of 3 female specimens of *Hemisus marmoratus* (PETERS 1854), kindly sent by Dr D. G. Broadley, from the neighbourhood of Umtali, Rhodesia.

The animals, treated with Colcemid (CIBA), were anaesthetized with MS 222 (Sandoz) and killed. Fragments of intestine, hypotonically treated, were fixed in 3:1 acetic alcohol for 1 h and placed in 45% acetic acid to soften them. Small fragments were squashed between two slides and stained with Mayer's acid haemalum or acetic orcein.

The chromosomes of the other species were studied again by using slides that A. Morescalchi kindly placed at my disposal, which he had prepared by the above-described technique; the results relative to these slides have already been the subject of previous reports (MORESCALCHI 1967, 1968*a*, 1968*b*). On my part, I made a more thorough karyometric study of *Breviceps* and *Kassina*, which had not previously been done in detail.

For this purpose, for each pair of homologues of the various species investigated, I determined the main morphometric characteristics: the relative length (rl), or the percentage length of each chromosome in relation to the total length of the chromosomes of the haploid set; the centromeric index (CI), namely the ratio between the length of the short arm and the total length of the chromosome; and the arm ratio (r), which expresses the ratio between the length of the long arm and that of the short arm.

As regards the nomenclature on chromosomes, I have conformed to that proposed by LEVAN *et al.* (1964), distinguishing the following types of chromosome: metacentric (m) with values of r ranging between 1 and 1.67 and values of CI between 50 and 37.5; submetacentric (sm) with values of r ranging from 1.67 to 3 and values of CI from 37.5 to 25; and subtelocentric (st) with values of r between 3 and 7 and values of CI between 25 and 12.5.

The measurements were carried out on the chromosomes of 7 intestinal metaphase plates of *Hemisus marmoratus* (♀ ♀) and on 6 spermatogonial or intestinal metaphase plates (in the ♀ ♀, only intestinal) of specimens of both sexes of *Breviceps gibbosus* and *Kassina senegalensis* (it may be mentioned that, in these species, there are no heterochromosomes).

RESULTS

The chromosome set of *Hemisus marmoratus* (Figs. 1 and 2) consists of 24 chromosomes. These I have divided schematically into two groups: large chromosomes and small chromosomes, according to whether the value of rl was greater or smaller than 7%. In the case of *Hemisus*, such a subdivision may seem arbitrary, since the differences that can be observed between the values of rl in the 5th pair and those in the 6th pair are greater than the differences between the corresponding values in the 6th and 7th pairs. Nevertheless, I have considered it useful to make this subdivision both because the

chromosomes belonging to the 7th to the 12th pairs show smaller differences in rl than those of the first 6 pairs (and therefore appear fairly homogeneous and easy to identify in the metaphase plates) and because it offers a more immediate criterion of comparison between the karyotype of this species and that of others, in some of which a similar difference appears more clearly marked.

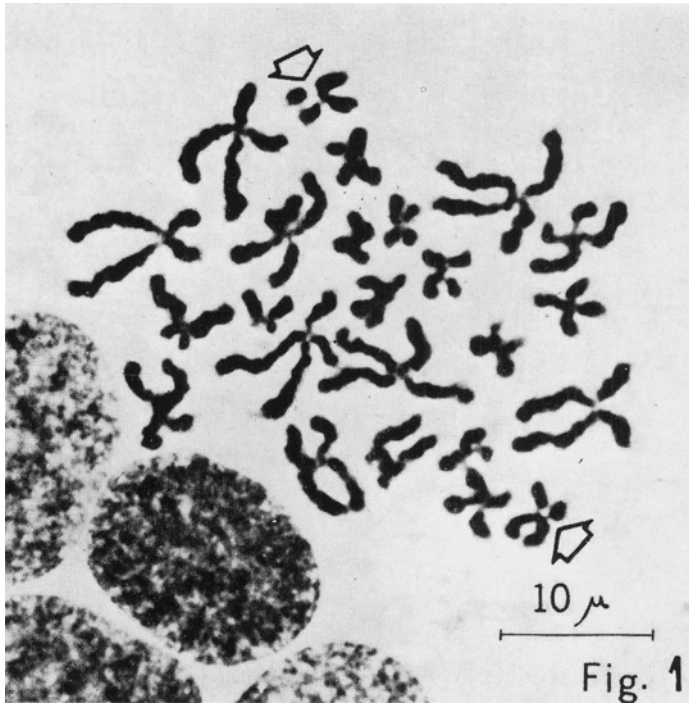

Fig. 1. — Intestinal metaphase plate of *Hemisus marmoratus* female. The arrows indicate the heterochromatic areas.

Among the large chromosomes it may be noted that those of the 1st, 4th and 5th pairs are m, those of the 2nd and 3rd pairs are sm, while those of the 6th pair are st. The small chromosomes, however, except those of the 12th pair which are sm, are all m. The chromosomes of the 8th pair show a heterochromatic area on the short arm, in the centromere region.

Regarding the karyotype of the species belonging to the families that may have affinities with *Hemisus*, the following preliminary remarks may be made.

Among the *Microhylidae*, the karyotypes that are known are those of *Kaloula pulchra* ($2n=28$), *Phrynomerus bifasciatus* ($2n=26$) and *Breviceps*

gibbosus ($2n=24$), the relations of which have already been discussed by MORESCALHI (1968*a*, 1968*b*); other karyotypes that are known are those of *Kaloula borealis* (*Cacopoides tornieri*, $2n=28$ (SATO 1936); *Dermatonotus mülleri* ($2n=22$) (RABELLO 1970) and *Gastrophryne carolinensis* ($2n=22$) (MORESCALCHI *et al.* 1970). Among these species, I investigated *Breviceps gibbosus*, an African burrowing microhylid having a diploid set of 24 chromosomes, like *Hemisus*.

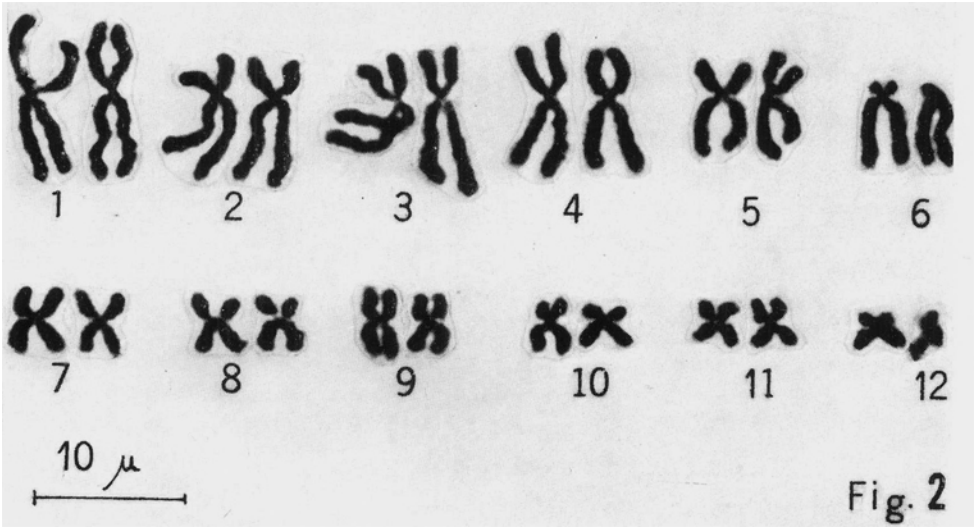

Fig. 2. — The karyotype of *Hemisus marmoratus*, reconstructed by pairing the homologues of an intestinal metaphase plate.

Among the *Hyperoliidae* so far studied, there are species with 24 and others with 26 chromosomes (cf. MORESCALCHI 1968*a*); among these, I studied again the chromosome set of a 24-chromosome species: *Kassina senegalensis*.

As regards the *Ranidae*, the results of various authors are known with respect to species having a diploid number of 26 and 24 chromosomes (cf. WICKBOM 1945; WITSCHI *et al.* 1958; KOBAYASHI 1962; SETO 1965; MORESCALCHI 1967 and ULLERICH 1967); among the latter I investigated *Rana arvalis*, a 24-chromosome species, which is one of the species most studied and of which I was able to make use of various slides.

Table shows the morphometric data that I collected on *Hemisus*, *Bre-*

TABLE

SPECIES		CHROMOSOME NUMBER											
		1	2	3	4	5	6	7	8	9	10	11	12
Hemisus marmoratus	rl	14.7	12.4	12.2	10.9	9.2	7.7	6.5	5.9	5.7	5.1	4.8	4.6
	CI	47.6	35.2	34.8	43.2	40.4	21.8	46.2	45.2	45.7	41.5	45.6	34.8
	r	1.10	1.84	1.87	1.31	1.43	3.58	1.16	1.21	1.19	1.37	1.19	1.87
Breviceps gibbosus	rl	15.5	12.9	12.3	11.2	9.7	8.8	5.7	5.7	5.2	4.8	4.3	3.8
	CI	42.5	35.2	29.9	25.7	36.6	42.9	44.6	40.6	36.2	45.7	45.8	24.4
	r	1.35	1.85	2.36	2.92	1.73	1.36	1.26	1.46	1.76	1.19	1.23	3.38
Kassina senegalensis	rl	14.1	11.2	10.2	9.8	8.9	7.7	6.7	6.7	6.5	6.2	6.1	5.7
	CI	42.9	33.7	37.5	32.4	41.7	47.1	40.7	44.3	45.1	46.8	42.2	47.2
	r	1.33	1.96	1.70	2.09	1.40	1.12	1.46	1.26	1.21	1.14	1.37	1.12
Rana arvalis	rl	15.8	14.1	12.2	11.5	9.6	8.5	6.2	5.4	4.9	4.4	3.9	3.3
	CI	45.0	41.0	35.0	40.0	43.0	45.0	42.0	21.0	24.0	40.0	33.0	28.0
	r	1.22	1.44	1.86	1.50	1.33	1.22	1.38	3.76	3.17	1.50	2.03	2.57

Relative length (rl), centromeric index (CI) and arm ratio (r) of the chromosomes of *Hemisus marmoratus, Breviceps gibbosus, Kassina senegalensis* and *Rana arvalis* (the last one after MORESCALCHI 1967).

viceps and *Kassina* and also the data on *Rana arvalis* taken from a previous report by MORESCALCHI (1967).

The diagram in Fig. 3 also shows a comparison between the haploid complement of *Hemisus* and those of the other three species. It represents

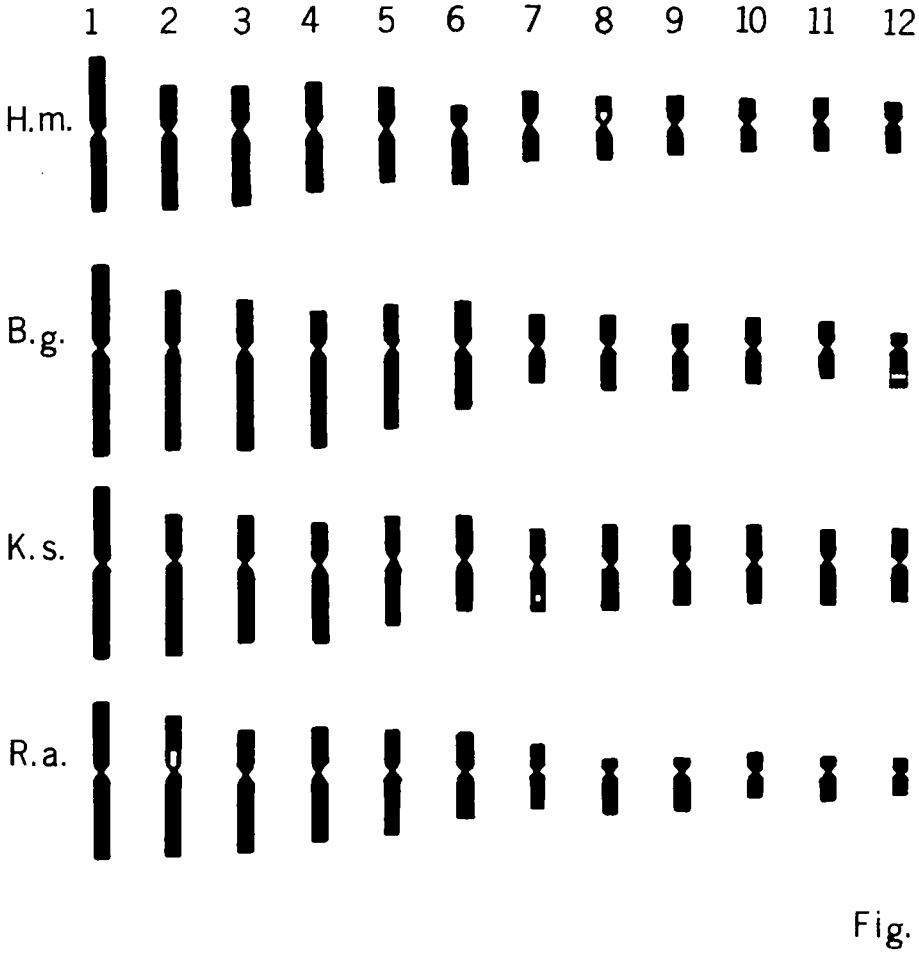

Fig. 3. — Diagram showing the idiograms of *Hemisus marmoratus*, *Breviceps gibbosus*, *Kassina senegalensis* and *Rana arvalis*. The clear outlines indicate the various heterochromatic areas.

the idiograms of the species studied, constructed by showing one chromosome for each pair of homologues of each species, the length of which has been obtained from the average of the values of absolute length caulculated on

the various metaphase plates examined, and the centromere position from the values of CI and r shown in Table, and by indicating the various heterochromatic areas with clear outlines.

Examination of the Table and the diagram and of the data of the literature relative to *Breviceps gibbosus*, *Kassina senegalensis* and *Rana arvalis*:

1) *Breviceps gibbosus* has 6 pairs of large chromosomes, which are sm, except those of the 1st and 6th pairs which are m, and 6 pairs of smaller chromosomes, which (unlike what is observed in *Hemisus*) differ markedly from the former with regard to their relative length. Among the latter, those of the 9th pair are sm, those of the 12th are st and all the others are m. The chromosomes of the 12th pair show an extensive heterochromatic area on the long arm, in an almost terminal position.

2) *Kassina senegalensis* reveals chromosomes that are somewhat difficult to subdivide into two groups, though there are some slight differences between the smaller chromosomes of the first group and the larger ones of the second. The chromosomes are all m, except those of the 2nd, 3rd and 4th pairs, which are sm. In the chromosomes of the 7th pair, a heterochromatic area may be noted on the long arm in a distal position.

3) *Rana arvalis* has 6 pairs of large chromosomes and 6 pairs of smaller ones, which are fairly sharply distinguishable from the former by having a shorter relative length. The large chromosomes, except those of the 3rd pair which are sm, are all m; among the small ones, those of the 7th and 10th pairs are m, those of the 8th and 9th pairs are st and those of the 11th and 12th pairs are sm. In this species it is often possible to find various heterochromatic areas in the colchicinized chromosomes, the most constant and extensive of these being situated on the short arm of the chromosomes of the 2nd pair, near the centromere.

DISCUSSION

I intend to make a brief comparison between the karyotype of *Hemisus* and that of the three above-mentioned species.

As regards *Hemisus* and *Breviceps*, appreciable differences may be noted, in the relative length and in the centromere position, between the chromosomes of the 6th pair in the two species (which are m and larger in *Breviceps* but are st in *Hemisus*); other differences may be found in the centromere position of the 4th pair (sm in *Breviceps* and m in *Hemisus*), while there are less marked differences in the centromere position of the chromosomes of the 5th and 9th pairs in the two species.

As regards *Kassina senegalensis*, it may be noted that the karyotype of this species differs from that of *Hemisus* as regards the relative length of the

chromosomes of the 3rd pair (shorter in *Hemisus*), the centromere position of those of the 6th pair (eccentric in *Hemisus*) and, in particular, the shape and sizes of the small chromosomes, which in *Kassina* are all m and generally of much greater relative length than the corresponding homologues in *Hemisus*.

Lastly, if the karyotype of *Hemisus* is compared with that of *Rana arvalis*, a certain similarity may be noted between the larger chromosomes in the two species, except between those of the 2nd and 6th pairs, which in *Hemisus* are smaller and are sm and st respectively, while in *Rana* they are m. However, there are greater differences to be noted between the small chromosomes of the two species, which in *Rana* are generally of shorter relative length and are m, sm and st, whereas in *Hemisus* they are all m, except those of the 12th pair, which are sm.

In general, therefore, the karyotype of *Hemisus marmoratus* is fairly similar to that of the other species examined, as regards the relative length and the centromere position of the large chromosomes, whereas greater differences may be noted between the four species as regards the morphological characteristics of the smaller chromosomes.

Furthermore, examination of the diagram in Fig. 3 shows that the chromosomes of *Hemisus* generally have a shorter absolute length than the corresponding chromosomes of *Breviceps* and *Kassina*, though it is fairly similar to that of the chromosomes of *Rana arvalis*.

It is extremely difficult to interpret the karyological relations of *Hemisus* with the species of the three families considered.

In fact, the resemblances and the differences between the karyotype of *Hemisus* and that of the other species occur in many different combinations, so that it is not possible to establish which of the three karyotypes is more similar to that of *Hemisus*. Moreover, it should be added that, even within the three above-mentioned families, greater differences are sometimes found between species of the same family than between species belonging to different families. For example, the karyotype of *Phrynomerus bifasciatus* (a specialized microhylid, with $2n=26$) is more similar to that of certain African *Ranidae* (*Mantella aurantiaca*) than to that of *Kaloula pulchra* or *Breviceps gibbosus*, even though the latter belong to the same family (cf. MORESCALCHI 1968*a*).

The fact that, in the *Microhylidae*, *Hyperoliidae* and *Ranidae*, it is possible to find species having karyotypes very similar to one another may also suggest the hypothesis that these forms of karyotype are the result of a converging karyological evolution in the three families.

This appears to be possible, since, according to WHITE's well-known principle of « homologous change » (WHITE 1954), there are some kinds

of endocellular factors that affect the structural chromosome changes, so that only some of all the possible types of rearrangement can actually establish themselves; consequently, in separate groups, sequences of structural changes of the same type may occur, giving rise to karyotypes that are morphologically similar to one another.

Furthermore, bearing in mind that, in all the three families considered, it is possible to find species with a 26-chromosome karyotype (a number which, since it is present in various species of all the families of higher Anura, is considered as basic for this large group of Amphibia), it would seem possible to assume that the complements of the species investigated here, with 24 chromosomes, may have arisen from 26-chromosome sets, by a mechanism possibly similar to that suggested by MORESCALCHI (1967) to explain the genesis of the karyotype of *Rana arvalis* from a 26-chromosome set, present in the older water frogs, through unequal translocations in two pairs of small chromosomes, which should have formed a single pair of larger chromosomes.

However, it is to be noted that also the 26-chromosome set of *Ranidae*, *Hyperoliidae* and *Microhylidae* is very similar in the three families.

Thus there remains the fact of the existence of a considerable karyological resemblance between species of families that, according to certain authors, may be systematically related to one another. In this respect, it should be mentioned that, while the various authors agree in assuming that the *Ranidae* and the *Hyperoliidae* have close affinities to one another (to the extent that they are sometimes grouped together in a single family, cf. POYNTON 1964), there is no agreement with regard to the affinities between the *Ranidae* and the *Microhylidae*: some authors consider them to be families phylogenetically alike (cf. NOBLE 1931; GRIFFITHS 1963; KLUGE and FARRIS 1969), whereas others maintain that the *Microhylidae* have had completely separate evolution from that of the *Ranidae* (cf. HECHT 1963; INGER 1967).

Consequently, if the karyology of *Hemisus* does not seem to offer definite data for a precise classification of this genus in one of the three families of Anura mentioned, the existence of such marked karyological similarities between *Hemisus* and species of the three families considered here may perhaps be an indication of a real relationship between all these forms.

REFERENCES

COCHRAN D. M., 1961. — *Living Amphibians of the world.* Doubleday & Co., Garden City, New York.

DE VILLIERS C. G. S., 1931. — *Some features of the cranial anatomy of Hemisus marmoratus.* Anat. Anz., 71: 305-331.

—, 1933. — *Breviceps and Probreviceps: comparison of cranial osteology of two closely related Anuran genera.* Anat. Anz., 75: 257-276.

GRIFFITHS I., 1963. — *The phylogeny of the Salientia.* Biol. Rev., 38: 241-292.
HECHT M. K., 1963. — *A reevaluation of the early history of the frogs. Part II.* Syst. Zool., 12: 20-35.
INGER R. F., 1967. — *The development of a phylogeny of frogs.* Evolution, 21: 369-384.
KLUGE A. G. and FARRIS J. S., 1969. — *Quantitative phyletics and the evolution of Anurans.* Syst. Zool., 18: 1-32.
KOBAYASHI M., 1962. — *Studies on reproductive isolation mechanisms in brown frogs. I. Hybrid sterility.* J. Sc. Hirosh. Univ., 20: 157-179.
LAURENT R., 1951. — *Sur la nécessité de supprimer la famille des Rhacophoridae mais de créer celle des Hyperoliidae.* Rev. Zool. Bot. Afr., 14: 116-122.
LEVAN A., FREDGA K. and SANDBERG A. A., 1964. — *Nomenclature for centromeric position on chromosomes.* Hereditas, 52: 201-220.
MORESCALCHI A., 1967. — *Le relazioni tra il cariotipo di Anuri Diplasioceli: I. Il corredo cromosomico di alcuni Ranidae.* Caryologia, 20: 65-85.
—, 1968*a*. — *Initial cytotaxonomic data on certain families of Amphibious Anura (Diplasiocoela after* NOBLE). Experientia, 24: 280-283.
—, 1968*b*. — *Hypotheses on the phylogeny of the Salientia, based on karyological data.* Experientia, 24: 964-966.
MORESCALCHI A., GARGIULO G. and OLMO E., 1970. — *Notes on the chromosomes of some Amphibia.* J. Herpet., 4: 77-79.
NOBLE G. K., 1931. — *The biology of the Amphibia.* McGraw-Hill Co., New York.
PARKER H. W., 1934. — *A monograph of the frogs of the family Microhylidae.* British Museum of Natural History, London.
POYNTON J. C., 1964. — *The Amphibia of Southern Africa: a faunal study.* Ann. Nat. Mus., 17: 1-334.
RABELLO M. N., 1970. — *Chromosomal studies in Brazilian Anurans.* Caryologia, 23: 45-59.
SATO I., 1936. — *Notes on the chromosomes of Cacopoides tornieri and Bufo radderi.* Zool. Mag., 48: 958-960.
SCHIØTZ A., 1967. — *The treefrogs (Rhacophoridae) of West Africa.* Spolia Zool. Mus. Haun., 25, Copenhagen.
SETO T., 1965. — *Cytogenetics studies in lower Vertebrates. II. Karyological studies of several species of frogs (Ranidae).* Cytologia, 30: 437-446.
ULLERICH F. H., 1967. — *Weitere Untersuchungen über Chromosomenverhältnisse und DNS-Gehalt bei Anuren (Amphibia).* Chromosoma (Berl.), 21: 345-368.
WHITE M. J. D., 1954. — *Animal cytology and evolution.* Cambridge University Press.
WICKBOM T., 1945. — *Cytological studies on Dipnoi, Urodela, Anura and Emys.* Hereditas, 31: 241-346.
WITSCHI E., KODANI M. and MIKAMO K., 1958. — *Comparative study of the chromosomes of European, American and Japanese frogs.* Anat. Rec., 131: 610-611.

SUMMARY

The karyotype of *Hemisus marmoratus*, an Anuran Amphibian of debated taxonomic position, consists of 24 chromosomes, with 6 pairs of large chromosomes (of which those of the 1st, 4th and 5th pairs are metacentric, those of the 2nd and 3rd pairs are submetacentric and those of the 6th pair are subtelocentric) and 6 pairs of smaller chromosomes, all metacentric, except those of the 12th pair, which are submetacentric.

Comparing the karyotype of *Hemisus* with that of species having the same chromosome number and belonging to the families which, according to the systematists, have the closest affinities with genus (*Microhylidae*, *Hyperoliidae* and *Ranidae*), considerable similarities may be noted between *Hemisus* and all the other species examined.

The existence of such marked karyological similarities between *Hemisus* and species of the three families considered suggests a real phyletic affinity between all these forms.